Abstracts

Explanation of symbols:
*No symbol = Member; * = Nonmember; ** = Emeritus or senior member*

1. Mapping the Sulfhydryl Groups of the Human Erythrocyte Membrane. RICHARD E. ABBOTT* AND DAVID SCHACHTER,** New York.

Using oriented membrane probes of the form *R-a-X*, where *R* = a membrane impermeant group, *a* = an arm of variable length, and *X* = a membrane-reactive substituent, we are mapping the –SH groups of human erythrocyte membranes. Two radioactive probes have been synthesized and used; in both *X* = a maleimide group and the arm lengths are small so that reaction is limited to the membrane surface. One probe, dextran-maleimide, has *R* = a dextran moiety, and the other, glutathione-maleimide, has *R* = S-alkylated glutathione. The –SH groups of the outer (exofacial) membrane surface have been quantified by reaction of [^{3}H]dextran-maleimide with intact erythrocytes and the value obtained is 7.5×10^3 (range: $3.9–11.6 \times 10^3$) –SH groups/μm^2 surface area. To estimate –SH of the inner (endofacial) surface, intact erythrocytes were treated first with non-radioactive dextran-maleimide. This was followed by hypotonic lysis and incubation of the ghosts obtained with [^{3}H]dextran-maleimide. The average value for endofacial –SH is 133×10^3 (range: $107–174 \times 10^3$) per μm^2 surface area. Further treatment of ghosts with [^{14}C]NEM yielded values for –SH in the membrane interior, and these are 1027×10^3 (range: 661–1521) per μm^2. Studies with [^{35}S]glutathione-maleimide give similar results. Specific proteins can now be located within the membrane on the basis of accessibility to the probes. Exofacial labeling yields minimal radioactivity in hemoglobin or "spectrin," but the glycoprotein fraction and a newly identified fraction precipitated by Triton X-100 treatment are very highly labeled. Exofacially or endofacially labeled proteins solubilized with SDS have been separated by Sephadex-gel filtration and polyacrylamide gradient gel electrophoresis. The endofacial label is more broadly distributed, and autoradiography of gels reveals at least 11 distinct bands. (Supported by NIH Grants AM04407 and AM01483.)

2. Dynamics of Dipeptide Metabolism and Its Effects on Free Amino Acid Pools, Glucose Levels, and Insulin Secretion. SIAMAK ADIBI, BARBARA KRZYSIK,* ALLAN DRASH,* AND JACQUELINE PETERSON,* Pittsburgh, Pa.

In view of the absence of any information on metabolism of dipeptides when they enter the body, studies were designed to investigate this problem. Either glycylglycine or glycylleucine (150 μmol/300 g body weight) was injected through jugular vein in rats. After 2 min the plasma levels of these dipeptides were 1.1 ± 0.1 and 0.8 ± 0.1 μmol/ml, respectively. The plasma half-life was longer for glycylglycine than for glycylleucine (245 vs. 126 s), but by 40 min both had completely disappeared from plasma. There were marked increases (0.9 to 1.1 μmol/ml) in plasma-free glycine and free leucine levels after glycylleucine injection. The rise and subsequent fall in the level of each of these amino acids were similar after glycylleucine or an equivalent mixture of free glycine and free leucine. However, the initial increase in plasma free glycine level was smaller after injection of glycylglycine than an equivalent amount (300 μmol/300 g body weight) of free glycine (2.3 vs. 3.0 μmol/ml, $P < 0.01$). Furthermore, there was a more rapid decline in plasma free glycine level after injection of free glycine than glycylglycine. Although neither of these dipeptides was detected in the liver, muscle, or kidney, the intracellular free amino acid pools in these tissues were markedly expanded after each injection. These expansions were similar whether dipeptide or its equivalent free amino acid mixture was administered. Although both glycylleucine and the corresponding free amino acid mixture stimulated insulin secretion to the same extent (maximal increases of 120 vs. 140 μU/ml, P = not significant), only the mixture of free glycine and leucine resulted in a marked hyperglycemia (an increase of over 100 mg/100 ml). It is concluded that dipeptides offer physiological advantages over free amino acids when administered intravenously. They lessen (*a*) the osmotic load, (*b*) the wide fluctuation in plasma free amino acid levels, and (*c*) hyperglycemia. (Supported by NIH Grants AM 15855 and AM 15861.)

3. The Prognostic Significance of Serum DNA-Binding Capacity in Patients with Lupus Nephritis. MARK K. ADLER,* ALEXANDER BAUMGARTEN,* BARRY HECHT,* AND NORMAN J. SIEGEL,* New Haven, Conn. (introduced by Stephen E. Malawista).

21 patients with systemic lupus erythematosus, aged 8–34 yr, were studied. All of these patients had a proliferative glomerulonephritis associated with subendothelial deposits, and all were treated with azathioprine (2–3 mg/kg/per day) and prednisone (10–20 mg/day). On the basis of multiple determinations of DNA-binding capacity of serial serum samples collected over many months to years, the patients were divided into two serological groups: (A) 13 patients in whom the initial DNA-binding capacity was variable (peak 94%), but in whom binding remained at or steadily decreased to levels below 60% within 6 months of the initial determination; and (B) 8 patients with DNA-binding capacity that remained over 70% for 9 consecutive months or more. The clinical course of each of the patients was independently evaluated. All patients had had the following studies at least every 6 months: urine analysis, blood urea nitrogen, serum creatinine, total serum protein, albumin:globulin ratio, antinuclear antibody, C3 globulin, and quantitative urinary protein excretion. At the end of follow-up, which ranged from 13 months to 5 yr, each patient's condition was categorized, on the basis of renal function and quantitative protein excretion, as either improved-stable or deteriorated. All of the 13 group A patients were considered improved-stable, while all of the 8 group B patients exhibited progression of their disease. The latter group includes the only three patients who died of lupus, and the only three who are presently on chronic dialysis. This study suggests that persistently elevated serum DNA-binding capacities may be of value in identifying patients who are having otherwise undetectable progression of their disease while on therapy. (Supported in part by Grants AM-5639 and AM-10493 from the NIH and grants from The Arthritis Foundation.)

4. Fibrinogen New York: an Abnormal Fibrinogen Associated with Thromboembolism. HAMID AL-MONDHIRY,* SOPHIE BILEZIKIAN,* AND HYMIE L. NOSSEL, New York.

A 54 yr old woman presented with a 23 yr history of repeated life-threatening thromboembolism. The presence of a qualitatively abnormal fibrinogen was suggested by demonstrating delayed and incomplete coagulation of plasma or partially purified fibrinogen by thrombin or reptilase. This defect was partially corrected by calcium. The plasma fibrinogen concentration was 0.50–1.64 mg/ml when estimated by heat turbidity, clottability,

or immunological techniques. The serum contained 80–820 μg/ml of unclottable fibrinogen-related materials even after 24 h exposure to thrombin. Ouchterlony double-diffusion immunoprecipitation showed no difference between normal fibrinogen or fibrin and that of the patient. The patient's plasma did not inhibit the coagulation of normal plasma. Plasma antithrombin III level was normal. Studies of the kinetics of thrombin action on fibrinogen demonstrated markedly impaired polymerization of preformed fibrin monomers. The patient's fibrinogen is tentatively designated fibrinogen New York; its possible identity with one of the previously described abnormal fibrinogens has not been excluded.

5. A Micropuncture Study of the Natriuresis Produced by Arterial Infusions of Substance P in the Rat. WILLIAM J. ARENDSHORST,* MARGARET A. COOK,* AND IVOR H. MILLS,* Chapel Hill, N. C. (introduced by William E. Lassiter**).

Substance P causes a natriuresis when infused into the renal artery of dogs, an effect associated with increased kallikrein excretion. To determine if this polypeptide affects sodium excretion and proximal salt and water reabsorption in the nondiuretic rat, late proximal collections of tubular fluid were performed in conjunction with clearance measurements. Saline was infused into the aorta above both renal arteries through a 30 gauge needle during the control period; then substance P in saline was administered. Two doses of substance P (50 and 10–20 pg/min) were given to two groups of animals. Substance P at 50 pg/min (six rats) increased urine flow (V) from 3.4 to 4.6 μl/min ($P < 0.001$) and sodium excretion ($U_{Na}V$) from 203 to 376 nEq/min ($P < 0.05$) with no significant change in glomerular filtration rate of single nephrons (SNGFR), 26.2 vs. 28.1 nl/min, or of the kidney (GFR), 0.98 vs. 1.00 ml/min. Mean tubular fluid to plasma inulin concentration ratio (F/P_{In}) in the last accessible proximal convolution fell from 2.30 to 1.82 ($P < 0.005$). Substance P had no effect on renal plasma flow ($RPF = C_{PAH}/E_{PAH}$) or arterial pressure. The presented values are for the micropunctured kidney. Similar increases in V and $U_{Na}V$ without a change in GFR were also observed in the contralateral kidney. Substance P at 10–20 pg/min (six rats) produced no statistically significant changes in any of the measured parameters: V = 3.3 and 3.3 μl/min; $U_{Na}V$ = 154 and 272 nEq/min; GFR = 0.93 and 0.86 ml/min; F/P_{In} = 1.94 and 1.91; SNGFR = 27.4 and 26.3 nl/min. In summary, aortic infusions of substance P at 50 pg/min produce a natriuresis and diuresis in the rat, and proximal fractional reabsorption is reduced while SNGFR, GFR, and RPF remain unchanged. The natriuresis after substance P infusion is thus due at least in part to the inhibition of sodium reabsorption in the proximal tubule. This effect might be mediated by intrarenal formation of bradykinin.

6. Secretion of Nerve Growth Factor by Cancer Cells. BARRY G. W. ARNASON,* JOEL OGER,* NICHOLAS J. PANTAZIS,* AND MICHAEL YOUNG, Boston, Mass.

Chromatographically, electrophoretically, and immunoelectrophoretically pure mouse submaxillary gland nerve growth factor (NGF) has been prepared and coupled covalently to bacteriophage T_4. When the resulting T_4-NGF conjugate is treated with monospecific rabbit antibody to NGF, the virus particles lose their infectivity for *E. coli*. Using this property, we have developed a phage immunoassay which detects concentrations of NGF as low as 1 ng/ml. Mouse L-cells (clone 929) injected into mice produce highly malignant sarcomas. L-cells are also known to be a source of factors ("conditioned tissue culture medium") which promote growth of other unrelated cells in culture. We now find that one of these factors is NGF. L-cells cultured together with chick embryonic sensory ganglia stimulate profuse neurite outgrowth, and the neurites grow toward the L-cells. Moreover supernatants of L-cell cultures contain considerable amounts of a protein which is immunochemically similar, if not identical, to NGF when assayed by the phage method. Similar studies demonstrate that 3T3 and SV40-transformed 3T3 fibroblasts also secrete NGF. Taken together, present evidence indicates that production of NGF is a general property of fibroblasts in culture. The biological function of NGF in vivo remains obscure, but a nerve growth-promoting factor is known to be present in granulation tissue, and a role for NGF produced by activated fibroblasts during wound healing is possible. Studies with human glioblastoma in culture have shown that these cells also produce a substance which reacts with antibody to mouse NGF. Since this tumor is known to produce "tumor angiogenesis factor" (TAF), there could be a relationship between NGF and TAF. (Supported by grants from NIH.)

7. Pathophysiology of Hemolytic Anemia in Man: Interaction of IgM Antibody, Complement, and Specific Macrophage Receptors. J. P. ATKINSON* AND M. M. FRANK, Bethesda, Md.

Purified human IgM isoagglutinins were utilized to sensitize ^{51}Cr-labeled erythrocytes with a known number of complement-fixing sites. These cells were then reinfused into the erythrocyte donor. A minimum of 20 C1-fixing sites per erythrocyte were required for decreased survival. As the amount of antibody coating the erythrocytes was increased, a larger percentage was sequestered. With 80 C1-fixing sites, more than 75% of the injected red cells were removed from the circulation within 10 min. In each case the clearance pattern consisted of rapid hepatic sequestration followed by a gradual return of a portion of the erythrocytes into the circulation where they survived normally. The mechanism of this previously undescribed clearance pattern was examined. Clearance was complement dependent since sensitized cells survived normally in angioedema patients with low levels of C4 and no detectable C2. Exposure of sensitized cells to fresh serum for 15 min led to the deposition of 550–800 C3b molecules per C1-fixing site. Such cells were immune adherence positive, agglutinated by anti-C3b, formed rosettes with human alveolar macrophages, and were sequestered in vivo, presumably due to binding to the C3b receptor on hepatic macrophages. After exposure to the C3b inactivator, the cells were immune adherence negative, agglutinated only by anti-C3d, did not form rosettes with macrophages, and survived normally in vivo despite being Coombs positive. Cleavage of cell-bound C3b to C3d may explain the release phase of the IgM clearance pattern. Whereas red cells coated with IgM antibody and complement were previously thought to be sequestered in the liver because of extensive membrane damage, these experiments suggest that clearance is determined by the interaction of complement with specific receptors on macrophages.

8. Altered Disposition of Diazepam in Liver Disease. GEORGE R. AVANT,* ULRICH KLOTZ,* GRANT R. WILKINSON,* ANASTACIO HOYUMPA,* AND STEVEN SCHENKER, Nashville, Tenn.

The pharmacokinetics of diazepam after single dose (0.1 mg/kg intravenously or 10 mg orally) administration were compared in drug- and alcohol-free normal volunteers, nine patients with alcoholic cirrhosis, and eight individuals with acute viral hepatitis. After intravenous injection, plasma levels of diazepam fell bi-exponentially, and the data was analyzed according to a two-compartment open model, allowing calculation of total plasma clearance ($\overline{Cl}$), volume of distribution of the central compartment (V_1), and terminal half-life ($t_{½(\beta)}$) of diazepam. In 35 normal controls there was a significant ($P < 0.001$) increase in $t_{½(\beta)}$ of diazepam with age, resulting in a fourfold increase in $t_{½}$ between ages 20 and 80. This was primarily due to an increase

in V_1 ($P = 0.003$); $\overline{Cl}$ and plasma protein binding did not change significantly with age. The data obtained in patients with alcoholic cirrhosis and acute viral hepatitis was compared with those from two age-matched control groups. The cirrhotic patients showed a more than twofold increase in $t_{1/2(\beta)}$ (mean value 105.4 h vs. 47.6 h, $P < 0.001$). This resulted primarily from a decrease in $\overline{Cl}$ (mean value 13.8 ml/min vs. 27.1 ml/min, $P < 0.001$). V_1 changed only marginally (mean value 0.305 liter/kg vs. 0.423 liter/kg, $P = 0.052$). Plasma protein binding of diazepam in cirrhosis was decreased to 95.3% from 97.5% in normals ($P < 0.001$). Subjects with viral hepatitis, during the acute phase of illness, had significant prolongation of $t_{1/2(\beta)}$ (75.4 h vs. 30.5 h, $P < 0.005$). Follow-up studies performed just as liver function studies returned to normal revealed $t_{1/2(\beta)}$ to have fallen almost to normal. Neither $t_{1/2(\beta)}$ nor $\overline{Cl}$ correlated with any liver function test. This impairment of diazepam elimination with hepatic dysfunction suggests that the drug should be used with caution in such patients.

9. Modification by Insulin of Adipocyte Phosphopeptide Metabolism. JOSEPH AVRUCH,* GUY LEONE,* AND DONALD B. MARTIN,** Boston, Mass.

We have studied [^{32}P]phosphopeptide metabolism in intact adipocytes and purified subcellular fractions prepared therefrom. Adipocytes incubated in Krebs-Ringer bicarbonate buffer, containing 0.5 mM $NaH_2{}^{32}PO_4$, incorporate ^{32}P into protein in all subcellular fractions studied (cytosol [C], plasma membrane [PM], endoplasmic reticulum [ER], mitochondria [M], nuclei [N]), attaining steady state between 1 and 2 h. Each subcellular fraction possesses a characteristic array of phosphopeptides when analyzed by SDS-gel electrophoresis, autoradiography, and scintillation counting. To evaluate acute effects of hormones on phosphopeptide metabolism, adipocytes were incubated for 2 h with $NaH_2{}^{32}PO_4$, exposed to hormone for 5 min, and disrupted. Epinephrine (1 μM) markedly enhanced the phosphorylation of a peptide, mol wt 68,000, located in the ER and cytosol. A phosphopeptide of mol wt 120,000 (ER, C) is also enriched, as are a host of minor phosphopeptides in the cytosol. The phosphopeptides of the PM are unaffected. Insulin (100 μU/ml) markedly enhanced the phosphorylation of a major PM phosphopeptide (mol wt 72,000), as well as the phosphopeptide of mol wt 120,000 (ER, C). Insulin plus epinephrine yielded further enhanced phosphorylation of these two peptides, whereas the phosphorylation of the 68,000 peptide (ER, C) was inhibited well below the level seen with epinephrine alone. We have demonstrated protein kinase (PK), active on endogenous substrates, in purified adipocyte PM, M, and cytosol. Cyclic adenosine monophosphate-(cAMP-) stimulated PK is restricted to the cytosol. The PM-PK is unresponsive to cAMP and cyclic guanosine monophosphate (cGMP), and its pH optimum is different from the cytosol activity. These findings demonstrate that insulin at physiologic levels can acutely alter protein phosphorylation in a manner not explained by alterations in cAMP metabolism. We propose that the effects of insulin on protein phosphorylation are mediated in part by another, yet undefined, second messenger. (Supported by NIH and The Hartford Foundation.)

10. The Influence of Cyclic Nucleotides on Granulocyte Superoxide Production. B. M. BABIOR, J. T. CURNUTTE,* AND R. S. KIPNES,* Boston, Mass.

Assembly of metabolically activated granulocytes is a feature of the inflammatory process. Earlier investigations showed that granulocytes produce superoxide (O_2^-), a compound possibly involved in oxygen-dependent bacterial killing. The O_2^- production rate appears to be a reliable index of granulocyte metabolic activity. We studied the effect of cyclic nucleotides on O_2^- production by granulocytes to obtain information regarding their influence on granulocyte metabolic activity. The effect of the nucleotide analogs N^6,$O^{2'}$-dibutyryl cyclic adenosine monophosphate (cAMP) and N^2,$O^{2'}$-dibutyryl cyclic guanosine monophosphate (cGMP) was investigated. With nucleotides omitted, O_2^- production by resting (no bacteria present) and stimulated (bacteria present) granulocytes was 8.8 ± 4.6 and 12.3 ± 0.6 nmoles/1.5 × 10^6 cells per 30 min, respectively. With 0.5 mM cAMP, resting O_2^- production fell to 45% of control ($P < 0.1$) and stimulated O_2^- production to 62% of control ($P < 0.05$). Lower concentrations inhibited to a smaller extent. Suppression of O_2^- production by cAMP was confirmed by findings that aminophyllin (0.5 mM) reduced stimulated O_2^- production to 29% of control ($P < 0.01$). (The effect of aminophyllin on resting cells was not significant.) With cGMP, results were inconclusive. At 0.5 mM, resting O_2^- production tended to rise and stimulated O_2^- production to fall, but differences were not significant. cGMP had little effect on cAMP-induced inhibition of O_2^- production. The parasympathomimetic agent carbachol, which increases tissue cyclic GMP levels, showed no consistent effect. Sympathomimetic catecholamines, which raise tissue cyclic AMP, were not tested because of interference with the assay. These results indicate that cyclic AMP influences granulocyte oxygen metabolism, suggesting that agents affecting intragranulocytic cAMP concentrations may thereby affect the inflammatory process. (Research supported by NIH and The Medical Foundation.)

11. Effect of Nitroglycerin on Distribution of Myocardial Blood Flow During Ischemia-Induced Coronary Vasodilation. ROBERT J. BACHE,* FREDERICK R. COBB,* AND JOSEPH C. GREENFIELD, JR., Durham, N.C.

Studies were performed to determine whether vasodilation distal to a flow-limiting coronary artery stenosis results in redistribution of myocardial blood flow with subendocardial underperfusion, and if this occurred, whether nitroglycerin could restore normal subendocardial perfusion. Eight awake dogs with electromagnetic flowmeters and hydraulic occluders on the left circumflex coronary artery were studied. A 5 s coronary artery occlusion resulted in reactive hyperemia (RH) with excess arterial inflow effecting 415 ± 20% (SE) repayment of the blood flow debt incurred during occlusion. When, after a 5 s occlusion, coronary inflow was restricted to the preocclusion rate for 20 s before complete release, the delayed RH was increased (debt repayment = 780 ± 75%, $P < 0.01$), suggesting regional myocardial ischemia continued during the interval of vasodilation when coronary inflow was held at the preocclusion rate. 5 min after nitroglycerin (0.015 mg/kg), a 5 s occlusion followed by a 20 s restriction of inflow to the preocclusion rate resulted in RH which was not augmented above the control response (debt repayment = 405 ± 70%), suggesting correction of regional ischemia by nitroglycerin. Distribution of myocardial blood flow was examined by injecting 7–10 μm radioactive microspheres into the left atrium. When the posterior left ventricular wall was sectioned into inner (I) and outer (O) layers, the control I:O ratio was 1.19 ± 0.03. When inflow was held at the preocclusion rate after a 5 s total occlusion, the I:O ratio was 0.68 ± 0.04, indicating subendocardial underperfusion. Nitroglycerin administration corrected this maldistribution of myocardial blood flow during ischemia-induced vasodilation, returning the I:O ratio to 1.25 ± 0.12. Thus, coronary vasodilation in the presence of a proximal coronary artery stenosis results in redistribution of myocardial blood flow with subendocardial ischemia, and nitroglycerin is capable of correcting this maldistribution. (Supported by NIH grant.)

12. Krebs Cycle-Generated Adenosine Triphosphate (ATP) As an Alternative Source of Energy for Phagocytosis by Polymorphonuclear Leukocytes (PMN). ROBERT L. BAEHNER, Indianapolis, Ind.

The energy required by PMN for phagocytosis is normally supplied by ATP generated from anaerobic glycolysis. Inhibition of glycolysis results in diminished phagocytosis. However, several Krebs cycle substrates were found to be capable of correcting defective phagocytosis induced by 5 mM 2-deoxyglucose (2-DG), a potent inhibitor of glycolysis. After incubation with 2-DG for 2 h (T_2), guinea pig PMN monolayers exhibited a markedly diminished rate of uptake of [^{14}C]*Staphylococcus aureus* coincident with a fall in ATP levels which reached 37.3 ± 7.9% of 0 h (T_0) control (compared to 90.3 ± 8.2% for control at T_2). Addition of 5 mM of either pyruvate, citrate, succinate, or L-alanine to suspensions of PMN containing 2-DG resulted in significant improvement of phagocytosis by PMN monolayers and restoration of ATP to levels between 70.8 and 93.7% of T_0 control. Moreover, the rate of evolution of $^{14}CO_2$ from [2-^{14}C]pyruvate, [^{14}C]citrate, [^{14}C]succinate, or [^{14}C] L-alanine was increased by 37.5–56.5% in the 2-DG-treated PMN. These studies indicate that the Krebs cycle can serve as an alternative source of ATP for PMN with defective glycolysis, provided the necessary substrates are available. (Research supported by NIH Grant AI 10892 and Riley Memorial Association.)

13. Apolipoprotein Composition and Lipoprotein Lipase (LPL) Activation in Experimental Diabetes Mellitus. HANOCH BAR-ON,* PAUL S. ROHEIM,* AND HOWARD A. EDER,** Bronx, N. Y.

Diabetes mellitus was produced by administration of streptozotocin to rats fed diets high in sucrose. Within 2 days, serum lipid concentrations rose with the mean triglyceride concentration rising to 950 mg/100 ml and cholesterol to 170 mg/100 ml. All lipoprotein classes increased, but the greatest increase was in the very low density lipoprotein. The composition of the subunit proteins of the apolipoproteins was determined by polyacrylamide-disc electrophoresis and isoelectric focusing. The concentration of the "C" proteins, the small molecular weight proteins present in both the very low and high density lipoproteins, was increased. The C-III proteins, which have proline as the carboxy terminal amino acid, increased disproportionally to the C-II proteins, which have glutamic acid in the carboxy terminal position. These proteins are known to affect the activity of lipoprotein lipase. Therefore, the activation of LPL prepared from rat adipose tissue was measured after the addition of increasing amounts of serum from control and from diabetic rats. With addition of increasing amounts of serum from the control rats, lipoprotein lipase activity increased and reached maximal levels with the addition of 0.2 ml of serum. With serum from diabetic rats, maximal activity was observed after the addition of ca. 0.01 ml of serum. However, the further addition of serum from diabetic rats produced marked inhibition of LPL activity. When increasing amounts of diabetic serum were added to an incubation mixture maximally activated by control serum, inhibition of this activation occurred. These findings may be due to the relative preponderance in diabetic serum of the C-III apoprotein, which has been shown to be an inhibitor of LPL. (Research supported by Grants HL 02965 and HL 14236 from NIH.)

14. A Molecular Basis for Early Events in Response to Thyroid Hormone. J. D. BAXTER,* M. A. CHARLES,* G. RYFFEL,* B. J. MCCARTHY,* AND K. M. MACLEOD,* San Francisco, Calif. (introduced by I. S. Edelmann**).

Several lines of evidence suggest that some responses to thyroid hormone depend upon its interaction with nuclear and chromatin binding sites. These sites are extractable and probably protein. In this study, high affinity ($Kd \sim 4 \times 10^{-10}$M), limited capacity (~4 pmol/ml DNA) binding of [^{125}I]triiodothyronine (T_3) by isolated nuclei or chromatin from rat liver or HeLa cells was demonstrated. The possibility that the DNA chromatin component binds T_3-"receptor" complexes was examined by incubating extracted T_3 complexes with purified rat DNA. When DNA was separated from free T_3 and DNA-free proteins by agarose filtration, T_3 eluted with DNA. In contrast, free T_3 and T_3 bound by plasma proteins did not associate with DNA. This suggests that DNA binds T_3 complexes. Most DNA in mammalian chromatin is not transcribed. Since chromatin containing DNA active in transcription can be separated from that which is inactive, the possibility that T_3 complexes are present in active chromatin was explored. Purified chromatin (protein-DNA ratio ~ 1.4) was sheared and separated by sucrose gradient sedimentation into its two major fractions. The slowly sedimenting fraction (~ 25% of the DNA) contains the active chromatin and has template activity using exogenous bacterial polymerase. The T_3-"receptor" sites were selectively concentrated in the active chromatin fraction (4-fold) when compared with the inactive fraction. These observations support the following hypotheses: (*a*) T_3 binds specifically to isolated chromatin; (*b*) DNA may be involved in chromatin localization of T_3 complexes; and (*c*) T_3-"receptor" complexes become preferentially localized in the chromatin subfraction active in transcription. The nonrandom distribution of complexes in chromatin is consistent with direct hormonal mediation of genetic expression. (Supported by NIH, NSF, and ACS.)

15. Increased 5-Phosphoribosyl-1-Pyrophosphate (PRPP) Synthetase Activity and Gout: Diversity of Structural Alterations in the Enzyme. MICHAEL A. BECKER,* LAURRENCE J. MEYER,* PAUL J. KOSTEL,* AND J. EDWIN SEEGMILLER, La Jolla, Calif.

Increased activity of 5-phosphoribosyl-1-pyrophosphate (PRPP) synthetase is a rare dominantly inherited cause of excessive purine production and clinical gout. In the two known families with this abnormality, different structural alterations have been demonstrated in PRPP synthetase, which catalyzes synthesis of the regulatory intermediate PRPP from ATP and ribose-5-phosphate (R5P). In partially purified extracts of the enzyme of affected members of one family, increased electrophoretic mobility on cellulose-acetate gel has been demonstrated as well as a lower isoelectric point and an increased immunochemical inactivation per unit of specific PRPP synthetase antiserum. These findings suggest that the enzyme has increased activity per molecule, and this is confirmed in comparative studies of purified normal and mutant erythrocyte PRPP synthetases. The enzymes, purified 4500-fold to near homogeneity and to comparable purity show: (*a*) 2.2-fold higher specific enzyme activity for the mutant PRPP synthetase; (*b*) 2.4-fold greater inactivation of mutant enzyme activity per unit of antiserum; (*c*) more rapid thermal inactivation of mutant enzyme (55°); (*d*) nearly identical affinity and inhibitory constants but markedly different maximal velocities; and (*e*) identical subunit molecular weights of 32,000 daltons. Increased enzyme specific activity provides a molecular mechanism for increased PRPP production which, in turn, provides a pathogenetic mechanism for the increased purine synthesis in this family. An unrelated patient with excessive purine production and gout but normal PRPP synthetase activity, when assayed at saturating substrate concentrations, has a PRPP synthetase with increased affinity for R5P. Fibroblasts cultured from this patient have diminished R5P and increased PRPP concentrations indicating still another distinct molecular alteration in this enzyme leading to purine overproduction. (Research supported by NIH Grants AM 13622, AM 05646, and GM 17702.)

16. Impaired Myocardial Uptake of Cardiac Glycosides After Coronary Reperfusion in Acute Myocardial Ischemia. GEORGE A. BELLER,* WILLIAM B. HOOD, JR., AND THOMAS W. SMITH, Boston, Mass.

The effect of coronary reperfusion on the myocardial uptake of [^{3}H]digoxin and [^{3}H]ouabain was studied in 36 dogs in which anterior wall ischemia was produced by snaring confluent branches of the left coronary system. Animals were reperfused 1, 2, and 6 h after occlusion. After 15 min of reperfusion, 1.0 mg of [^{3}H]digoxin or 0.5 mg of [^{3}H]ouabain was given intravenously, and 30 min (ouabain group) or 2 h (digoxin group) later the hearts were excised. Subendocardial (endo) and subepicardial (epi) samples from ischemic, border, and nonischemic zones were analyzed for [^{3}H]glycoside concentrations. Regional myocardial blood flow was also measured in animals receiving [^{3}H]ouabain utilizing 15-μm radioactive microspheres. In six dogs occluded for 6 h and reperfused, mean [^{3}H]digoxin concentration in the ischemic zone was markedly reduced to 15 ± 2% (SE) of the nonischemic concentration in endo and to 40 ± 4% in epi regions ($P < 0.05$). Border zone uptake was decreased to 46 ± 11% of nonischemic uptake in endo and 64 ± 4% in epi layers ($P < 0.05$). In six dogs reperfused after 2 h of occlusion, mean [^{3}H]digoxin concentrations were reduced to 46 ± 5% in endo and 65 ± 6% in epi layers of the ischemic zone ($P < 0.05$). In five dogs occluded for 1 h and reperfused, [^{3}H]digoxin uptake was comparable in endo and epi layers of all three zones. Similar reductions in [^{3}H]ouabain uptake were observed after reperfusion. Diminished cardiac glycoside binding could *not* be accounted for by the residual impairment of regional myocardial blood flow in ischemic and border zones. These findings indicate that restoration of coronary blood flow after 2–6 h of coronary occlusion is associated with altered digoxin and ouabain binding by reperfused ischemic myocardium consistent with ischemia-induced structural or functional alterations in the putative digitalis receptor, $(Na^+ + K^+)$-ATPase.

17. Renal Tubular Effects of Acute Unilateral Renal Denervation (AURD). ELSA BELLO,* ROMULO E. COLINDRES,* ELEANOR M. LIPHAM,* ENRIQUE PASTORIZA,* AND CARL W. GOTTSCHALK,** Chapel Hill, N.C.

Micropuncture studies were undertaken to delineate the renal responses to acute unilateral renal denervation (AURD) and the factors involved in these responses. AURD was produced in anesthetized nondiuretic rats by application of phenol to the left renal artery. Before and after this procedure the following measurements were performed: (*a*) inulin clearance (GFR), PAH clearance and extraction (RPF), urinary volume (V), and urinary sodium excretion ($U_{Na}V$); (*b*) single nephron filtration rate (SNGFR) and F/P inulin in late proximal tubules; and (*c*) hydrostatic pressures in proximal and distal tubules and postglomerular capillaries (servo-null method). The same studies were performed in sham-denervated animals. Denervation increased V (4.5 to 9.6 μl/min; $P < 0.001$) and $U_{Na}V$ (341 to 1699 nEq/min; $P < 0.001$) from the left kidney, without significant changes from the right. GFR and RPF remained unchanged. After denervation late proximal F/P inulin decreased (2.22 to 1.45; $P < 0.001$) while SNGFR remained unchanged (31 and 33 nl/min, P = not significant). In denervated kidneys free flow hydrostatic pressure increased in proximal (11.90 to 13.93 mm Hg; $P < 0.001$) and distal (6.5 to 8.59 mm Hg; $P < 0.001$) tubules. Peritubular capillary pressure increased (9.78 to 10.66 mm Hg, $P < 0.01$). Glomerular capillary pressure (estimated from the stop flow pressure and plasma oncotic pressure) remained unchanged. There were no changes in sham-denervated animals. In summary, AURD produces a diuresis and natriuresis associated with a decrease in proximal fluid reabsorption. The response appears to be unrelated to changes in renal hemodynamics, and no evidence for redistribution of glomerular filtrate was found. These results show an effect on proximal tubular function mediated by the renal nerves.

18. Evidence for Humoral Factor Responsible for the Hypercalciuria of Phosphate Depletion. CLARA BEN-ISAAC,* SHAUL G. MASSRY, SHELDON ROSENFELD, * CHARLES R. KLEEMAN,** AND MIRIAM BICK,* Los Angeles, Calif.

Phosphate depletion (PD) causes hypercalciuria due to decreased tubular reabsorption of Ca. The hypercalciuria is not prevented by thyroparathyroidectomy, administration of parathyroid extract, or intrarenal infusion of phosphate. A humoral factor may be responsible for hypercalciuria. This possibility was tested in in vitro perfusion of rabbit kidneys. With PD, produced in rabbits by adding $Al(OH)_3$ to diet for 2–3 months, serum P fell from 6.5 ± 0.08 (SE) to 1.5 ± 0.04 and serum Ca rose from 13.1 ± 0.08 to 15.7 ± 0.09 mg/dl without change in percent diffusibility; fractional excretion of diffusible Ca rose from 10.1 ± 0.73 to 29.1 ± 1.39%. Perfusion experiments included: (*a*) normal kidney + normal blood (control); (*b*) PD kidney + normal blood; (*c*) normal kidney + PD blood; (*d*) normal kidney + PD blood + phosphate to raise serum P to normal; and (*e*) normal kidney + normal blood + $CaCl_2$ to raise serum Ca to levels seen in PD. FCaE in perfusion studies were: control, 1.24 ± 0.33; PD kidney, normal blood: 8.1 ± 2.67 ($P < 0.01$); normal kidney, PD blood: 5.6 ± 1.47 ($P < 0.01$); normal kidney, PD blood phosphate: 4.5 ± 0.88 ($P < 0.01$); normal kidney, normal blood, $CaCl_2$: 1.6 ± 0.24 (P = NS). High FCaE of 8.9 ± 0.64 and 9.2 ± 1.88% was noted when perfusion of normal kidneys with a mixture of normal and PD blood with latter constituting 60% and 40% of the mixture, respectively. In most perfusion experiments with PD blood FNaE also increased. The data are consistent with the following. (*a*) Hypercalciuria of PD is not due to the fall in serum P or rise in serum Ca, per se. (*b*) A humoral factor is present in blood of PD animals and is responsible for hypercalciuria. (*c*) This factor is present in adequate concentration since smaller amounts of PD blood produced significant hypercalciuria. (*d*) The humoral factor may be attached to PD kidney and cause hypercalciuria during perfusion of such kidney with normal blood. (*e*) Intrinsic alteration in PD kidney may also contribute to hypercalciuria.

19. Determinants of Glomerular Filtration in Experimental Glomerulonephritis. C. M. BENNETT,* D. A. MADDOX,* T. M. DAUGHARTY,* R. J. GLASSOCK,* T. KWONG,* D. KNUTSON,* W. M. DEEN,* C. R. ROBERTSON,* AND B. M. BRENNER, San Francisco and Los Angeles, Calif.

Pressures and flows were measured in surface glomerular capillaries, efferent arterioles, and proximal tubules of 20 Wistar rats in the early autologous phase of nephrotoxic serum nephritis (NTN). Linear deposits of rabbit and rat IgG were demonstrated by immunofluorescence. Light microscopy revealed proliferative glomerulonephritis, and proteinuria was present. Although whole kidney and single nephron GFR in NTN (0.8 ml/min ± 0.1 SE and 27 nl/min ± 2, respectively) remained unchanged from values in nine weight-matched normal hydropenic control rats (0.8 ± 0.1 and 28 ± 2), important alterations in glomerular dynamics were noted. Mean transcapillary hydraulic pressure difference ($\overline{\Delta P}$) averaged 41 ± 1 mm Hg in NTN versus 34 ± 1 in controls ($P < 0.005$). Oncotic pressures at the afferent (π_A) end of the glomerular capillary were similar in both groups (~16 mm Hg) but increased much less by the efferent end (π_E) in NTN (29 ± 1 mm Hg) than in controls (35 ± 1, $P < 0.025$). Hence, equality between $\overline{\Delta P}$ and π_E, denoting filtration pressure equilibrium, obtained in control but not in NTN. While glomerular plasma flow rate was moderately higher in NTN (88 ± 8 nl/min) than in controls (66 ± 4, $P < 0.001$), the failure to achieve filtration equilibrium in NTN was primarily the consequence of

a marked fall in the glomerular capillary ultrafiltration coefficient, *Kf*, to a mean value of 0.03 nl/s·mm Hg), considerably lower than that found recently for the normal rat (0.08 nl/s·mm Hg). Thus, despite extensive glomerular injury, evidenced morphologically, and by the low *Kf*, GFR remained normal. This maintenance of GFR resulted from increases in $\overline{\Delta P}$ and glomerular plasma flow, both of which increase the net driving force for filtration, and thereby compensate for the reduction in *Kf*. (Supported by VA and NIH.)

20. Long-Term Lymphoid Cell Lines in the Study of Genetic Disease: Fucosidosis, Homocystinuria, and Gaucher's Disease. NICHOLAS BERATIS,* LYNN FLEISHER,* BRIAN TURNER,* CESARE DANESINO,* RICCARDO LONGHI,* GERALD GAULL,* AND KURT HIRSCHHORN,** New York.

We have established lymphoid lines from homozygotes and heterozygotes for fucosidosis, homocystinuria (cystathionine synthase deficiency), and Gaucher's disease. We have studied five enzymes in these and in normal lines under optimal assay conditions. Activity of arylsulfatase A and B in normal lines was unaffected by time in culture. Arylsulfatase A activity (mean ± SD) in 13 lines assayed from 20 days to 3 months after establishment was 47.4 ± 26.5 nmoles 4-nitrocatechol per mg protein per hour, while that of 11 lines maintained in culture from 12 to 25 months was 46.8 ± 20.6. Five of these lines were studied at both time intervals (51.0 ± 15.4 vs. 51.7 ± 28.1). Arylsulfatase B remained stable for at least 10 months (28.3 ± 6.5 vs. 28.9 ± 5.7). α-L-Fucosidase activity in lines from 11 normals, 1 heterozygote and 2 homozygotes for fucosidosis was 323.5 ± 91.2, 160.9, and 1.6 nmoles *p*-nitrophenol per mg protein per hour, respectively, with no overlap between the groups. Cystathionine synthase activity in lines from 10 normals, 3 heterozygotes, and 1 homozygote for homocystinuria was 9.49 ± 3.41, 3.21, and 0.8 nmoles cystathionine per mg protein per hour, respectively, with no overlap. β-Glucosidase activity in lines from 23 normals, 2 heterozygotes, and 1 homozygote for Gaucher's disease was 744.2 ± 724.3, 376.7, and 128.9 nmoles 4-methylumbelliferone per mg protein per 30 min, respectively, with overlap between adjacent groups on multiple determinations. These data together with previous studies in Lesch-Nyhan, citrullinemia, metachromatic leukodystrophy, and cystic fibrosis indicate that lymphoid lines retain their genotype and maintain quantitative enzymatic stability over long periods. Also their vigorous proliferation and apparent permanence make them preferable to other tissue culture systems in the study of genetic disorders.

21. Unique Relationship Between Bilirubin Turnover and Bilirubin Clearance in Type I Congenital Nonhemolytic Jaundice (CNJ). PAUL G. BERK,* BRUCE F. SCHARSCHMIDT,* JEANNE G. WAGGONER,* AND STEVEN C. WHITE,* Bethesda, Md. (introduced by Nathaniel I. Berlin).

Using a multicompartmental model of erythrokinetics, we have previously predicted that repeated phlebotomy would decrease bilirubin turnover (BRT) by skewing RBC age distribution toward young cells (1973 *J. Clin Invest.* **52:** 8a). If bilirubin clearance (CBR: ml/min per kg) remains constant, decreasing $\overline{BRT}$ should produce a proportional fall in plasma bilirubin concentration ($\overline{BR}$). Studies in patients with congenital spherocytosis confirmed that a marked fall in $\overline{BRT}$ after splenectomy produced no change in CBR, so that $\overline{BR}$ fell as expected. Accordingly, a 20 yr old woman with CNJ and kernicterus was phlebotomized 500 ml/wk for 4 months. Control values for total body hemoglobin (CO space), circulating red cell mass (^{51}Cr), and mean red cell lifespan (calculated from [^{3}H]bilirubin kinetics) were incorporated into the model, which predicted the following changes during phlebotomy: a 35% fall in BRT; a 230% increase in marrow precursor hemoglobin; a 45% fall in [^{51}Cr]RBC t½; and a characteristic alteration of the shape of the RBC survival curve after [^{14}C] glycine labeling. Measurements during phlebotomy confirmed each of these predictions. In particular, BRT fell by 31%. Unexpectedly, $\overline{BR}$ remained unchanged at 25.5 mg/100 ml. Analogous results were observed in phlebotomized Gunn rats. Kinetic studies demonstrated that the failure of $\overline{BR}$ to fall resulted from prolongation of the terminal radiobilirubin t½ and a fall in CBR in every instance. These studies indicate that (*a*) in CNJ, CBR is uniquely influenced by BRT, possibly because some of the alternate pathways of bilirubin metabolism in this disease may function only at a very high $\overline{BR}$; (*b*) kinetic studies and compartmental modeling are useful tools in clarifying the changes produced by therapeutic maneuvers; and (*c*) "the best laid schemes in rats and men gang aft a-gley."

22. In Vivo Evidence for an Antagonism Between Vasopressin (VP) and Prostaglandin (PG) in the Mamalian Kidney. T. BERL,* R. J. ANDERSON, K. M. MCDONALD, AND R. W. SCHRIER, Denver, Colo.

These studies were undertaken to examine whether an antagonism between VP and PG occurs in vivo in the mammalian kidney. Two groups of experiments were performed in steroid-replaced, hypophysectomized dogs undergoing a water diuresis. In each group the response to intravenous VP (100 mU) was examined on two occasions. In the control group (Cont) the second VP injection was preceded by an injection of the carrier solution for indomethacin (Indo), while in the experimental group (Exp) the second VP injection was preceded by the injection of Indo, an inhibitor of PG synthesis. In the Cont versus Exp the first VP injection increased urinary osmolality (Uosm) to a similar degree (94 ± 6 to 249 ± 22 mOsm, $P < 0.001$ versus 85 ± 6 to 227 ± 19 mOsm, $P < 0.001$). Free-water clearance (C_{H_2O}) also decreased comparably in the Cont (1.4 ± 0.4 to 0.2 ± 0.1 ml/min, $P < 0.001$) and Exp (1.9 ± 0.2 to 0.3 ± 0.1 ml/min, $P < 0.001$). The second VP injection, however, produced markedly different effects on Uosm and C_{H_2O} in the Cont and Exp. In the Cont the second VP injection increased Uosm from 111 ± 8 to 205 ± 11 mOsm ($P < 0.001$) as C_{H_2O} decreased from 1.6 ± 0.3 to 0.5 ± 0.1 ml/min. However, in the Exp the second VP injection increased Uosm from 106 ± 14 to 702 ± 69 mOsm ($P < 0.001$) as C_{H_2O} decreased from 1.3 ± 0.3 to −0.6 ± 0.1 ml/min ($P < 0.001$). These changes in Uosm ($P < 0.001$) and C_{H_2O} ($P < 0.05$) after inhibition of PG with Indo were significantly greater than after the second VP injection in the Cont. These results implicate a physiological role of PG in modulating the hydro-osmotic effect of VP in the mammalian kidney.

23. Evidence for Multiple Sodium Pumps in the Isolated Perfused Rat Kidney. ANATOLE BESARAB,* PATRICIO SILVA,* AND FRANKLIN H. EPSTEIN,** Boston, Mass.

Several studies suggest more than one mechanism of sodium reabsorption by the kidney. The isolated perfused rat kidney is an ideal model for the investigation of these pumps because the perfusion medium can be altered at will and inhibitors can be added at maximal concentration without fear of extrarenal toxicity. In kidneys perfused with bovine albumin-Krebs-Ringer-bicarbonate solution the addition of 25 mM ouabain to completely inhibit Na-K-ATPase decreased fractional reabsorption of sodium from 95% to 54% of the filtered load. Subsequent addition of 1 mM acetazolamide reduced sodium reabsorption even further to 34%. Ouabain alone reduced sodium reabsorption to 30% in kidneys perfused without bicarbonate. Restoration of bicarbonate in the

medium to 25 mEq/liter was accompanied by a rise in fractional reabsorption to 53%; the further addition of acetazolamide produced a drop back to about 35%. Ethacrynic acid, ethacrynic-cysteine, or furosemide (2 mM) decreased fractional sodium reabsorption only slightly when given after ouabain. Perfusing the kidney at 11°C instead of 37°C reduced sodium reabsorption to 10% of GFR without further depression when ouabain and acetazolamide were added. Rewarming the kidney to 32°C increased sodium reabsorption to almost 30%. It appears that there are at least three mechanisms for sodium reabsorption: one pathway dependent on Na-K-ATPase that is inhibited by ouabain and partly blocked as well by ethacrynic acid and furosemide, a second sensitive to acetazolamide and involving bicarbonate reabsorption, and a third residual mechanism sensitive to temperature and contributing approximately one-third of sodium reabsorption.

24. The Effects of a Porphyrinogenic Drug on Steroid Metabolism in Man. DAVID R. BICKERS,* H. LEON BRADLOW,* ALVITO P. ALVARES,* AND ATTALLAH KAPPAS,** New York.

Patients with acute intermittent porphyria (AIP) manifest three enzymic abnormalities: elevated hepatic δ-aminolevulinate synthetase (ALAS); decreased uroporphyrinogen synthetase (UROS); and decreased steroid 5α-reductase. Decreased steroid 5α-reductase activity leads to a compensatory shift of endogenous hormone metabolism from the 5α to the 5β pathway resulting in the disproportionate generation of 5β-metabolites. These metabolites are potent inducers of ALAS, and their excessive production after puberty probably contributes to the activation of AIP. AIP patients are sensitive to drugs which induce ALAS and experimental hepatic porphyria. In this study, the possibility was explored that the porphyrinogenic drug phenobarbital could alter steroid metabolism in normals so as to lead to the generation of disproportionate fractions of 5β hormone metabolites, as is characteristic of AIP patients. [4-^{14}C] testosterone and [1,2-^{3}H] 11-OH androstenedione (11-OHAD) were administered to four normal volunteers before and after 3 wk of phenobarbital (2 mg/kg per day) and their 5α and 5β metabolism determined. Phenobarbital effects on drug metabolism were assessed with phenylbutazone and antipyrine. Control 5β/5α metabolite ratios from [4-^{14}C] testosterone were normal (0.8:1-1:1) in all subjects. Phenobarbital markedly shifted the ratios in three subjects to a preponderance of 5β metabolite. Concurrently, antipyrine and phenylbutazone metabolism was accelerated. Studies with 11-OHAD demonstrated that phenobarbital diminished steroid 5α-reductase activity and produced derangements of hormone metabolism like those in AIP. One volunteer showed no phenobarbital effect on drug or steroid metabolism. These studies demonstrate that phenobarbital causes abnormalities in steroid metabolism in normal subjects analogous to those found in active AIP patients. These drug-induced aberrations of hormone biotransformation may represent one mechanism by which exogenous chemicals provoke exacerbations of AIP in the latent, genetically susceptible (UROS deficient) individual. (Research supported by grants from the NIH.)

25. Uptake and Metabolism of Lipoprotein Particles by Cultured Aortic Smooth Muscle Cells. EDWIN L. BIERMAN, OLGA STEIN,* SHLOMO EISENBERG,* AND YECHEZKIEL STEIN,* Seattle, Wash., and Jerusalem, Israel.

Multipotential aortic smooth muscle cells (SMC) proliferate and become lipid laden during atherogenesis. Preparation of homogeneous cultures of SMC provides a tool for direct testing of their interaction with different lipoproteins. SMC with characteristic morphology were cultured from intimal-medial strips of rat thoracic aorta and incubated with ^{125}I-labeled homologous high density lipoproteins (HDL), very low density lipoproteins (VLDL), and "remnants" of lipolysis of VLDL (produced from [^{125}I]VLDL by incubation with lipoprotein lipase-rich plasma 1 h at 37°C). All lipoprotein fractions were rapidly taken up intracellularly. Although a portion of lipoprotein radioactivity was apparently surface bound and trypsin releasable, autoradiography revealed cytoplasmic labeling. More HDL than VLDL protein was taken up by SMC (55 vs. 31 mμg/100 μg lipoprotein protein added per 10^6 cells in 24 h; 90 vs. 57 in 48 h). In comparison, uptake of remnants (100 mμg/100 μg protein added per 10^6 cells in 24 h) far exceeded that of the larger parent VLDL (mean particle diameters 427 Å vs. 234 Å) and also was more than twice that of the smaller (110 Å) HDL. Lipoprotein degradation was assessed by 48 h incubation of SMC in unlabeled medium after exposure to [^{125}I] lipoprotein, trypsinization, and replating. 32 ± 6% (mean ± SD) of HDL radioactivity taken up by SMC released was TCA precipitable, suggesting egress of lipoprotein protein by exocytosis. Only a small fraction of labeled protein in cells was released as TCA soluble, ^{125}I-labeled breakdown products (HDL, 1.7 ± 1.4%; remnants, 0.5%). Thus, although cultured SMC can readily take up small and large lipoproteins, particularly VLDL "remnants," catabolism of lipoprotein protein is slow, suggesting a mechanism for progressive lipid accumulation in SMC in vivo. (Supported by grants from NIH and Guggenheim Foundation.)

26. The Ionic Control of 1,25-Dihydroxy-Vitamin D_3 Production by Isolated Chick Renal Tubules. DANIEL D. BIKLE,* Philadelphia, Pa. (introduced by James J. Ferguson**).

Isolated renal tubules prepared from vitamin D-deficient chicks catalyze the 1α-hydroxylation of 25-hydroxy-vitamin D_3 ($25OHD_3$) in vitro. The rate of synthesis of the product, 1,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$), was found to be regulated by the interrelated effects of the calcium, phosphate, and hydrogen ion concentrations in the medium. At pH 7.4, increasing the medium calcium concentration from 0 to 0.5–1.0 mM caused an increase in 1α-hydroxylation. Higher concentrations of calcium either had no effect or produced an inhibition depending upon medium phosphate concentration: the higher the phosphate concentration, the greater the inhibition produced by a high medium calcium concentration. At pH 6.7, calcium as low as 0.1 mM profoundly inhibited $1,25(OH)_2D_3$ production with little effect seen by further increasing the calcium concentration. No difference in $1,25(OH)_2D_3$ synthesis could be demonstrated between tubules incubated at pH 7.4 and 6.7 when calcium was omitted from the medium. Increasing medium phosphate concentration led to increasing rates of $1,25(OH)_2D_3$ synthesis in the absence of calcium. When the concentration of medium calcium was 4 mM, changes in phosphate concentration had little effect upon hydroxylase activity. However, at both 1 and 2 mM calcium, increasing phosphate concentration from 0 to 6.0 mM had a biphasic effect: low phosphate concentrations were inhibitory, but as the phosphate concentration was increased, this inhibition was reversed. However, at no point was $1,25(OH)_2D_3$ production greater in the presence of phosphate than in its absence when the medium contained calcium. This biphasic effect of phosphate in the presence of calcium was seen whether the incubation was carried out at pH 7.4 or at pH 6.8. (Research supported by a fellowship from NIH-5F03 AM52376-02.)

27. Cyclic Adenosine Monophosphate Controls Bile Salt and Hydroxy Fatty Acid-Induced Colonic Electrolyte Secretion. HENRY J. BINDER,* New Haven, Conn. (introduced by H. M. Spiro**).

Perfusion of the colon with dihydroxy bile salts (BS) and hydroxy fatty acids (OHFA) results in secretion of water and electrolytes. Previous indirect studies have suggested that cyclic

adenosine monophosphate (cAMP) may control BS-mediated secretion. To determine directly the effect of BS and OHFA on colonic cAMP, cAMP levels were determined by a protein binding assay in rat colonic mucosa incubated with 2 mM taurochenodeoxycholic acid (TCDC), 2 mM taurocholic acid (TC), and 0.5 mM ricinoleic acid (RiA). In control mucosa, cAMP content was 3.44 ± 0.29 pmoles/mg protein; in TCDC-incubated tissue, cAMP levels were significantly increased to 6.08 ± 0.74 ($P < 0.01$). In contrast, incubation with 2 mM TC, a trihydroxy BS which does not produce secretion in vivo or alter ion transport in vitro, did not alter cAMP content. Incubation of colonic mucosa with RiA, an OHFA, also resulted in a significant increase in mucosal cAMP levels (5.27 ± 0.55). In this experimental system, 2 mM theophylline increased colonic cAMP levels by 3.75 pmoles/mg protein. To test the ability of TCDC to mediate another cAMP-controlled process, the effect of TCDC on lipolysis in rat epididymal fat cells was studied. 2 mM TCDC significantly increased lipocyte glycerol production by 30%. These results indicate that intestinal electrolyte secretion induced by both dihydroxy BS and OHFA is associated with increases in colonic mucosal cAMP. These studies support the proposal that cAMP is the major determinant in the control of intestinal ion secretion. (Supported by grants from John A. Hartford Foundation, Inc., and NIH.)

28. Dual Action of Phentolamine on Insulin Release from Isolated Rat Islets. WILLIAM G. BLACKARD AND SAMUEL S. ANDREWS,* New Orleans, La.

Isolated islets were prepared by collagenase digestion of pancreas from male albino Wistar strain rats weighing 300–400 g. 7–10 islets were incubated in 5 ml KRB medium containing 0.5% bovine serum albumin, 2500 K.I. units Trasylol, and experimental additives. The incubation flasks were placed in a metabolic shaker at 37°C and 80 oscillations per min. After 60 min the medium was removed and assayed for immunoreactive insulin (IRI). The maximal inhibitory effect of diazoxide and norepinephrine on glucose-induced IRI release was observed at 10^{-3} and 10^{-6} concentrations, respectively. Phentolamine produced maximal reversal of diazoxide-induced insulin inhibition at 10^{-3} M concentrations and of norepinephrine-induced insulin inhibition at 10^{-4} M concentrations. In addition, phentolamine at 10^{-3} M concentrations reduced by more than half glucose-induced insulin secretion from islets incubated with glucose and phentolamine alone. Inhibition of glucose-induced IRI release was not observed at lesser concentrations of phentolamine (10^{-4} or below). The enhanced IRI release caused by the alpha blocker phentolamine in the presence of alpha receptor stimulants (norepinephrine and diazoxide) occurred at the same concentrations of the drug which inhibited glucose-induced IRI release. Phentolamine at 10^{-3} concentrations did not inhibit glucagon- or tolbutamide-stimulated insulin release. The dual opposite effects of phentolamine on IRI release may explain the lack of complete reversal of diazoxide and norepinephrine inhibition of glucose-induced IRI release from islets by phentolamine. In addition, the restriction of the inhibitory effect of phentolamine on IRI release to its effect on glucose-induced IRI release and not on glucagon- or tolbutamide-induced IRI release indicates different modes of action of these insulinotrophic agents. (Supported by NIH Grant AM-10151.)

29. Influence of Acute Viral Hepatitis on Diphenylhydantoin (DPH) Elimination in Man. T. F. BLASCHKE,* P. J. MEFFIN,* A. J. RICE,* M. ROWLAND,* AND K. L. MELMON, San Francisco, Calif.

Although the microsomal enzyme systems of the liver are involved in the elimination of many drugs, little is known about the effects of hepatic dysfunction on drug metabolism. Previous investigators have suggested that DPH elimination, which is dependent on metabolism by mixed-function oxidases in the liver, may be impaired in patients with acute viral hepatitis (1971 *Ann. N.Y. Acad. Sci.* **179**:704). The pharmacokinetics of DPH was examined in five patients with either type A or B acute viral hepatitis (AVH) while liver function tests were abnormal (SGOT range 77–1356 U) and again when the patients were clinically and biochemically healthy (H). Thus each patient could be used as his own control to eliminate genetic and environmental variation. DPH was measured in plasma by a specific GLC assay, and protein binding was determined by ultracentrifugation of labeled DPH added to plasma. The data could be adequately described by a single exponential equation, and the Vd(L), plasma half-life ($t_{1/2}$ in hours), and plasma clearance (CL, liters/h) of DPH were calculated from the log-linear plot. Surprisingly, it was found that DPH elimination was unaltered by acute viral hepatitis. There was no significant difference (using a paired t test) in any of the pharmacokinetic parameters calculated [$t_{1/2}$ (AVH)-13.2, $t_{1/2}$ (H)-13.6; Vd (AVH)-42.4, Vd (H)-40.0; CL (AVH)-2.55, CL (H)-2.19]. Although plasma albumin concentrations were normal in all subjects during AVH, the unbound fraction of DPH was increased (AVH, 12.7%; H, 9.9%). No correlates were noted between DPH CL and any of the liver function tests obtained, or the percent unbound DPH. When these findings are considered along with results of other investigators using different drugs in AVH (such as diazepam and antipyrine), they suggest that alterations in hepatic drug clearance capacity produced by inflammatory liver disease may be more specific than previously suspected.

30. Transformation Antigens on Stimulated Lymphocytes. AVRUM A. BLUMING,* MICHAEL LYNCH,* AND MAUREEN KAVANAH,* Boston, Mass. (introduced by Robert S. Schwartz).

Immunologically mediated regulation of lymphoproliferation requires a self-recognition mechanism. This was sought by measuring the ability of blood lymphocytes to recognize transformed, autologous lymphocytes. Human blood lymphocytes incubated with phytohemagglutinin (PHA) for 72 h, followed by mitomycin-C treatment, induced blast transformation of autologous lymphocytes from 20/20 healthy adults. Blastogenesis was measured by reactor cell incorporation of [^{3}H] thymidine and was uniformly greater at 72 than at 48 h. The contribution of PHA bound to the stimulating cells was assessed in several ways. The supernatant of washed, PHA-transformed lymphocytes did not stimulate normal autologous lymphocytes. Lymphocytes incubated with PHA for 1 h or for 72 h before mitomycin treatment bound equivalent amounts of [^{131}I]PHA; cells treated for 1 h did not transform and did not stimulate autologous lymphocytes. By contrast, cells incubated for 72 h did transform and stimulated autologous lymphocytes. Lytic sonication of PHA-transformed lymphocytes abolished their stimulating capability. An identical result was observed in allogeneic mixed lymphocyte reactions after lytic sonication of the stimulating cells. PHA itself maintained its stimulatory capability after sonication. *N*-acetyl-D-galactosamine (NAGAL) competes with PHA for lymphocyte membrane binding sites. Incubation of reactor lymphocytes with NAGAL did not diminish their response to PHA-transformed autologous lymphocytes. These results strongly suggest the presence of autorecognition determinants on membranes of transformed lymphocytes. The relatively rapid reaction to these determinants is consistent with a prior exposure to them. (Research supported in part by a grant from the National Leukemia Association.)

31. Sulfonylurea Action on Adenylate Cyclase. HANS BODE,* PATRICIA MEARA,* HELEN JONES,* AND JOHN CRAWFORD,** Boston, Mass.

Sulfonylureas augment cyclic AMP-mediated hormonal responses in several tissues. Phosphodiesterase inhibition, demonstrable for chlorpropamide and tolbutamide in cell-free systems, is an unlikely mechanism because: concentrations 10-fold therapeutic serum levels are required; diazoxide, a competitive antagonist of sulfonylurea effect in the hormonal systems, acts like sulfonylureas on phosphodiesterase activity; and in vivo, neither diazoxide nor tolbutamide penetrates the cell membrane to reach intracytoplasmic phosphodiesterase. Failure of penetration also rules against a direct action of sulfonylureas on cyclic AMP. The effect of sulfonylureas on adenylate cyclase activity was studied in rat renal cortical and medullary homogenates stimulated with parathyroid hormone (PTH) 3 U/ml, and vasopressin (VP) 3–33 mU/ml. In vitro addition of tolbutamide or chlorpropamide failed to accelerate PTH or VP-induced adenylate cyclase. In rats prefed the compounds (200 mg/kg per day), however, adenylate cyclase responses of cortex to PTH and medulla to VP were significantly increased. The possibility that sulfonylureas stimulate adenylate cyclase by opposing the action of prostaglandins was suggested by the similarity of tolbutamide and indomethacin effects on toad bladder; both augment vasopressin influence on water flux but negate the inhibitory effect of prostaglandin E_1. In homogenates and incubated slices of renal medulla, adenylate cyclase activity and cyclic AMP concentrations were measured after simultaneous addition of VP 1 mU/ml and PGE_1 10^{-8} M. PGE_1 inhibited vasopressin-stimulated adenylate cyclase activity and cyclic AMP accumulation in control animals. In sulfonylurea-pretreated rats PGE_1 inhibition was negated. We conclude that sulfonylureas may require intact cellular membranes for exhibition of their augmentation of adenylate cyclase in mammalian kidney, but in the two renal systems studied, their action is exerted at the PGE_1-sensitive site. (Research supported by Hood Foundation, King Trust, and NIH.)

32. Oxygen Affinity-Independent Effects of pH on Sickling and Gelation. ROBERT M. BOOKCHIN,* RONALD L. NAGEL, AND TANIA BALAZS,* Bronx, N. Y.

It is known that small reductions in pH promote sickling by lowering the O_2 affinity of Hb, but there is uncertainty as to whether $[H^+]$ affects sickling by a mechanism apart from the Bohr effect. The effects of varied pH on RBC sickling and on gelation were studied in fresh blood and in hemolysates from persons with sickle cell anemia (SS) and sickle trait (SA). In SA blood (37°C,pCO_2 = 40 mm Hg) at plasma pH 7.35, sickling began at oxyHb (saturation) levels below 15% and fully deoxy blood had 10–20% sickling; at pH 7.0 (HCl), sickling began at 50% oxyHb and about 60% of RBC sickled when fully deoxygenated. Similar effects were seen in SS blood, e.g., at pH levels of 7.65, 7.30, and 7.15, respectively: $p^{50}O_2$ = 26, 36.5, and 47 mm Hg; sickling began at oxyHb levels of 55%, 86%, and 90%; new sickling of half the RBC occurred at oxyHb levels of 26%, 46%, and 58%. The minimum gelling concentrations (MGC) on deoxygenation were determined on SS and SA hemolysates dialyzed against 0.15 M PO_4 with pH from 7.8 to 7.0. With stepwise reduction in pH, the MGC of SS fell from 31 to 20 g/100 ml and that of SA fell from 39 to 29 g/100 ml (pH levels in RBC and Hb solutions were 0.2–0.3 units below those in plasma or buffer). Thus sickling and gelation of both SS and SA blood vary with changes in $[H^+]$ to a much greater extent than can be explained by the Bohr effect. Since elevation of the level of intracellular organic phosphates lowers intracellular pH, and since lowered pH increases the binding of 2,3-DPG to hemoglobin, the interrelationships between these factors play important roles in determining red cell sickling. (Research supported by the New York Heart Association, AHA, and NIH.)

33. Delineation of the Luteinizing Hormone (LH) and Cortisol (F) Abnormalities in Anorexia Nervosa (AN). ROBERT M. BOYAR,* SHELDON KAPEN,* JORDAN W. FINKELSTEIN,* DAVID K. FUKUSHIMA,* ELLIOT D. WEITZMAN,* AND LEON HELLMAN,** Bronx, N.Y.

We have previously described the synchronization of augmented LH secretion in pubertal boys and girls. This pubertal LH secretory "program" gradually yields to the adult pattern of pulsatile LH secretion of equal magnitude during waking and sleep periods after the completion of sexual maturation. 24 h, 20 min interval plasma sampling with polygraphic monitoring of sleep was performed in seven amenorrheic (two primary, five secondary) girls with AN (24–39% below ideal weight) and 18 women with other types of amenorrhea. Plasma LH (RIA), F (CPB), $[^{14}C]$ F disappearance, F production rate (F-PR), and cortisol binding globulin (CBG) were measured. Four of the seven patients with AN showed the typical pubertal LH pattern which was inappropriate for their chronological age (17–23). The other three showed the prepubertal LH pattern: low levels (1.5–4.0 mIU/ml) during both waking and sleep. One patient restudied after normalization of body weight showed "maturation" of her LH pattern to the normal adult type. 24 h mean F was elevated in six of seven patients with AN (5.7–13.7 μg/100 ml), $[^{14}C]$ F disappearance was prolonged in five of seven (80–110 min), F-PR was increased in six of seven (20–38 mg/g creatine per day), while CBG was normal. These types of LH and F abnormalities were not found in any of the 18 patients with amenorrhea (Stein-Leventhal, amenorrhea-galactorrhea, and "post-pill" amenorrhea). These data indicate that AN is associated with (*a*) a highly specific, reversible abnormality of LH secretion, and (*b*) a rather specific abnormality in F metabolism which has not yet been shown to be reversible.

34. The Role of Bile Canalicular Membrane Enzymes, Na^+,K^+-Activated ATPase, and Sodium Transport in Secretion of Bile Salt-Independent Canalicular Flow (BSIF). JAMES L. BOYER,* DONNA RENO,* THOMAS LAYDEN,* AND JOSEPH SCHWARZ,* Chicago, Ill. (introduced by J.B. Kirsner**).

A major fraction of canalicular bile is secreted independently of the osmotic effects of bile salt transport. To study the role of sodium transport in this process, plasma membranes enriched in bile canaliculi were isolated from male rat hepatocytes. Canalicular membrane enzymes were assayed under a variety of conditions which alter BSIF in the bile fistula rat or isolated perfused rat liver (IPRL), including changes in temperature, administration of cardiac glycosides, ion substitution for sodium, thyroid treatment, and phenobarbital administration. Electron microscopy and analysis of enzyme markers revealed a purified membrane fraction enriched in bile canaliculi containing Na^+,K^+-ATPase, Mg^{++}ATPase, and 5′-nucleotidase (10.2 ± 2.9, 48.8 ± 12.4, and 59.3 ± 9.6 μmoles Pi/mg protein per hr, respectively. Temperature optima for Na^+,K^+-ATPase but not Mg^{++}ATPase were identical with those for bile secretion in the IPRL (40–41°C). Canalicular membrane Na^+,K^+-ATPase was selectively inhibited by ouabain (10^{-3}M) and scillaren (10^{-4}M to 5×10^{-4}M) in concentrations that produced maximum inhibition of bile secretion. Iso-osmotic substitution of lithium or choline for sodium resulted in no significant change in Mg^{++}ATPase activity but abolished Na^+,K^+-ATPase and reduced BSIF in the IPRL. Triiodothyronine doubled Na^+,K^+-ATPase and increased

BSIF from 1.9 to 2.6 μl/min per g liver without significantly altering either Mg^{++} ATPase or 5'-nucleotidase activity. Phenobarbitol administration increased BSIF, the yield of membranes, and the relative activity of Na^+,K^+-ATPase compared to Mg^{++}ATPase and 5'-nucleotidase. The results of these experiments show that both bile canalicular membrane Na^+,K^+-ATPase and content correlate with the magnitude of bile secretion as altered by diverse physical and chemical factors, and support the hypothesis that sodium transport and Na^+,K^+-ATPase are determinants of BSIF.

35. Cyclic Nucleotide-Mediated Secretion of Glucagon and Gastrin in Monolayer Culture of Rat Pancreas. JAN T. BRAATEN,* ANTOINETTE SCHENK,* MICHAEL J. LEE,* JAMES E. MCGUIGAN, AND DANIEL H. MINTZ, Miami and Gainesville, Fla.

Pancreatic monolayer cultures were established by enzymatic dissociation of 3-day-old rat pancreases. Primary and secondary cultures were maintained for more than a month with continuous secretion of insulin, glucagon, and gastrin. This is the first known observation of pancreatogenous gastrin secretion (PGS) in vitro. The present study was undertaken to resolve the controversy concerning the effects of cyclic AMP (cAMP) on glucagon secretion and to define its role in PGS. Immunoreactive glucagon (IRG) secretion (mean ng/28 cm^2 dish) from 6-h incubations in KRB, 95% O_2, 5% CO_2 at 1.67 mM glucose (0.67), was stimulated by cAMP, 5 mM (1.31, $P < 0.001$); dibutyryl cAMP (dbcAMP), 5 mM (1.38, $P < .001$); and theophylline, 1 mM (1.58, $P < 0.001$). At 16.7 mM glucose, IRG secretion was suppressed (0.21, $P < .001$) and again stimulated by cAMP, 5 mM (0.42, $P < 0.001$); dbcAMP, 5 mM (0.42, $P < 0.001$); and theophylline, 1 mM (0.64, $P < 0.001$). The stimulating effect of dbcAMP, 1 mM on IRG (from 0.35 to 1.04, $P < 0.001$) was markedly enhanced by theophylline, 2 mM (4.24, $P < 0.001$). Gastrin secretion was also stimulated from nondetectable levels to 139 (pg/dish), by theophylline, 2 mM, and further enhanced by adding cAMP, 0.5 mM (245 $P < 0.05$). The stimulatory effect of cyclic nucleotides on insulin secretion was also confirmed. It is concluded that (*a*) the secretion of the three hormones identified to date from pancreas is stimulated by increasing the intracellular concentration of cAMP or dbcAMP; (*b*) the suppression of alpha cell function by glucose resides in a mechanism independent of the effects of cAMP in the secretory process; and (*c*) the monolayer culture of pancreas provides a unique in vitro system for simultaneous study of factors influencing the secretion of insulin, glucagon, and pancreatogenous gastrin.

36. Effect of a Heptapeptide (2–8) Analog on Angiotensin-Mediated Aldosterone Biosynthesis. EMMANUEL L. BRAVO,* MAHESH C. KHOSLA,* AND F. MERLIN BUMPUS,* Cleveland, Ohio (introduced by Harriet P. Dustan).

Recent studies have shown that the heptapeptide (2–8) fragment of angiotensin (A-II) has a similar stimulatory effect on aldosterone secretion (ASR) as does its octapeptide progenitor. Because there is little heptapeptide in arterial blood, it has been suggested that A-II could act to stimulate ASR via local production of heptapeptide. This hypothesis was examined by comparing the effect of an antagonist of A-II ([Sar¹, Ile⁸] A-II) to that of the heptapeptide ([Des-Asp¹, Ile⁸] angiotensin) on ASR during A-II infusion. Experiments were performed in 26 bilaterally nephrectomized, ACTH-suppressed male mongrel dogs. Infusates were given into peripheral veins, and timed samples of adrenal venous effluent for steroid determinations were collected from the left lumboadrenal vein. ASR was determined during infusions of (*a*) saline, (*b*) A-II, (*c*) antagonists, and (*d*) antagonists + A-II. A-II was infused at constant doses of 20 ng/kg per min and the antagonists at 80–3000 ng/kg per min. Both antagonists had slight agonistic activity on ASR. [Sar¹, Ile⁸] A-II inhibited ASR only when given at doses of 3000 ng/kg per min ($P < 0.01$). By contrast, [Des-Asp¹, Ile⁸] angiotensin inhibited ASR in doses as low as 200 ng/kg per min ($P < 0.01$). These results indicate that [Des-Asp¹, Ile⁸] angiotensin is a more specific antagonist of the aldosterone-stimulating activity of A-II, and provides additional evidence for an important role of the heptapeptide (2–8) fragment of A-II on aldosterone biosynthesis.

37. Effects of Acute Exercise on Arterial Insulin and Glucose Concentration in Obese and Normal Men. GEORGE A. BRAY, BRIAN J. WHIPP,* SANKAR N. KOYAL,* AND KARLMAN WASSERMAN, Torrance, Calif.

Chronic physical training reduces plasma insulin concentration in obese subjects, but the effects of acute exercise have not been systematically examined. We exercised 10 lean ($\overline{BW}$ = 72.8 kg) and 10 moderately obese volunteers ($\overline{BW}$ = 103.3 kg) on a cycle ergometer at 50, 100, and 150 W each for 15 min, allowing recovery between tests. Exercise at comparable levels of O_2 uptake was also performed at three work rates on a treadmill. Arterial blood samples were obtained before and at the end of each work period. At rest, IRI was 62 μU/ml in the obese and 22 in the lean subjects. During the 50 W exercise, insulin fell to 26 μU/ml in the obese and 11 in the lean subjects. During the recovery phase, IRI rose significantly ($P < 0.05$) to 47 μU/ml in the obese and to 16 in the lean subjects. At the intermediate work rate, insulin dropped to 27 μU/ml in the obese and 7 in the lean subjects, and did not rise during the recovery phase. No further fall was noted at the highest work rate. Treadmill exercise invoked a similar pattern of insulin response. Resting glucose was 99 mg/100 ml in the obese and 80 in the lean subjects, decreasing to 73.5 and 74 after the lightest cycle ergometer exercise. With recovery, glucose rose to 92 mg/100 ml in the obese and 83 in the lean. During the moderate work, glucose fell to 73 mg/100 ml and 75, and did not rise during the recovery. No further fall was observed at the highest work rate. Similar findings were observed for treadmill exercise. In summary, arterial insulin and glucose decreased in obese and lean subjects with exercise. However, even severe exercise did not lower insulin concentrations in the obese subjects to the resting level as in the normal subjects. (Research supported by grants from NIH.)

38. Characterization of an Angiotensin II Receptor from Adrenal Glomerulosa Cells. PETER I. BRECHER,* HAE YUNG PYUN,* AND ARAM V. CHOBANIAN, Boston, Mass.

Characterization of the hormonal receptor for angiotensin II (AII) may provide important information concerning the adrenal and vascular effects of the hormone. In the current study, tritiated AII was found to bind to a macromolecular substance solubilized from glomerulosa cells of the rat adrenal gland. The receptor was localized in the particulate fractions of the tissue homogenate, primarily in the 10,000 *g* pellet. The substance was soluble in buffered 0.4 M KCl but aggregated in solutions of low ionic strength. Bound AII was separated from free hormone by several techniques, including Sephadex-gel chromatography, sucrose density gradient ultracentrifugation, and protein precipitation. The binding observed was both saturable and specific. Excess amounts of unlabeled AII, [1 sar, 8 ala]-angiotensin, or the heptapeptide analog reduced completely the binding of labeled AII, whereas angiotensin I, the pentapeptide analog, or synthetic ACTH had only minimal effect as inhibitors. Adrenal glomerulosa cells contained much more of the binding substance than did fasciculata and reticularis cells. Other tissues, including liver, diaphragm, kid-

ney, uterus, and skeletal muscle, were without binding activity. The specific binding was temperature dependent, occurring more readily at 25°C than at 0°C. The binding was reversible since bound AII could be displaced by the addition of excess hormone. Sucrose density gradient ultracentrifugation of the AII-macromolecular complex in 0.4 M KCl indicated a sedimentation constant of about 9S. These studies suggest the presence of a specific and physiologically significant receptor for AII in the membranous fraction of rat adrenal glomerulosa cells. (Research supported by NIH Grants HL14358 and HL07299.)

39. The Effect of Zinc on Hemoglobin Binding by Red Blood Cell Membranes. GEORGE J. BREWER, SUMITRA DASH,* AND FRED J. OELSHLEGEL, JR.,* Ann Arbor, Mich.

Zinc may play an important role in sickle cell anemia (SCA), first because many patients are zinc deficient, and second, because of two, possibly independent, pharmacological effects. By binding to hemoglobin, zinc increases its oxygen affinity, which may be of benefit in inhibiting sickling provided high enough levels of red cell zinc can be obtained. In addition, however, zinc improves the filterability of sickle cells at a zinc/hemoglobin ratio of only 0.03. This result at such a low zinc concentration (which should be readily obtainable in vivo) suggests an effect on the membrane of the sickle cell. Studies with one-stage red cell ghosts revealed that 1.5 mM zinc decreased hemoglobin retention of such ghosts by 86%. Further, zinc partially blocked the marked hemoglobin retention induced in ghosts by 1.0 mM calcium (57% reduction in hemoglobin retention). Studies elsewhere have suggested that calcium accumulation is intimately involved in the production of irreversibly sickled cells. We postulate that one effect of zinc on sickle cells may be to counteract the deleterious effect of calcium on the membrane, perhaps by preventing cross-linking between hemoglobin and membrane which would tend to make the membrane more rigid. Thus, zinc therapy in SCA may: (*a*) reverse symptomatology related to zinc deficiency (e.g., growth retardation, hypogonadism, leg ulcers); and (*b*) pharmacologically decrease vascular plugging which results from loss of red cell deformability. (Research supported by Contract NHLI 72-2918-B from NIH.)

40. Gelation of Methemoglobin S. ROBIN W. BRIEHL, Bronx, N. Y.

Gelation of hemoglobin S depends on deoxygenation and on the T (deoxy) as opposed to the R (oxy) quaternary structure. Therefore, gelation might be used to indicate conformation in solution. Most hemoglobin derivatives favor R or T state strongly, but indirect evidence indicates that aquomethemoglobin exists in more balanced equilibrium; it has been crystallized in T state under constraints (1973 *J. Mol. Biol.* **79**:495). Concentrated solutions of aquomethemoglobin S were sedimented to equilibrium at 19160 rpm for 1 wk in the analytical ultracentrifuge as previously described for deoxyhemoglobin (1973 *J. Mol. Biol.* **80**:445). When gelation occurs, there is a sol to gel phase change at a minimal gelling concentration (MGC). At 20°C and pH 6.2 in 0.05 M *bis* Tris with 0.1 M NaCl, aquomethemoglobin S exhibits MGC of about 0.37 g/ml in 10 mM inositol hexphosphate (IHP). Aquomethemoglobin A in these studies (as oxy- or deoxyhemoglobin A and oxyhemoglobin S in previous experiments) shows no phase change. Aquomethemoglobin S also gels in 10 mM 2,3-diphosphoglycerate and when stripped, but at higher MGC's, up to 0.50 g/ml, than in IHP. A pH dependence of gelation occurs, MGC rising to 0.47 g/ml in IHP as pH is raised to 7.7. Aquomethemoglobin S, unlike oxyhemoglobin S, gels, but MGC's are higher than for deoxyhemoglobin. Thus the R-T equilibrium for aquomethemoglobin S in solution, and almost certainly for aquomethemoglobin A, lies in an intermediate position. This conclusion further justifies use of partially oxidized tetramers in studying reciprocal relation between quaternary conformation on the one hand, and spin and location of heme iron in relation to the heme plane on the other. It also suggests that the presence of methemoglobin could exacerbate sickle cell disease. (Research supported by NIH grant.)

41. Neural Control of Hepatic Glycogenolysis During Glucopenia in Man. ROBERT G. BRODOWS,* F. XAVIER PI-SUNYER,* AND ROBERT G. CAMPBELL,* Rochester and New York, N. Y. (introduced by Sami A. Hashim).

Previous studies have shown that complete disruption of the sympathetic outflow tract is associated with an inability to mobilize glucose in response to 2-deoxyglucose-induced glucopenia, despite increased cortisol and growth hormone levels (1973 *J. Clin. Invest.* **52**:1841). Liver glycogen stores may be mobilized during hypoglycemia by release of adrenomedullary catecholamines, by direct stimulation of hepatic sympathetic efferent fibers, and by glucagon. To clarify the independent contribution of direct hepatic sympathetic stimulation to glycogenolysis, 2-deoxyglucose (2DG), a competitive inhibitor of glucose metabolism, was infused (50 mg/kg) for 20 min to nine normal and four adrenalectomized (adrenx) subjects and the counter-regulatory response evaluated for periods up to 180 min. Adrenx subjects received oral cortisone (37.5 mg) 60 min before 2DG infusion. In controls, mean percent changes from basal levels for plasma free fatty acids (FFA) (142%), catecholamines (359%), and growth hormone (600%) were maximal at 60 min; glucose (148%), lactate (132%), and cortisol (92%) peaks occurred between 90 and 180 min; insulin remained unchanged. In adrenx subjects, despite no change in plasma FFA, lactate, catecholamines, and glucagon, glucose rose 20% by 150 min; the growth hormone rise was similar to normals. No change in plasma glucose was found with cortisone alone. A significant increase in glucose response from 60 min onward was found in the 2DG infusion studies when compared to cortisone alone. Thus, during glucopenia, plasma glucose rose in spite of unchanged adrenomedullary catecholamine and glucagon levels. The data indicate that glycogenolysis due to direct hepatic sympathetic stimulation occurs during glucopenia in man. (Research supported by grants from NIH.)

42. Identification of the Molecular Defect in Familial Hypercholesterolemia. MICHAEL S. BROWN* AND JOSEPH L. GOLDSTEIN,* Dallas, Tex. (introduced by Daniel W. Foster).

Cultured fibroblasts from homozygotes with familial hypercholesterolemia (FH) overproduce cholesterol because of genetic resistance to low density lipoprotein (LDL)-mediated suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (reductase), the rate-controlling enzyme in cholesterogenesis (Goldstein and Brown. 1973. *Proc. Natl. Acad. Sci. U.S.A.* **70**: 2804). We now report that this defective regulation is due to a marked deficiency of a hitherto unidentified cell surface receptor for LDL. In cell lines from 13 controls, biologically active [^{125}I] LDL (labeled in protein) bound to membranes with high affinity (apparent K_m = 10 μg/ml), high specificity (displacement by LDL, VLDL, normal serum, but not by HDL or abetalipoproteinemic serum), and saturability (maximal binding: 24×10^4 molecules per cell). Suppression of reductase was directly related to the amount of [^{125}I] LDL bound. Bound [^{125}I] LDL was degraded proteolytically to small peptides and amino acids. Treatment of the surface of normal cells with pronase inactivated the LDL receptor, decreased LDL degradation, and prevented suppression of reductase. In cells from five FH homozygotes, maximal LDL binding was only 3.6% of

normal (range, 0–19%). As a result, [^{125}I] LDL degradation (LDL concentration, 5 μg/ml) was markedly reduced: 40 (range, 0–95) ng/mg cell protein per 6 h vs. 989 (639–1470) in 13 controls, and suppression of reductase did not occur. Cells of 11 heterozygotes showed intermediate levels of [^{125}I] LDL binding, degradation, and enzyme suppression. We conclude that in FH a primary deficiency in LDL receptors results in overproduction of cholesterol and decreased degradation of LDL, both of which may be important in producing high levels of serum LDL. This cell culture system provides a unique opportunity to study the mechanism by which a mutation in a regulatory protein causes a dominant disorder in man. (Supported by grants from NIH and American Heart Association.)

43. Epidemiology of Euthanasia in an Extended Care Facility. NORMAN K. BROWN,* MARIA A. BROWN,* AND DONOVAN J. THOMPSON,* Seattle, Wash. (introduced by Robert H. Williams**).

With the increasing capability and cost of highly complex medical technology in prolonging life (PL), the decisions made by physicians in delivering health care to the dying have been under social pressure to select more often the path of negative euthanasia (NE) (i.e., halting treatments to allow death). Our goal in this study was to identify, quantify, and characterize NE decisions in an extended care facility. From pilot studies we learned that patients who were on the nurses' "poor" list or febrile (≧ 101°F) experienced high mortality (50%). Accordingly, we defined the physician's decision to hospitalize such a patient as PL, and *not* to hospitalize as potential NE. Of 761 patients admitted by 284 community physicians, 179 sustained a "poor" and/or febrile episode. Of these, 46 were hospitalized (PL), while 139 were left in the facility (potential NE), 95 going on to die. Evidence that the physician intended NE was prospectively identified from entries in the medical records of 53 patients (definite NE). Among multiple social and medical comparisons between the PL and definite NE groups, the presence of cancer or mental impairment most strongly foretold the physician's decision for NE, while age, sex, and other diagnoses made little difference. It is concluded not only that NE is occurring frequently in this extended care facility, but also that certain patient characteristics set the stage for this action. NE is a significant and evidently rational mode of delivering care to certain patients, and warrants further physician/public education. (Research supported by grant from NIH.)

44. Nuclear Phospholipid Metabolism in Myocardial Ischemia. THOMAS A. BRUCE,* Oklahoma City, Okla. (introduced by Leonard P. Eliel).

The quantity of ischemic cells which surrounds an infarcted area of myocardium potentially determines the clinical course of the illness. The function of these damaged cells is likely dependent on the integrity and performance of the individual subcellular components. Phospholipid depletion in ischemic mitochondrial and microsomal membranes seems directly related to decreased capacity for oxidative metabolism and calcium transport, respectively, and the addition of exogenous phospholipids experimentally reverses the abnormalities. In this study the focus has been on the nuclear membranes and their capacity for protein synthesis. Nuclear phospholipid metabolism has been measured in ischemic, infarcted, and healthy myocardium of dogs after coronary ligation. The findings have been compared with parallel studies of [^{14}C]glycine incorporation into protein as an index of protein synthesis. Total nuclear phospholipid content dropped to a third of the control myocardial levels within 30 min of severe ischemia, and to half the control levels after infarction developed. Specific activity of lecithin (the largest phospholipid component, with 46% of total phosphorus content) dropped modestly, but specific activity of lysolecithin and sphingomyelin rose markedly. During a concomitant period there was significant overall depression of protein synthesis in the ischemic muscle, but [^{14}C]glycine incorporation into nuclear ribosomes already had increased. Within 4 days after coronary ligation, both protein and phospholipid synthesis were markedly elevated in the infarcted area and to a lesser degree in the borderline ischemic tissue. (Research supported by a grant from the VA Research Program.)

45. Effect of Propranolol upon Morphine Metabolism in Man. S. FRED BRUNK,* MARGRIETA DELLE,* AND WILLIAM R. WILSON,** Iowa City, Ia.

Propranolol, a beta-blocking agent which acts both centrally and peripherally, has been studied in man to determine whether it affects morphine metabolism since studies in animals have shown that morphine interacts with the adrenergic system. 12 men were studied with morphine alone (C) and had repeat morphine metabolism studies while receiving propranolol, 20 mg (I) or 40 mg (II) every 6 h. Plasma and urine levels of free and conjugated morphine were determined after [*N-methyl*-^{14}C] morphine sulfate, 5.75 mg/m^2, intravenously. Plasma samples were obtained at 15–360 min after injection. The plasma levels of free morphine at 15 min averaged 50 mμg/ml in C, 53 in I, and 48 in II. An equilibrium plateau was reached at 1 h when the plasma level was 23 in C, 24 in I, and 23 in II; at 3 h the level was 12 in C, 13 in I, and 12 in II ($P > 0.05$). The biological half-lives of plasma free morphine were similar. Plasma levels of conjugated morphine and the biological half-lives of conjugated morphine in C, I, and II were similar. 24 h urinary excretion of free morphine (per cent of administered dose) was 10.9% in C, 12.5 in I, and 9.7 in II ($P > 0.05$); cumulative excretion of conjugated morphine was 49% in C, 49 in I, and 48 in II ($P > 0.05$). *N*-demethylation, as measured by expired $^{14}CO_2$, was similar in C, I, and II. The findings indicate that propranolol does not affect the metabolism of morphine in man.

46. Cancer Patients' Lymphocytes: Clinical and Experimental Factors Affecting Responses to Tumor Antigen. DAVID M. BULL* AND RICHARD A. HELMS,* Boston, Mass. (introduced by Ronald A. Arky).

For clinical immunodiagnosis and immunoprognosis in colon cancer, we showed (1973. *Science.* 181:957) that lymphocytes and monocytes separated from whole blood using Ficoll-hypaque and sucrose gradients are more reliable in tumor antigen-induced inhibition of migration than are crude leukocytes. Using isologous antigen, crude leukocyte migration correlates incompletely with clinical state, and in the potentially clinically useful homologous system, little or no correlation is seen. We have studied clinical and experimental factors affecting leukocyte migration which may facilitate practical application of migration inhibition studies. In 85 experiments using a variety of leukocyte populations prepared by combinations of sedimentation in dextran, defibrination, separation through Ficoll-hypaque mixtures, and layering in sucrose gradients, we found that: (*a*) platelets are toxic to migrating cells giving drastically reduced and variable migration patterns, while granulocytes block lymphocyte responsiveness to antigen; (*b*) isologous and homologous cancer sera block antigen responsiveness and reverse migration inhibition; (*c*) multiple washes of migrating cells give larger and more reproducible migration patterns, a factor which may add to the efficacy of the Ficoll-hypaque preparative method; (*d*) disseminated cancer is associated with reduced migration inhibition that is independent of the effect of chemotherapy and is associated in some cases with depressed cutaneous responses to Candida, mumps, and tuberculin; and (*e*) certain cancer patient sera are capable of imparting tumor

antigen-responsiveness to the migrating cells of cancer-free individuals. Understanding of these and other factors affecting leukocyte migration may allow expanded exploitation of this relatively simple assay of human tumor immunity. (Supported by grants from NIH.)

47. The Synandrogenic Action of Progestins in Mouse Kidney: a Model for Multiple Hormonal Interactions. LESLIE P. BULLOCK,* MOMCILO MILJKOVIC,* AND C. WAYNE BARDIN, Hershey, Pa.

Steroids with minimal biological activity may modify the effects of other hormones on their end organs. Several progestins, including progesterone caproate, medroxyprogesterone acetate (MPA), cyproterone acetate, and megesterol acetate, had little or no androgenic action on mouse kidney. However, when administered with testosterone, small doses of these agents potentiated androgen action, an effect designated as synandrogenic. Interestingly, larger doses of the latter two steroids were antiandrogenic. All these progestins were inactive in the kidney of androgen-insensitive (tfm/y) mice with defective androgen receptors, suggesting that androgens and progestins share a common mechanism of action in this tissue. To investigate this possibility, a radioactive synandrogenic progestin of high specific activity was needed. Of the above four steroids we chose to synthesize [^{3}H]MPA by selectively tritiating 17α-acetoxy-6α-methyl- $\Delta^{1,4}$-pregnadien-3,20-dione. In vivo studies indicated that [^{3}H]MPA was not metabolized to a potent androgen and that [^{3}H]MPA was concentrated by kidney nuclei of normal but not tfm/y mice. These latter observations along with in vitro competitive binding studies indicated that the cytoplasmic and nuclear receptor components for MPA were identical with those for androgens. Furthermore, physical properties of the androgen receptor, including sedimentation velocity and isoelectric point, were similar with either MPA or testosterone as ligand, even though the binding constants for these steroids differed. We conclude that in mouse kidney the biological activities of testosterone and MPA are mediated through a common effector molecule, the androgen receptor. Allosteric interaction of progestins with the androgen receptor may explain the synandrogenic action of this class of steroids. (Supported by Contract NIH-NICHD-72-2730.)

48. Effects of Isolated Left Anterior Descending Coronary Artery Stenoses on Coronary Blood Flow. IVAN BUNNELL,* FRANCIS KLOCKE, DAVID GREENE,* DJAVAD ARANI,* RAVINDER TANDON,* RENE OLIVEROS,* AND STEPHEN WITTENBERG,* Buffalo, N. Y.

Previous studies from our laboratory have shown a systematic difference in average left ventricular flow per unit mass (F/W) at rest between normal individuals and patients with double and triple vessel coronary artery disease (70 ± 13 ml/min per 100 g, n = 20 vs. 54 ± 11 ml/min per 100 g, n = 26; $P < 0.01$). However, effects on flow of single vessel obstruction have not been quantified. Accordingly, 14 patients with isolated obstruction of the left anterior descending coronary artery (LAD) were studied using inert gas desaturation and selective sampling from the area supplied by the LAD through a catheter advanced deep into the great cardiac vein (GCV). Seven patients had 90–100% LAD occlusions; F/W ranged from 39 to 48 and averaged 44 ml/min per 100 g. In contrast, in the previous normal group, only 4 of 20 values were below 60 ml/min per 100 g and none below 50. In seven patients with 60–75% LAD occlusions, F/W ranged from 42 to 88 and averaged 63 ml/min per 100 g; three values were clearly normal (> 70 ml/min per 100 g). The importance of selective GCV sampling was demonstrated by sampling simultaneously from the more proximal coronary sinus (CS). In four of five patients in whom GCV flow was < 50 ml/min per 100 g, CS flow was 21–33% higher, i.e., in the "overlap" range between normal and abnormal. We conclude the following. (*a*) LAD stenoses of > 90% are associated with reductions in local F/W even at rest. (*b*) LAD stenoses of < 90% may be associated with normal, intermediate, or abnormal resting F/W. (*c*) measurement of local F/W by selective GCV sampling allows functional evaluation of < 90% obstructions in a manner not possible from the arteriogram alone. (Supported by NIH Grant HL-15194.)

49. Isolation of a Novel B_{12}-Binding Protein Associated with Adolescent Hepatomas. ROBERT L. BURGER,* SAMUEL WAXMAN,* HARRIET S. GILBERT,* AND ROBERT H. ALLEN,* St. Louis, Mo., and New York. (introduced by Stuart Kornfeld).

Recent studies have documented the presence of a previously undescribed acidic B_{12}-binding protein (B_{12}BP) in the serum of three adolescents with hepatomas and extraordinary elevations of serum B_{12} (15–53 ng/ml; normal 0.2–0.9 ng/ml) and serum B_{12}-binding ability (20–533 ng/ml; normal 1–2 ng/ml). Using affinity chromatography on B_{12} Sepharose, we have now isolated the hepatoma-related B_{12}BP in homogeneous form from the pleural fluid of one patient (S. A.) and the plasma of another patient (M. M.). The hepatoma B_{12}BP's belong to the "R-type" class of B_{12}BP's since rabbit anti-hepatoma (S. A.) B_{12}BP antiserum does not precipitate human intrinsic factor or transcobalamin II on immunodiffusion but does give lines of complete identity with the two hepatoma B_{12}BP's and the "R-type" B_{12}BP's isolated from human milk and saliva. The four related proteins are indistinguishable in terms of amino acid composition and molecular weight (63,000–67,000), but differ in their carbohydrate composition. The type of sugar and the indicated number of residues (moles/mole B_{12}) for the hepatoma (S. A.), hepatoma (M. M.), milk and saliva B_{12}BP's, respectively, are as follows: sialic acid (16, 19, 6, 7); fucose (10, 9, 20, 35); galactose (44, 37, 37, 45); mannose (24, 27, 22, 21); galactosamine (2, 5, 3, 5); and glucosamine (38, 43, 36, 41). Metastatic tumor from patient M. M. has a markedly elevated B_{12} binding ability (> 99% due to "R-type" B_{12}BP) of 400 ng/g wet weight compared with < 3 ng/g for normal liver and uninvolved liver from M. M. These studies suggest that adolescent hepatomas associated with elevated levels of serum B_{12} and B_{12}-binding ability may represent an important subtype in which the tumor synthesizes and secretes an "R-type" B_{12}BP with a distinctive (high sialic acid, low fucose) carbohydrate composition. (Supported by NIH and ACS.)

50. Leucine: a Possible Regulator of Protein Synthesis in Muscles. MARIA G. BUSE* AND S. SANDRA REID,* Charleston, S.C. (introduced by Joseph C. Ross**).

The branched-chain amino acids, leucine, isoleucine, and valine, are catabolized mainly extrahepatically. Their oxidation by muscles is under metabolic and hormonal regulation and is stimulated by fasting. The intracellular or compartmental concentration of one or more of the branched-chain amino acids in muscles could act as a signal modulating the rate of protein turnover. Rat hemidiaphragms were incubated for 1 h in balanced salt solution containing 5.5 mM glucose, with or without the three branched-chain amino acids, 0.3 mM each. They were then transferred to identical media containing in addition 1 mU/ml insulin and 0.1 mM [U-^{14}C] lysine and incubated for 2 h. Muscles incubated with branched-chain amino acids incorporated 20% more [^{14}C] lysine into proteins than controls ($P < 0.05$). When the effect of each branched-chain amino acid (0.5 mM) on [^{14}C]lysine incorporation into proteins was tested separately, valine was ineffective and isoleucine inhibitory ($P < 0.01$), but leucine stimulated significantly (+ 25%, P

< 0.01). Leucine also stimulated ^{14}C incorporation into proteins when [^{14}C]acetate was used as a tracer. Leucine-induced stimulation of [^{14}C]lysine incorporation into proteins was observed using hemidiaphragms from fed or fasted rats, incubated with or without insulin. Hemidiaphragms preincubated for 1 h with 0.5 mM leucine without the radioactive tracer incorporated more [^{14}C]lysine into proteins during the next hour in the absence of leucine than the controls which were preincubated and incubated without leucine ($P < 0.001$). Experiments with actinomycin D and cycloheximide suggest that leucine stimulates translation and not transcription. Modulation of protein turnover in muscles by leucine may play a role in the transition to negative N_2 balance and accelerated gluconeogenesis during fasting, stress, or uncontrolled diabetes. (Research supported by Grant AM-02001 from NIH.)

51. Methylprednisolone Fails to Inhibit Primary and Secondary Antibody Responses but Causes Marked Suppression of On-Going Antibody Formation in Man. WILLIAM T. BUTLER, ROBERT B. COUCH, ROGER D. ROSSEN,* AND EVAN M. HERSH,* Houston, Tex.

The specific effects of corticosteroids on immunity in man are poorly understood despite extensive administration of high doses of these agents. We administered 96 mg methylprednisolone (MP) for 3 or 5 days (16 mg orally every 4 h) to 17 normal male volunteers and studied its effect on immunoglobin metabolism and on antibody responses to antigens given during or 1 day after MP administration. Results were compared to the antibody responses in 12 control volunteers immunized and studied simultaneously but who did not receive MP. We reported previously that significant decreases in serum IgG occurred in 86%, in IgA in 43%, and in IgM in 14% of treated volunteers within 2–4 wk after MP, whereas no significant changes occurred in untreated controls (1973. *J. Clin. Invest.* 52:2629). Despite these decreases in circulating immunoglobulin concentrations, the primary antibody responses to keyhole limpet hemocyanin were similar in MP-treated and control volunteers. Likewise, MP treatment did not significantly alter the secondary antibody responses to diphtheria toxoid, adenovirus 1, adenovirus 5, and influenza A. In contrast, naturally acquired neutralizing antibody to herpesvirus decreased in the majority of MP-treated volunteers in proportion to the decrease in serum IgG. Thus although MP causes inhibition of on-going immunoglobulin and antibody synthesis, high doses of MP fail to inhibit antigen processing or primary and secondary antibody responses. These results can be explained by the hypothesis that MP does not affect the cells involved in the induction of the immune response, but that its primary effect is on a plasma cell or plasma cell precursor which is crucial for the maintenance of the on-going antibody synthesis.

52. Detection of Tumor-Specific Immunity in Normal Household Contacts of Tumor-Bearing Patients. VERA S. BYERS,* ALAN S. LEVIN,* AND H. HUGH FUDENBERG, San Francisco, Calif.

Our group is presently treating a variety of patients with tumor-specific transfer factor derived from healthy household contacts who have been found to have tumor-specific immunity directed against cells of the histologic type of cancer of the patient. About 20% of all nontumor bearing household contacts have significant cell-mediated immunity specifically directed to the tumor type of the cancer patient, but not to other tumors. Cytotoxicity is measured by the capacity of donor lymphocytes to lyse all three osteogenic sarcoma tumor tests cell lines but not matching fibroblasts nor tumor cell lines from three other tumor types with their matching fibroblasts using ^{51}Cr release from the target cells as the assay. 10 of 46 household contacts tested had tumor-specific immunity against osteogenic sarcoma, while only 1 of 100 normal controls had this reactivity. 3 of 12 household contacts of a hypernephroma patient had tumor-specific immunity to this tumor, while no normal controls had this reactivity. These data suggest that these cancers may be caused by an infectious agent which causes malignant disease in some individuals (presumably genetically predisposed) and cellular immunity without disease in others exposed to the same antigen. No correlation existed between cellular immunity and the presence or absence of antibodies (as measured by quantitative immunofluorescence) in patients or donors.

53. P_{CO_2} Gradients Between Blood and CSF During Acute and Prolonged Alterations of Acid-Base Balance. JOHN J. CARONNA,* BO K. SIESJO,* AND FRED PLUM,** Lund, Sweden, and New York.

Theoretical considerations based on the Krogh tissue diffusion model suggest that the P_{CO_2} gradient between CSF and arterial blood ($P_{(csf\text{-}a)CO_2}$) in the steady state should vary inversely with cerebral blood flow (CBF), a high CBF being reflected by a narrow gradient and vice versa. However Davies and Gurtner have reported that the $P_{(csf\text{-}a)CO_2}$ varies directly with arterial hydrogen ion activity ($_a[H^+]$) and with CBF due to the presence of the so-called Wien effect. To test this hypothesis we have measured pH and P_{CO_2} of arterial and cerebral venous blood and the P_{CO_2} of CSF in rats during acute respiratory acidosis and alkalosis and after 24–72 h of NH_4Cl-induced metabolic acidosis. Although the Wien effect, if present, should produce considerable changes in these experimental situations, the results provide no such evidence. $P_{(csf\text{-}a)CO_2}$ varied inversely with both $_a[H^+]$ and $Paco_2$ during hyper- and hypocapnia, and therefore must have varied inversely with CBF. $P_{(csf\text{-}a)CO_2}$ did not change during metabolic acidosis. In these experiments the P_{CO_2} gradient between CSF and arterial blood was not different from the P_{CO_2} gradient between arterial and cerebral venous blood. In all acid-base categories tested the observed P_{csfCO_2} was not different from the value predicted by the formula based on the tissue diffusion model:

$$P_{csfCO_2} = \tfrac{1}{2}\,(Paco_2 + Pvco_2) + 1$$

We conclude that $P_{(csf\text{-}a)CO_2}$ varies inversely with CBF and does not vary directly with $_a[H^+]$. These observations are contradictory to the hypothesis that the Wien effect governs the physiological relationship between arterial and CSF P_{CO_2} but are consistent with the values derived from the classical Krogh diffusion model. (Research supported by grants from USPHS and the Swedish government.)

54. Antagonism Between Chloramphenicol and Penicillin in Streptococcal Endocarditis in Rabbits. J. CARRIZOSA,* W. D. KOBASA,* AND D. KAYE, Philadelphia, Pa.

Antagonism between chloramphenicol (C) and procaine penicillin (P) was studied in rabbits with streptococcal endocarditis. Polyethylene catheters were inserted into the heart through the carotid artery and 72 h later 10^7–10^8 *Streptococcus viridans* were injected intravenously. P (100 mg/kg) or P (100 mg/kg) plus C (90 mg/kg) were injected intramuscularly twice a day starting either 6 or 72 h after injection of the streptococci. In these experiments C was always administered 1 h before P. In untreated animals aortic valve vegetations contained mean log number 6.2 colony-forming units of streptococci per gram 6 h after infection and mean log number 8.0 streptococci per gram 72 h after infection. In animals treated after 6 h of infection, vegetations contained mean log numbers of 3.0 in P animals and 3.7 in P + C animals ($P > 0.05$) after

24 h of treatment, and 2.4 in P animals and 4.3 in P + C animals ($P < 0.01$) after 48 h of treatment. After 72 h of treatment most vegetations from P animals were sterile and the mean log number per gram in P + C animals was 3.5. In animals treated after 72 h of infection, vegetations contained mean log numbers 3.8 in the P animals and 5.2 in the P + C animals ($P < 0.01$) after 3 days of treatment, and 1.7 in P animals and 2.5 in P + C animals ($P < 0.05$) after 5 days of treatment. After 7 days of treatment most of the vegetations in both groups were sterile. In other experiments when C was injected 1 h after P, animals receiving P + C had higher numbers of streptococci in vegetations than P animals. These studies demonstrate that C antagonizes P in this model of streptococcal endocarditis.

55. Coagulation Abnormalities in Hyperbetalipoproteinemia and Their Modification with Therapy. ANGELINA CARVALHO,* ROBERT W. COLMAN, AND ROBERT S. LEES, Boston, Mass., and Philadelphia, Pa.

Clinical sequelae and death in hyperbetalipoproteinemic patients are usually from thrombotic complications of atherosclerosis. We have investigated platelet function and intravascular coagulation in 17 such patients to determine whether increased coagulability might occur in and complicate hyperbetalipoproteinemia (type II hyperlipoproteinemia). All tests were compared with results from 26 normal subjects. Platelets from untreated type II patients and from those treated with diet alone were markedly more susceptible to aggregation in vitro than normal platelets, whether tested with epinephrine (25 times normal sensitivity, $P < 0.01$), ADP, or collagen (three times normal sensitivity, $P < 0.01$). Furthermore, nucleotide release by aggregated platelets from type II subjects was 4- to 6-fold increased ($P < 0.01$), suggesting that thrombus propagation in vivo might be increased. In addition, intravascular coagulation, measured by the plasma concentration of high molecular weight fibrinogen derivatives, was increased 10-fold ($P < 0.01$). In these patients, plasma prekallikrein, kallikrein inhibitor, and factor XII were significantly lower than normal ($P < 0.01$), suggesting that activation of the intrinsic clotting pathway may be the mechanism responsible for intravascular coagulation. Treatment with clofibrate (Atromid-S) returned platelet sensitivity nearly to normal, although it did not affect platelet nucleotide release. Clofibrate abolished the activation of the intrinsic pathway and decreased intravascular coagulation in type II patients. These changes occurred despite the lack of significant plasma lipid response to clofibrate therapy. The data suggest that increased atherogenesis in type II hyperlipoproteinemia is accompanied by increased coagulability and platelet reactivity, which may further predispose these patients to arterial thrombosis. Clofibrate may exert its putative beneficial effect in part through its action on coagulation mechanisms. (Research supported by NIH Grants HL-14209, HL-48075, and RR-88.)

56. Evidence for an Alternate Complement Pathway Defect Other Than C3 Proactivator (C3PA) Deficiency in Sickle Cell Disease (SCD). JAMES CASPER,* SUSAN KOETHE,* AND GLENN RODEY,* Milwaukee, Wis. (introduced by Richard Aster).

Sickle cell disease (SCD) patients are deficient in a heat-labile, pseudoglobulin component of the alternate complement pathway necessary to opsonize pneumococci for phagocytosis (Johnston, R.B. 1973. *N. Engl. J. Med.* 288:803). Since C3PA is both heat labile and a pseudoglobulin, it has been proposed that SCD patients are deficient in C3PA. The following studies were undertaken to further define the complement abnormality in patients with SCD. 17 normal and 22 SCD sera containing 0.01 M EGTA to block the classical complement pathway were incubated at 37°C with either saline or zymosan. Residual complement activity of saline-serum mixtures was 104 ± 27 and 110 ± 12 CH_{50} units in normal and SCD patients, respectively ($P > 0.05$). Residual complement activity of zymosan-serum mixtures were 3 ± 4 and 23 ± 31 CH_{50} units in normal and SCD patients, respectively ($P < 0.01$), confirming that complement consumption via the alternate pathway is defective in SCD. Mean serum C3PA levels determined by quantitative immunodiffusion were 278 ± 64 and 310 ± 85 μg/ml in normal and SCD patients, respectively ($P > 0.05$), indicating that the quantity of C3PA in SCD patients is normal. When functional activity of C3PA was measured in 17 SCD patients and 17 controls using a cobra venom factor (CoVF) assay (Hunsicker, L. G. 1973. *J. Immunol.* 110:128), reciprocal serum dilutions required to lyse 50% of sheep erythrocytes (CoVF activable $hemolysis_{50}$) were 10.7 ± 32 and 8.52 ± 3.08 CoVF AH_{50} units/ml in normal and SCD patients, respectively ($P < 0.05$). The slight, albeit statistically significant, decrease in CoVF activity in SCD patients makes it unlikely that a qualitative C3PA deficiency explains pronounced alternate pathway abnormality in SCD. We conclude that the complement abnormality in SCD, and possibly susceptibility to infection manifested by SCD patients, is related to other factor(s) necessary for alternate pathway activation. (Supported by Contract HL-3-2928B.)

57. The Isolation of a Nuclear "Precursor" to a Specific Steroid Hormone-Induced Messenger RNA. L. CHAN,* J. ROSEN,* S. HARRIS,* A. MEANS,* AND B. O'MALLEY, Houston, Tex.

We have previously demonstrated that estrogen induces accumulation of the messenger RNA (mRNA) for the specific oviduct protein, ovalbumin, as quantitated in an in vitro translation system. A complementary DNA copy ($[^3H]cDNA_{ov}$) of purified mRNA was prepared using viral reverse transcriptase. Hybridization of $[^3H]cDNA_{ov}$ to whole chick DNA reveals that the ovalbumin gene is represented once per haploid genome. We now report that a high molecular weight precursor for ovalbumin mRNA (pre-mRNA) can be isolated from oviduct nuclei and is under estrogen control. Whereas no translatable ovalbumin mRNA could be extracted from oviduct nuclei of immature chicks, administration of estrogen resulted in induction of ovalbumin mRNA extractable from these nuclei. RNA extracted from highly purified hen oviduct nuclei was also found to contain ovalbumin mRNA activity assayed in the translation system. Fractionation of this nuclear RNA on formamide gradients (which prevents aggregation) revealed that some of the pre-mRNA was present in a form >30S. Similar treatment of cytoplasmic polysomal RNA demonstrated that all mRNA activity was in the 18S form, consistent with the previously determined size of purified ovalbumin mRNA. Repeated fractionation of hen oviduct nuclear RNA on formamide gradients resulted in RNA which was free of lower molecular weight forms as confirmed by electrophoresis on acid-urea agarose gels. Hybridization of the highly purified >30S nuclear RNA to $[^3H]cDNA_{ov}$ confirmed that specific ovalbumin mRNA sequences were present at a concentration ~3% that of total cell ovalbumin mRNA. These data constitute the initial demonstration of the hormonal induction of a high molecular weight nuclear RNA which will subsequently be processed to yield a cytoplasmic mRNA for a specific hormone-regulated protein.

58. The Effect of Glucagon on the Contribution of Alanine to Hepatic Glucose Production in Man. J. L. CHIASSON,* J. E. LILJENQUIST,* B. C. SINCLAIR-SMITH,* AND W. W. LACY,* Nashville, Tenn. (introduced by O.B. Crofford).

The association of high glucagon levels and increased hepatic alanine extraction in fasting man has suggested that alanine is a major glucose precursor and that glucagon plays a significant role in the control of gluconeogenesis. The present study was undertaken (*a*) to measure the contribution of alanine to hepatic glucose production, and (*b*) to determine the effect of glucagon on this process. In five normal postabsorptive men undergoing hepatic vein catheterization, [U-^{14}C] alanine was infused continuously for 80 min. After the arterial alanine specific activity had stabilized (40 min), the study consisted of a 20 min basal period followed by a 20 min glucagon infusion period (50 ng/kg per min). Arterial alanine specific activity (4922 ± 362 cpm/μmol) and net splanchnic alanine uptake (96.8 ± 5.1 μmol/min) remained constant, while arterial alanine concentration declined from 241 ± 6 to 204 ± 8 μmol/liter during the glucagon infusion. This indicates an increased uptake of alanine at some extrasplanchnic site(s). Net splanchnic glucose production (NSGP) increased after glucagon from 151 ± 10 to 504 ± 81 mg/min. The basal rate of conversion of arterial alanine to glucose was 30 ± 2 μmol/min, or 33% of its net splanchnic extraction. Of the basal NSGP only 1.7% was derived from arterial alanine. During glucagon infusion, net splanchinic [^{14}C] glucose production doubled, and the conversion of alanine to glucose increased 93% to a maximum of 58 ± 9 μmol/min. The data indicate either that arterial alanine is not a major precursor of glucose or that gluconeogenesis contributes little to hepatic glucose production after a 14 h fast. Glucagon acutely increases the conversion of alanine to glucose by intrahepatic diversion to glucose rather than by increasing its uptake. (Supported by NIH Grants HL 08195, RR95, and AM 17026.)

59. Radioimmunoassay of 3,3′,5′-Triiodothyronine (Reverse T_3). INDER J. CHOPRA,* Los Angeles, Calif. (introduced by D. H. Solomon**).

Highly specific antibody to reverse T_3 (rT_3) was prepared by immunization of a rabbit with rT_3 conjugated to human serum albumin with carbodiimide. The antiserum diluted 1:250 was employed in a simple, precise, and reproducible double-antibody radioimmunoassay (RIA) capable of detecting 10 pg of nonradioactive rT_3. The relative potency of various thyroid analogues (on a weight basis) to inhibit the binding of [^{125}I] rT_3 to antibody was: rT_3, 100; *l*-T_3, 0.02; *d*-T_3, 0.006; triiodothyroacetic acid, 0.003; *l*-thyroxine (T_4) (two reagent preparations and three lots of Synthroid), 0.06–0.09; *d*-T_4, 0.045; tetraiodothyroacetic acid, 0.034; 3,5-*l*-diiodothyronine (T_2), < 0.002; 3,3′-T_2, 10.0; 3,5-diiodothyropropionic acid, 0.005; 3-monoiodothyronine, 0.24; *l*-thyronine, < 0.002; DIT, 0.03; MIT, < 0.02; potassium iodide, < 0.0003. RIA was employed to measure rT_3 concentration in ethanol extracts of human serum. Recovery of extraction of [^{125}I] rT_3 added to serum was (mean ± SE, n = 10) 89 ± 1.5%. Recovery of nonradioactive rT_3 added to serum averaged 93 ± 1% (n = 16). Serum rT_3 concentrations were [mean ± SD (No.), ng/100 ml]: euthyroid, 41 ± 10 (27); hyperthyroid 103 ± 49 (22); hypothyroid, 19 ± 9 (12); pregnancy or estrogen treatment, 54 ± 7 (5); hypothyroid on replacement therapy with Synthroid, 55 ± 19 (12); newborn cord serum, 136 ± 29 (7). It is proposed that: (*a*) rT_3 is a normal component of human serum; (*b*) peripheral metabolism of T_4 is an important source of rT_3 in serum; (*c*) the combination of high serum rT_3 and the previously reported low serum T_3 in cord sera may signify that the metabolism of T_4 to T_3 or rT_3 is not necessarily a random process. (Supported by NIH Grant AM 16155.)

60. Skeletal Muscle Intracellular Oxygen Tension ($ICPo_2$) During Exercise. B. J. CLARK* AND R. F. COBURN, Philadelphia, Pa.

We have adapted to man our method of estimating $ICPo_2$ in "red" muscle (*Am. J. Physiol.* **220**:66, 1971; **224**:876, 1973). The method depends on competitive binding of O_2 and CO to myoglobin. The mean Po_2 in "equilibrium" with myoglobin is computed from MBCO measurements. Since over 95% of body CO is bound to hemoglobin and myoglobin, changes in MBCO can be detected from changes in HBCO at constant body CO. Calculations show for decreases in $ICPo_2$ sufficient to limit aerobic metabolism (assumed < 1 mm Hg), CO will shift into muscle from blood resulting in HBCO 80–85% of control. 11 normal, nontrained human subjects, 21–41 yr old, exercised at varying work loads on a bicycle ergometer. The subjects respired in a closed system which prevented pulmonary CO exchange. Venous blood was drawn before and 15–30 s after 1.5- to 2.0-min exercise periods. Exercise at 60–70% of $\dot{V}O_2MAX$, breathing 21% O_2, resulted in HBCO of 99.16 ± SE 0.86% of control ($P > 0.05$). Exercise, breathing 21% O_2, at 110–120% of the work load which just resulted in $\dot{V}O_2MAX$ (supermax) resulted in fall in HBCO to 95.24 ± SE 1.20% of control ($P < 0.01$). Supermax exercise, breathing 13–14% O_2, resulted in HBCO of 83.08 ± SE 2.79% of control ($P < 0.01$). Animal and theoretical studies showed decrease in blood and muscle pH or temperature increase could cause a small CO shift into muscle. We conclude the following. Small shifts of CO into muscle with short duration, supermax exercise, breathing air, are probably explained by pH and temperature changes; data appear to indicate $ICPo_2$ is near normal and oxygen delivery per se does not limit oxygen uptake in "red" muscle under these conditions. Aerobic capacity during supermax exercise, with 14% O_2, appears limited by oxygen transport since large CO shifts into muscle indicate a critical fall in $ICPo_2$.

61. Preservation of Jeopardized Myocardium by Collateral Blood Flow. MICHAEL V. COHEN,* B. LEONARD HOLMAN,* JAY LEVINE,* JAMES M. DOWNEY,* PER ELDH,* EDMUND H. SONNENBLICK, AND EDWARD S. KIRK,* Boston, Mass.

Myocardial integrity depends on the balance between supply and demand, but their relative importance after coronary occlusion is poorly understood. To show the importance of collateral blood flow, left anterior descending coronary arteries in 12 closed-chest dogs were embolized with fluoroscopically placed plugs. Infarcts examined 1 wk later involved 0–37% of the left ventricle and varied inversely with collateral flow measured with radioactive microspheres at the time of occlusion ($r = 0.91$). Collateral flow can develop rapidly and protect ischemic myocardium as demonstrated by hollow emboli which became obstructed with thrombi within hours but produced significantly smaller infarcts than did solid plugs. Increased demand caused by adrenergic stimulation was compensated by increased collateral flow in 10 acute experiments. Thus norepinephrine, by raising blood pressure, enhanced collateral flow measured by ^{133}Xe clearance and caused minimal changes in epicardial electrograms. In contrast, isoproterenol with similar inotropic effects decreased collateral flow and greatly increased myocardial injury. Coronary steal caused by coronary vasodilation contributed to the detrimental effect. 3 wk after occlusion collateral vessels maintained normal myocardial function and under conditions of increased demand or jeopardized supply could be dilated by nitroglycerin to preserve function. In hearts with well developed collaterals revascularization of the occluded artery was able to restore cardiac function after sudden occlusion of the remaining normal vessels. Thus retrograde collateral flow can preserve large areas of myocardium. These studies show that myocardial injury, infarct size, and ultimate function after coronary occlusion are primarily determined by collateral blood flow. Accordingly, means to enhance collateral circulation should be considered in clinical efforts to preserve jeopardized myocardium. (Research supported by grants from NHL.)

62. Adrenal Cortical Response to Acute Volume Depletion in Anephric Man. C. ROBERT COOKE,* JOHN S. HORVATH,* MICHAEL A. MOORE,* TURNER BLEDSOE,* AND W. GORDON WALKER,** Baltimore, Md.

Recent studies on the effect of acute volume and sodium depletion on plasma aldosterone concentration (PA) in anephric man have failed to completely resolve the question whether aldosterone secretion is responsive to volume-related stimuli in the absence of the renin-angiotensin system. To resolve this issue, six anephric patients were studied during 7 h of hemodialysis resulting in an average weight reduction of 2.4 kg and during a comparable period of dialysis in which normal saline was infused to keep the body weight constant. Plasma [Na] was unchanged and plasma [K] decreased ($P < 0.005$) in both the volume-depletion (VD) and volume-stable (VS) studies. Mean (± SEM) PA at the beginning and after 1, 4, and 7 h of dialysis was 5.6 ± 1.7, 5.7 ± 2.2, 6.4 ± 1.7, and 3.5 ± 0.8 ng/100 ml in VD and 3.9 ± 1.0, 4.4 ± 1.0, 5.2 ± 2.0, and 3.3 ± 1.0 ng/100 ml in VS. Plasma cortisol concentration (PC) was increased ($P < 0.05$) after 7 h of dialysis in VD but not in VS. In similar studies performed during dexamethasone suppression (six patients), PA remained constant and failed to increase in one patient whose weight decreased 6.4 kg, even though PC increased from < 2.0 to 10.1 μg/100 ml. The results of these studies indicate that aldosterone secretion in anephric man is unresponsive to acute volume depletion when plasma [Na] is unchanged despite increased ACTH elaboration. The low PA at 7 h could in part be related to the reduction in plasma [K]. (Supported in part by NIH Grants HL 3303 and RR 35.)

63. Persistence of Capsular Polysaccharide Antigenemia After Pneumococcal Pneumonia in Man. J. D. COONROD* AND H. PARKER,* Lexington, Ky., and Milwaukee, Wis. (introduced by Ward E. Bullock).

We have observed persistent antigenemia with pneumococcal capsular polysaccharide (PCP) in 6 of 75 patients with pneumococcal pneumonia. The kinetics of this antigenemia in four patients was compared with the antigenemia produced in rats by an immunologically "paralyzing" dose of pneumococcal capsular polysaccharide vaccine (PCPV). Antigen was detected by counterimmunoelectrophoresis or latex agglutination. After pneumococcal pneumonia, PCP persisted in the circulation from 3 to 17 wk, decreasing at a constant and exponential rate in each case. The mean half-life ($t_{½}$) was 8 days (range 2–14 days). In rats, circulating PCPV was present for > 17 wk. Clearance was exponential and constant during the first 4 wk ($t_{½}$ = 10 days) but decreased thereafter ($t_{½}$ = 38 days at 17 wk). The mean molecular weight of circulating antigen and its immunoreactivity in gel diffusion tests did not change with time. Therefore, persistence of circulating PCP or PCPV could not be attributed to the presence of degraded polysaccharide. There was antigenuria during the entire period of antigenemia in rats and after pneumonia. However, antigenuria did not account directly for the decline in circulating antigen since urine polysaccharide had a lower molecular weight and had only partial identity in gel with circulating antigen. In summary, (*a*) persistent antigenemia occasionally follows pneumococcal pneumonia in man; and (*b*) this antigenemia resembles but is not identical with that produced by a "paralyzing" dose of polysaccharide in rats. (Research supported in part by Grant CC 00579, Center for Disease Control, USPHS.)

64. Variable Response to Increased Methyltetrahydrofolate in Plasma in Pernicious Anemia. B. A. COOPER AND T. ABE,* Montreal, Canada.

Folinic acid administered by mouth is converted almost completely to methyltetrahydrofolate in the intestinal wall. Folinic acid was fed to six patients with pernicious anemia in relapse to determine if elevation of methyltetrahydrofolate in plasma would correct the megaloblastic abnormality. One of two patients receiving 0.8 μg/day of *dl*-folinic acid showed evidence of partial response of bone marrow morphology, some reticulocyte response, and increase of serum cholesterol and urate, whereas in the other, thrombocytopenia was partially corrected, but no other change occurred in her megaloblastic picture. One of four patients receiving 6 mg/day of *dl*-folinic acid responded completely to this therapy, whereas no significant change was observed in the megaloblastic status of the others. The patient who responded partially to 0.8 μg/day had the highest pretreatment serum folate of the group (40 ng/ml), whereas the one who responded completely to 6 μg/day had the lowest (6.1 ng/ml). Fractionation of B12 coenzymes in plasma, erythrocytes, and bone marrow cells did not reveal changes in distribution of these coenzymes during administration of folinic acid. After injection of cyanocobalamin, there was a prompt increase of 5′-deoxyadenosyl B12 in bone marrow cells, with much small increase of methyl-B12 (the coenzyme presumed to be lacking in megaloblastic anemia due to B12 deficiency). 1000 μg of cyanocobalamin was much more effective in converting these coenzymes than was 100 μg. These studies indicate that some patients with B12 deficiency respond to increase of serum methyltetrahydrofolate level by conversion of megaloblastic to normoblastic bone marrow, but most will not. Response is not directly related to folate level or to presumed folate deficiency. In some patients, increased serum folate level will increase platelets, neutrophils, or reticulocytes without converting megaloblastic to normoblastic bone marrow. Increase of serum methyltetrahydrofolate level does not alter the relationship of B12 coenzymes in bone marrow cells, whereas injection of cyanocobalamin does this promptly. Reappearance of normal levels of methyl-B12 in cells is much faster after injection of 1000 than of 100 μg of cyanocobalamin.

65. Immobilization of the Lipid Bilayer: the Primary Spur Cell Defect. RICHARD A. COOPER, STEVEN FISCHKOFF,* ELIZABETH ARNER,* AND JANE VANDERKOOI,* Philadelphia, Pa.

Spur cells are cholesterol-rich red cells acquired in severe hepatocellular disease. Because their membranes are rigid, spur cells are "conditioned" and later destroyed in the spleen. To understand this rigidity, the mobility of lipids within the membrane bilayer was assessed in natural spur cells and in spur cells prepared by incubating normal red cells with cholesterol-rich cholesterol:lecithin dispersions. 12(9-Anthroyl) stearic acid (AS) is a fluorescent probe of the lipid bilayer. It dissolves into the hydrophobic portion of membranes. Mobility within the membrane bilayer is indicated by a low polarization of the fluorescence of AS after its polarized excitation. The presence of cholesterol out of proportion to phospholipid in both natural and experimental spur cells caused a 10% increase in AS fluorescence polarization at 37°C. Thus, excess cholesterol decreases the mobility of lipids within the membrane bilayer. Cold compresses the lipid bilayer. This increases the polarization of AS fluorescence and decreases membrane surface area, as calculated from osmotic fragility. Lowering the temperature from 37°C to 10°C increased AS polarization 50% in normal red cells but only 22% in cholesterol-rich red cells. Similarly, lowering the temperature from 37°C to 0°C decreased membrane surface area in normal cells but caused no change in cholesterol-rich red cells. Thus, excess cholesterol interferes with the cold-induced compression of the lipid bilayer. Cholesterol extends the surface area of red cell membranes. These studies demonstrate that, in addition, cholesterol causes the bilayer of cholesterol-rich red cell membranes to be relatively fixed and immobilized in this extended form. They suggest that this effect of cholesterol

on the bilayer is the primary defect responsible for the rigidity of spur cells and for their premature destruction in vivo. (Research supported by Grants AM 15441 and GM 12202 from the NIH.)

66. Collagen Gene Expression in the Remaining Lung After Unilateral Pneumonectomy. MORTON J. COWAN* AND RONALD G. CRYSTAL,* Bethesda, Md. (introduced by Martha Vaughan**).

After left pneumonectomy (PX) in the rabbit, the right lung almost doubles its total mass within 4 wk. This growth response includes a significant increase in the total number of lung cells. During normal development, collagen gene expression is transiently, but significantly, enhanced in the neonatal period. Just before birth, 3% of the total lung protein synthesis is devoted to collagen synthesis. This increases 4-fold in the neonatal period and then falls to 3% in adulthood. To see if the growth response of lung to PX in the adult includes a return to the newborn pattern of collagen gene expression, 40 2-month-old rabbits underwent PX, while 40 littermate controls underwent left thoracotomy (TH) without PX. At 1, 3, 7, 14, and 28 days after surgery, lung slices were incubated in Dulbecco's modified Eagle medium with [^{14}C]proline. Collagen synthesis per cell (nmoles [^{14}C]proline incorporated into [^{14}C]hydroxyproline per mg DNA·h) was significantly greater in the PX group than the TH group on day 7 (1.78 ± 0.98 vs. 0.62 ± 0.17; mean $\pm$ SD, $n = 18$) and on day 14 (2.65 ± 0.47 vs. 0.93 ± 0.36; $n = 10$) but returned to control levels by day 28 (0.88 ± 0.63 vs. 0.63 ± 0.22; $n = 24$). Expressed as percent total protein synthesis, collagen synthesis was normal ($3.0 \pm 0.6\%$) in the TH group at days 1–28. In the PX group, however, the percent collagen synthesis on days 1, 3, 7, 14 and 28 was, respectively, 3.4, 2.8, 5.8, 6.0, 2.1. The increase at days 7 and 14 is significant to $P < 0.01$. It appears that collagen gene expression during the growth response after PX is similar to the normal collagen gene expression in the newborn period when there is a relative shift toward collagen synthesis.

67. Chenodeoxycholic Acid (CDC) and Phenobarbital (PB) Effects on Hepatic Cholesterol and Bile Acid Synthesis in Man. M. J. COYNE,* G. G. BONORRIS,* L. I. GOLDSTEIN,* AND L. J. SCHOENFIELD, Los Angeles, Calif.

The effects of CDC, 750 mg/day and PB 90–180 mg hs, individually, combined, and placebo on biliary lipid composition and on the rate-limiting hepatic enzymes of cholesterol and bile acid synthesis were determined in patients with cholelithiasis. The aim was to study the mechanisms whereby CDC and PB decrease the cholesterol saturation of bile. Nine patients with cholelithiasis in a double blind study of gallstone dissolution underwent liver biopsy, fasting at 9:00 a.m. The microsomal fraction from 50 mg of liver was assayed in duplicate (agreement 3–10%) for HMG CoA reductase and 7 α-hydroxylase (Schoenfield et al. 1973. *J. Lab. Clin. Med.* 82:858–868). The proportions of bile acid, lecithin, cholesterol, and individual bile acids in duodenal bile were determined before and after 6 months treatment. CDC caused a significant decrease in HMG CoA reductase and 7 α-hydroxylase and a significant increase in CDC and in the ratio of bile acid plus lecithin to cholesterol in bile. The lipid ratio and CDC in bile were significantly increased by PB and both. PB, however, significantly increased HMG CoA reductase and did not effect 7 α-hydroxylase. With both, HMG CoA reductase did not change, while 7 α-hydroxylase activity significantly decreased. We conclude the following. (*a*) CDC caused increased CDC in bile and decreased hepatic cholesterol synthesis, both of which may contribute to reducing the cholesterol saturation of bile. (*b*) PB caused increased CDC and decreased cholesterol saturation in bile despite increased hepatic cholesterol synthesis and unchanged bile acid synthesis. (Supported by NIH Grant AM 15631.)

68. Regulation of Complement-Derived Inflammatory Mediators by Factors in Human Serum. RICHARD DATA,* PETER A. WARD, AND GERD TILL,* Farmington, Conn.

The third (C3) and the fifth (C5) components of human complement can each be cleaved to produce chemotactic and anaphylatoxin activities. These have been produced by trypsin treatment of purified preparations of C3 and C5 or by incubation at 37°C of human serum containing 1 M ϵ-aminocaproic acid in the presence or absence of yeast particles. By the use of antibodies it has been demonstrated that the biological activities generated in human serum are C3 or C5 products. Normal human serum has been fractionated by a combination of protein precipitation by salt, gel filtration, and ion-exchange chromatography. Two fractions, A and B, have been obtained, each having the capacity to inactivate the bacterial chemotactic factor derived from *Escherichia coli*. Fraction A, a β-globulin with a sedimentation velocity of $\sim$ 7S, inactivates the C3 chemotactic fragment as well as anaphylatoxin activities from both C3 and C5. Fraction B, an α-globulin with a sedimentation velocity of $\sim$ 4S, preferentially inactivates the C5 chemotactic and the C3 anaphylatoxin fragments. These data indicate a heterogeneity in chemotactic and anaphylatoxin inactivator activities in human serum and suggest that the biologically active fragments from a given complement component may not be structurally identical. (Supported in part by NIH Grants AI-09651 and AI-10155.)

69. Pancreatic Beta Cell Sensitivity to Glucose and Tissue Sensitivity to Insulin: Quantitation of the Disturbances in Uremia. RALPH A. DEFRONZO,* JORDAN D. TOBIN,* JOHN W. ROWE,* DANIEL G. SAPIR,* KARL J. KRAMER,* AND REUBIN ANDRES,** Baltimore, Md.

The relative contributions of impaired insulin secretion and of tissue insensitivity to insulin to the carbohydrate intolerance of uremia were investigated in eight uremic patients. Two types of glucose-clamp experiments were performed in each patient before and after 10 wk of hemodialysis. In both types, arterial blood glucose (G) is set by a servo-controlled glucose infusion using a negative feedback formula. *Hyperglycemic clamp:* G is acutely raised 125 mg/100 ml above basal level and is maintained for 2 h. The glucose infusion rate is an index of glucose metabolized (M) and increased after dialysis in all patients (mean Δ M = 49% $\pm$ 3.9, SEM, $P < 0.001$). The biphasic plasma insulin response (I), a measure of beta cell sensitivity, did not change consistently postdialysis (three increased, one no change, four decreased). The M/I ratio, an index of tissue sensitivity to insulin, increased postdialysis in all subjects (Δ M/I = 78% $\pm$ 22.2, $P < 0.01$). *Euglycemic clamp:* plasma insulin concentration is acutely raised by a primed-continuous insulin infusion; hypoglycemia is prevented by clamping G at the basal level. M/I, again a measure of tissue sensitivity, increased in all patients postdialysis (Δ M/I = 66% $\pm$ 20.8, $P < 0.02$). The dominant carbohydrate defect in uremia is thus a decrease in tissue sensitivity to insulin. The surprising apparent lack of consistency in beta cell response postdialysis can be explained by the strong inverse correlation between beta cell sensitivity and tissue sensitivity ($r = -0.9169$, $P < 0.001$): those individuals who showed striking improvement in tissue sensitivity to insulin actually decreased their serum insulin responses to hyperglycemia; those whose improvement in tissue

sensitivity was more modest showed increases in beta cell responses.

70. Triiodothyronine Binding to Hepatic Cell Nuclei In Vitro. LESLIE J. DEGROOT AND JANINE TORRESANI,* Chicago, Ill., and Marseille, France.

Triiodothyronine (T_3) given to rats in vivo binds to saturable receptor protein macromolecules. The T_3-protein complex is solubilized by 0.4 M KCl, and is destroyed at 37°C or by PCMB. We studied these possible T_3 receptors in rat liver nuclei prepared by tissue homogenization in 0.32 M sucrose + 1 mM $MgCl_2$ + 2 mM $CaCl_2$ + 10 mM pH 7.4 Tris (SMCT). Incubated nuclei were washed twice in SMCT + 5 mg/ml albumin. Specific binding was considered that displaced by 100-folds excess unlabeled T_3. Nuclear binding of tracer $^{125}T_3$ (5 pM) was 0.5 fmole per nuclei from 0.25 g liver (250 μg DNA). Calcium ions improved nuclear stability and 1 mM EGTA inhibited T_3 binding. Binding occurred at 0–2°C, was maximal at 20–30°C, and was time dependent over $0^{-\infty}$ min. Nuclear T_3 was extracted by 0.4 M KCl. The KCl-extracted T_3 was in a macromolecular protein complex since it resisted binding by exchange resin, and was destroyed by Pronase. This T_3-protein complex was destroyed by 37°C and PCMB. Liver cytosol inhibited nuclear binding of T_3. Cyanide, azide, puromycin, and actinomycin were without effect. Analysis by saturation with T_3 demonstrates a single set of binding sites with capacity of 1.5 ng/g liver nuclear equivalent and Kd of 1.5×10^{-9}M. The nuclear binding of T_3 in vitro is comparable in capacity and affinity to that observed in liver in vivo, and the T_3-macromolecular complex formed behaves similarly during KCl extraction and during heat or PCMB treatment. Our data suggests that T_3 binding differs from systems described for steroid hormones. In this in vitro system free T_3 binds to a set of limited capacity nuclear sites, but binding occurs even at 0°–2°C and without apparent requirement for a cytosol receptor.

71. Acyl Hydrolases in Human Platelets. ARIE DERKSEN,* RICHARD D. NORLIN,* WALTER C. PICKETT,* AND PHIN COHEN, Boston, Mass.

In previous studies (*J. Clin. Invest.* 49:128) we noted an increase in stainable free fatty acids (FA) after incubation with disrupted as compared with intact platelets; the present studies seek to define the apparent lipolytic activity. FA and triglycerides (TG) of fresh platelets were isolated on thin-layer chromatography (TLC) and quantified by gas-liquid chromatography (GLC). Lipid extract equivalent of 10 mg platelet protein contained 1.5–2 μg FA (almost exclusively 16:0 and 18:0) and 30–40 μg TG (16:0, 31%; 18:0, 18%; 18:1, 37%; 18:2, 14%). In timed studies with intact platelets in glucose-poor, 300 mOsm buffer at pH 7.4, FA levels fell for 60–90 min, then linearly rose to control levels by 3 h. By contrast, in media containing 5 mM glucose, FA levels doubled in 2 h, probably reflecting a sparing effect of glucose on FA oxidation. The FA levels of disrupted platelets rose linearly exceeding control levels up to 5-fold after 3 h, probably related in part to the negative effect of diluted (-)-carnitine levels on FA oxidation. With a 3 h incubation time, disrupted platelets showed 2 pH optima for FA release. At pH 4.0 a marked rise in 18:1 as compared with saturated FA was noted. This was accompanied by a fall in TG levels and a release of free glycerol. At pH 9.0, release of saturated FA predominated over 18:1, and there was neither reduction of TG nor release of glycerol. We conclude that human platelets have at least two acyl hydrolases: an acid hydrolase acting on TG, and an alkaline hydrolase acting mainly on the 1-position of phospholipids. (Supported by Grants HE 13584 and HE 13802 from NIH and U.S. Army grant DADA 17-70-C-0083.)

72. Protein Kinase Activation in Thyroid Slices by Thyroid-Stimulating Hormone (TSH) and Prostaglandin E_1 (PGE_1). FRED DERUBERTIS* AND JAMES B. FIELD,** Pittsburgh, Pa.

TSH regulates thyroid gland function by increasing cyclic adenosine monophosphate (cyclic AMP). Although cyclic AMP activates purified thyroid protein kinase, such stimulation has not been demonstrated in intact thyroid cells. Pig thyroid slices incubated with and without TSH and PGE_1 were homogenized and assayed for protein kinase using histone as substrate, with and without exogenous cyclic AMP (5×10^{-6}M). Results were correlated with cyclic AMP concentrations in slices and exogenous [^{3}H]cyclic AMP (1 pmole) binding in homogenates. TSH increased slice cyclic AMP content sixfold over control and decreased [^{3}H]cyclic AMP binding by homogenates 50% (control 1550 ± 35 cpm/mg, TSH 752 ± 54). Protein kinase activity from TSH-treated slices was significantly greater (4.7 ± 0.3 pmoles/mg per 5 min) than controls (2.5 ± 0.2). Exogenous cyclic AMP increased enzyme activity from control slices to 5.8 ± 0.3 but had negligible effects on that from TSH-treated slices, suggesting complete enzyme activation by the hormone-mediated increases in endogenous cyclic AMP. 1 min incubation of slices with TSH significantly increased protein kinase activity ratio (−CAMP/ + CAMP) from 0.45 to 0.65 and to 0.92 by 10 min. Appropriate changes occurred in cyclic AMP concentrations and [^{3}H]cyclic AMP binding. As little as 0.13 mU/ml TSH significantly increased the protein kinase activity ratio. 2.5 mU/ml TSH maximally activated protein kinase (activity ratio of 1), yet larger concentrations caused further elevation of cyclic AMP in slices and additional inhibition of [^{3}H]cyclic AMP binding. Thus, protein kinase may be rate limiting in thyroid slices, or all TSH-induced cyclic AMP is not available to activate protein kinase. PGE_1 produced similar but quantitatively smaller changes. These studies demonstrate protein kinase activation in intact thyroid slices which could mediate the effects of TSH and PGE_1. (Research supported by NIH grant.)

73. Induced Reversal of Compensatory Renal Hypertrophy. C. M. DIJKHUIS,* HERO VAN URK,* AND RONALD A. MALT, Boston, Mass.

Cross-circulation of a normal rat with an anephric rat produces compensatory renal hypertrophy (CRH) so far indistinguishable from that produced by unilateral nephrectomy in a single rat. Since the humoral stimulation can be stopped, cross-circulation allows the stability of CRH to be tested. 89 pairs of unrestrained rats connected by carotid-jugular shunts protected by skin bridges were disconnected after 48 h of cross-circulation. In normal rats connected to anephric rats for 48 h, mg kidney per g body was 17% greater than shams ($P < 0.001$), and micrograms renal RNA per microgram DNA was + 12% ($P < 0.002$). 6 h after disconnection, renal mass was + 10% ($P < 0.02$) and after 12 h was normal (+ 5%, $P > 0.20$). RNA/DNA at 6 and 12 h were, respectively, + 21% ($P < 0.005$) and + 6% ($P > 0.40$). Results in the two groups were almost coincident from 12 to 48 h after disconnection. Water contents were constant at all times. Carotid-jugular shunts within a single rat invariably decreased renal mass and RNA/DNA contents. Kidneys that had immediately recovered from cross-circulation-induced CRH responded normally to CRH provoked by unilateral nephrectomy. Early compensatory hypertrophy appears to require the presence of its regulator for maintainence. Reversal of CRH seems to be even faster than atrophy of an immobilized or denervated muscle. Concurrent studies support the view that variations in catabolism are important regulators of CRH. In patients with renal transplants, if dialysis removes the stimuli, rapid reversal of

CRH could be an undesired concomitant. (Supported by NIH Grant AM-12769.)

74. A New Human Endogenous Pyrogen. CHARLES A. DINARELLO* AND SHELDON M. WOLFF, Bethesda, Md.

Human peripheral blood leukocytes release a protein (leukocytic pyrogen, LP) after appropriate in vitro activation, such as phagocytosis. This protein is pyrogenic when injected into rabbits. Monocytes and neutrophils were separated by Hypaque-Ficoll gradients from buffy coats of blood obtained from normal humans. After incubation with heat-killed staphylococci, monocyte preparations released 20 times more LP into the supernatant media than did an equal number of neutrophils. During purification of these pyrogens it was discovered that these cell preparations each produced a distinct and different LP. The LP obtained from neutrophils had a mol wt of 15,000 after Sephadex G-75 gel filtration, an isoelectric point of 6.9, and could be precipitated and recovered from 50% ethanol at −10°C. In contrast, the LP derived from monocyte preparations had a mol wt of 38,000, an isoelectric point of 5.1, and was destroyed in cold ethanol. Both molecules were unaffected by viral neuraminidase but biologically destroyed at 80°C for 20 min and with trypsin at pH 8.0. The febrile peak produced by partially purified neutrophil pyrogen occurred at 40 min, while that from monocytes was at 60 min ($P < 0.05$). In addition, monocyte pyrogen produced more sustained fevers for the same peak elevation as neutrophil pyrogen. Thus, these studies demonstrate for the first time two chemically and biologically distinctive pyrogens derived from circulating human white blood cells. These findings have important implications for our understanding of the pathogenesis of fever in man.

75. Inhibition of the Steroidogenic Effects of Cholera and Heat-Labile *E. Coli* Enterotoxins by Ganglioside. SAM T. DONTA* AND JOHN P. VINER,* Iowa City, Ia. (introduced by Robin D. Powell).

Cholera enterotoxin (CT) and the heat-labile enterotoxins of *E. coli* (ECT) have been shown to induce morphogenic and steroidogenic changes in adrenal cell tissue cultures. The enterotoxic effects of CT are inhibited by prior incubation of the toxin with the specific ganglioside G_{M1}, whereas those of ECT require 1000 times or more ganglioside to be inhibited. The purpose of this investigation was to determine if gangliosides could inhibit the toxins' effects on adrenal cells, and if so, whether the degree of inhibition differed between the two toxins. For this purpose, three different ganglioside preparations were preincubated with CT or ECT, then the mixture added to Y1 adrenal cells in monolayer tissue culture and the subsequent morphologic and steroidogenic effects noted. Only with G_{M1} ganglioside was any inhibition of the toxins' effects noted. With CT, the morphogenic and steroidogenic effects were inhibited by a ganglioside:toxin ratio of 5:1 on a weight basis. Concentrations of the crude *E. coli* toxin preparation that gave similar morphogenic and steroidogenic effects as CT were inhibited by the same amount or less of G_{M1} as that required to inhibit the effects of CT. These findings indicate that at least for Y1 adrenal tumor cells, G_{M1} may be the receptor for both CT and ECT. The reasons for the lack of equal inhibition of the enterotoxic effects of CT and ECT remain to be clarified. (Supported by NIH Grant AI 11416-01.)

76. Factors Determining the Response of Maximum Expiratory Flow to Changes in Gas Density. J. DOSMAN,* F. BODE,* R. R. MARTIN,* AND P. T. MACKLEM, Montreal, Canada.

We have previously studied the effects of breathing an 80% helium-20% oxygen mixture on the maximum expiratory flow-volume curve in 61 nonsmokers and 46 smokers and have demonstrated that the percent of vital capacity where flow became identical with that on air (iso-flow volume; $V_{isov\dot{V}}$) provided the most sensitive indicator of early small airways obstruction in smokers with normal maximum expiratory flow ($\dot{V}_{max}$). In order to determine if loss of recoil (P_{el}) or increase in upstream resistance (R_{us}) were responsible for the increase in $V_{iso\dot{V}}$ with age we calculated V_{max}, P_{el}, and R_{us} at $V_{iso\dot{V}}$. Although $V_{iso\dot{V}}$ rose with age ($P < 0.001$) in nonsmokers, there was no change with age of V_{max}, R_{us}, or P_{el} at $V_{iso\dot{V}}$. In smokers $V_{iso\dot{V}}$ increased more steeply with age ($P < 0.001$) and R_{us} at $V_{iso\dot{V}}$ was similar to nonsmokers. $\dot{V}_{max}$ and P_{el} at $V_{iso\dot{V}}$ were higher in smokers ($P < 0.001$ for both). This indicates that the higher $V_{iso\dot{V}}$ cannot be attributed entirely to loss of recoil in smokers. However, the rise in $V_{iso\dot{V}}$ with age in nonsmokers is probably due to loss of elastic recoil. The fact that $\dot{V}_{max}$ and P_{el} at $V_{iso\dot{V}}$ did not change with age in nonsmokers but did in smokers is further evidence that increased $V_{iso\dot{V}}$ in smokers is due in part to airway disease. In addition, we studied 10 people who stopped smoking and, while P_{el} was unchanged after cessation, $V_{iso\dot{V}}$ was reduced, indicating an improvement in small airways obstruction. (Research supported by grants from the NIH, 73-2902r, and Medical Research Council of Canada.)

77. Retardation of Bone Marrow Colony Growth Using Sera and Ig Fractions from Patients with Felty's Syndrome and Systemic Lupus Erythematosus (SLE). DEWEY J. DUCKHAM,* BONNIE J. MACWILLIAMS,* FREDRICA E. SMITH,* AND RALPH C. WILLIAMS, JR.,**, Albuquerque, N. M.

In vitro culture of mouse bone marrow on semisolid agar was used to study the effects of serum and isolated Ig fractions on growth of granulocyte and monocyte colonies. Mouse bone marrow was used in the presence of human colony-stimulating factor isolated from pooled normal human urine. 19 sera from patients with Felty's syndrome, 30 sera from patients with active SLE, 36 sera from patients with rheumatoid arthritis, and 28 normal controls were studied. No effect on marrow culture growth patterns was noted if serum or Ig fractions were added 24 or 48 h after cultures were begun. Therefore, serum or isolated Ig fractions were mixed with agar at the time of initiation of bone marrow culture and numbers of colonies counted at 3, 6, 9, and 12 days. Concentrations of 1×10^5 cells/ml produced optimal conditions for clear, reproducible colony enumeration. In individual experiments rate of growth for all cultured cells was similar and each experiment was compared with serum or Ig from normal controls. 16 of 19 sera (84%) from patients with Felty's syndrome showed marked retardation (50–90%) of numbers of cultured marrow colonies. Similar effects were noted with respect to colonies of both granulocytic and monocyte precursors. Retardation of numbers of marrow colonies was most pronounced during the first 3–8 days of culture. Clear evidence was obtained that DEAE-isolated IgG fractions from Felty's sera contained bone marrow colony-suppressive effects. No such effect was demonstrated in any of 28 normal sera or Ig fractions. 40% of sera from 30 patients with SLE showed marrow colony growth retardation; a similar though less marked effect was also detected in 10% of 36 patients with uncomplicated rheumatoid arthritis. (Supported by Grants AMAI 13824-04 and AM 13690-04 from the NIH.)

78. Successful Correlation of Immunological Function Tests and the Adequacy of Immunosuppressive Therapy in Renal Transplantation. JOHN M. DWYER,* RICHARD J. MANGI,* FREDRIC FINKELSTEIN,* KEN FISHER,* AND ERNESTO HINDLER,* New Haven, Conn. (introduced by Elisha Atkins**).

Immunosuppressive therapy for renal graft recipients is modified by results of renal function studies and white cell counts rather than by measuring direct effects on lymphocytes, the targets of the therapy. Such theoretically desirable studies have become practical with microassay systems for lymphocyte stimulation and readily applicable markers for thymus-derived (T) and bone-marrow derived (B) lymphocytes. We applied these techniques to measure immunological function in 19 subjects on long-term hemodialysis and 18 subjects who received renal allografts (15 from cadavers). Delayed hypersensitivity skin responses were sought with four antigens, peripheral blood T and B lymphocytes were enumerated, and DNA synthesis was measured in lymphocytes stimulated in vitro with phytohemagglutinin, concanavalin A, pokeweed, *Candida albicans,* and pooled irradiated homologous lymphocytes. Subjects for transplantation were studied 3–12 h after preoperative administration of a single dose of 4 mg/kg of azathioprine and at specific time joints after transplantation. Results indicate that (*a*) immunological function is normal during chronic hemodialysis; (*b*) preoperative azathioprine significantly impairs immune functioning by the time of actual transplantation; (*c*) subjects transplanted 3 yr before this study had a marked reduction in circulating T lymphocytes (4–26% versus normal value of 55–65%); and (*d*) without clinical information nine subjects were considered to have normal immunological function (seven of these patients experienced chronic rejection episodes, the other two experienced acute rejection crisis). One subject profoundly immunosuppressed as judged by the above tests was clinically diagnosed as having an acute rejection episode, a diagnosis subsequently shown to be incorrect. Increased immunosuppression was followed by a fatal candidiasis. We feel these tests can significantly improve the safety and success of renal graft management. (Research supported by Grant PHS AI 11785.)

79. Triiodothyronine, Starvation, and α-Glycerophosphate Dehydrogenase. TOM DYKMAN,* LAWSON GLOVER,* AND JAMES WYNN,** Little Rock, Ark.

Thyroid hormone-treated animals (T) resemble starved animals (S). Liver glycogen (1933. *Am. J. Physiol.* **105:** 103) is decreased. Plasma amino acids (1967. *Endocrinology.* **85:** 1166), liver gluconeogenesis (1967. *Biochem. J.* **104:** 45P), and plasma fatty acids (1966. *N. Engl. J. Med.* **274:** 426) are increased. Liver mitochondrial (LM) α-glycerophosphate dehydrogenase activity (GPD) is increased in T (1959. *J. Biol. Chem.* **234:** 3051). Skeletal muscle mitochondrial (MM) GPD and liver and muscle cytoplasmic (LC and MC) GPD are unchanged in T (1965. *J. Biol. Chem.* **240:** 1427). In S LM-GPD, MM-GPD and MC-GPD are not reported and LC-GPD is decreased (1967. *J. Nutr.* **91:** 489). This study was designed to evaluate whether GPD changes in T may be related to starvation. Charles River rats, 325–400 g, were (*a*) fed controls (C); (*b*) triiodothyronine treated, 1 mg/kg body weight intraperitoneally, fed, and sacrificed at 60 h (T3); or (*c*) starved 24 h (S). GPD's are expressed as average moles α-glycerophosphate oxidized per milligram total protein per hour. LM-GPD's were C-11.48, T3-22.78, and S-16.00. S and T > C ($P < 0.05$). MM-GPD's were C-203, T3-175, and S-194 (no significant differences). Although T3 LC-GPD and MC-GPD < C ($P < 0.05$) and S LC-GPD and MC-GPD were not different from C, these cytoplasmic differences may reflect soluble protein decreases in S (1967. *J. Nutr.* **91:** 489) and increases in T (1939. *Endocrinology.* **25:** 899). Therefore, total cytoplasmic GPD is possibly decreased in both T and S. Increased LM-GPD, possibly decreased LC-GPD and MC-GPD, and unchanged MM-GPD in both S and T support the thesis that catabolism in hyperthyroidism is an adaptation to starvation. (Supported by NIH T01-AM05625 and 5-R01-AM12452.)

80. Preferential Cutaneous Infiltration by Neoplastic Thymus-Derived Lymphocytes: Morphologic and Functional Studies. R. EDELSON,* C. KIRKPATRICK,* E. SHEVACH,* T. YOSHIDA,* M. LUTZNER,* AND I. GREEN,** Bethesda, Md., and Buffalo, N.Y.

To investigate whether neoplastic lymphocytes which preferentially infiltrate the skin share common features, we studied the abnormal lymphocytes in 10 patients with lymphoproliferative disorders distinguished by extensive cutaneous involvement. The neoplastic circulating cells in each of seven patients with lymphocytic leukemia and erythroderma (four of which had the Sezary syndrome) could be identified as thymus-derived (T) cells by their formation of spontaneous rosettes with sheep erythrocytes and the presence of specific membrane antigens. In addition to their T cell markers, the leukemic cells from one of these patients had complement receptors, a characteristic of bone marrow-derived (B) lymphocytes. The abnormal cells infiltrating erythrodermic skin in the seven leukemic patients and cutaneous plaques in three patients with mycosis fungoides were also shown to have T cell membrane properties. The failure of lymphocytes from three patients with T cell leukemia to respond to mitogens correlated with their unresponsiveness to allogeneic lymphocytes in mixed leukocyte cultures (MLC). Leukemic T cells from one patient failed to stimulate allogeneic lymphocytes in MLC despite having a full complement of HL-A antigens, supporting the contention that the products of these two histocompatibility loci are separately expressed. Sera and lymphocyte supernates from each of four tested patients with T cell leukemia contained large amounts of macrophage migration inhibitory factor. These results indicate that neoplastic lymphocytes preferentially infiltrating the skin frequently have T cell membrane features but otherwise comprise a functionally and morphologically heterogeneous group. Investigation of abnormal lymphocytes in such disorders should significantly contribute to an improved understanding of immunopathologic mechanisms and facilitate the development of more specific therapeutic approaches for certain lymphoproliferative processes.

81. Correction of Intracellular Acidosis in Ischemic Myocardium with Alkaline Infusions. RICHARD M. EFFROS,* BUNYAD HAIDER,* PHILIP O. ETTINGER,* HENRY A. OLDEWURTEL,* AND TIMOTHY J. REGAN,** Newark, N.J.

Although it is assumed that myocardial cells become acidotic during acute ischemia, in vivo measurements of myocardial cell pH (MCpH) have not been obtained. In this study MCpH has been determined in intact anesthetized dogs by an indicator dilution approach. 1 ml of blood containing vascular ([^{125}I]albumin), extracellular ([^{51}Cr]EDTA), water (THO), and pH ([^{14}C]DMO) indicators was injected through a catheter into the left anterior descending coronary artery, and samples of blood were collected at 5-s intervals from the great cardiac vein. Correction for recirculation was made by deconvoluting arterial from venous concentrations, and

MCpH was calculated from indicator mean transit times, plasma pH, and the pK_a of DMO. Myocardial perfusion was determined both from ^{85}Kr and THO washout curves. Myocardial ischemia was induced by inflating a balloon on the arterial catheter proximal to the injection port. Myocardial perfusion decreased by an average of 57%, and MCpH declined from 6.95 ± 0.02 to 6.64 ± 0.08 (SEM, $n = 10$, $P < 0.001$) over a 15- to 45-min period. Systemic infusions of 0.4 M Na_2CO_3 (5–10 ml/min for 15–60 min) increased arterial pH to 7.50–7.60 and raised MCpH to the normal range (6.89 ± 0.02, $n = 5$). Myocardial perfusion remained unchanged, and serum osmolality increased by an average of 10 mosmol/kg. It is concluded that alkaline infusions can be used to reverse the intracellular acidosis of the myocardium which characterizes acute ischemia. (Supported by NHLI 72-2970.)

82. A New Type of Regulation of Hormone Action by Fatty Acids. GEORGE S. EISENBARTH* AND HAROLD E. LEBOVITZ, Durham, N.C.

We have recently demonstrated that unsubstituted fatty acids (0.5–2.0 mM) specifically block somatomedin stimulation of cartilage macromolecular synthesis, but have no effect on unstimulated (basal) synthesis. The present study was designed to determine the mechanism of this fatty acid effect. Experiments utilized pelvic cartilage from 10-day chick embryos. Incubations were done in a pH 7.45 Tris buffer (electrolytes, essential amino acids, and glucose added). Pooled rat sera was the source of somatomedin. Somatomedin action on cartilage persists hours after exposure. Utilizing this property, we showed that fatty acids block the initial events of somatomedin interaction with cartilage rather than the intracellular synthesis of cartilage macromolecules. Cartilages preincubated with media containing 5% rat serum, then transferred to fresh media containing [^{3}H]leucine, incorporate more (39.8 ± 7.2%) leucine than control cartilages (no serum in preincubate). If *n*-butyrate (1 mM) is present during the serum preincubation, it completely blocks serum stimulation (7.8 ± 7.2%). If *n*-butyrate is added to the incubation after the serum preincubation, it has no effect on stimulated macromolecular synthesis (45.0 ± 7.8%). Additionally, we showed that there was no correlation between fatty acid effects on cartilage intermediary metabolism and the inhibition of serum-stimulated macromolecular synthesis (e.g., *n*-butyrate and octanoate inhibit both glucose oxidation and somatomedin stimulation of macromolecular synthesis; isobutyrate inhibits only somatomedin stimulation of macromolecular synthesis; and ketones inhibit only glucose oxidation). There was no relationship between the rate of *n*-butyrate and isobutyrate oxidation and their dose-related inhibition of somatomedin-stimulated macromolecular synthesis. These studies suggest a new model of fatty acid regulation of hormone action that involves inhibition of the initial events of the interaction of a hormone and its responsive tissue. (Supported by NIH.)

83. Effect of Diuretics on O_2 Consumption Rate (QO_2) of Renal Cortical (C) and Outer Medullary (OM) Mitochondria of Dogs. G. EKNOYAN,* H. SAWA,* S. HYDE,* A. SCHWARTZ,* AND W. SUKI, Houston, Tex.

An effect of diuretics on cellular metabolism has been shown. To examine further the direct effect of diuretics on mitochondria, the major source of cellular energy, we measured their effect on isolated C and OM mitochondrial respiration. QO_2 was measured in a Gilson oxygraph utilizing either glutamate-malate or succinate as substrate. QO_2 was always higher in C than OM: 142 ± 3.3 vs. 123 ± 2.4, $P < 0.001$, with glutamate-malate and 187 ± 6.2 vs. 133 ± 6.4, $P < 0.001$, with succinate. A dose-response curve was constructed for each of the following: ethacrynic acid (EA), furosemide (F), chlorothiazide (CTZ), acetazolamide (A), and chlormerodrin (Hg). EA had a greater effect on C than on OM, 50% inhibition occurring at 5.9×10^{-4}M in C and 8.9×10^{-4} in OM. Neither cysteine nor dithiothreitol inhibited this effect. All other diuretics inhibited C and OM equally. The 50% inhibitory molar concentration for F was 2.2×10^{-3}; for CTZ 8.4×10^{-3}; for A 1.4×10^{-2}; and for Hg 4.4×10^{-5}. The effect of Hg was abolished by cysteine. EA, F, and CTZ have no effect on mitochondrial cytochrome oxidase measured with a recording spectrophotometer at 550 mμ. These results confirm the difference between C and OM mitochondria and indicate that all diuretics examined exert a direct inhibitory effect on mitochondrial respiration. Mercurials are the most potent inhibitors and presumably exert their effect by reacting with sulfhydryl groups. They are followed in potency by EA, F, CT, and A.

84. Determinants of the Deranged Sodium Homeostasis in Decompensated Cirrhosis. MURRAY EPSTEIN,* DAVID PINS,* AND NEIL SCHNEIDER,* Miami, Fla. (introduced by E. Reiss**).

Despite documentation of excessive secretion of aldosterone (Aldo) in patients with decompensated cirrhosis, the relationship between the increased levels of Aldo and the abnormal Na retention is not clear. Since in normals the redistribution of blood volume induced by water immersion to the neck (NI) results in ↑ $U_{Na}V$ independent of alterations in GFR or Aldo (1973. *Circ. Res.* **32**: 228), it was of interest to utilize NI to assess the role of Aldo in the Na retention of cirrhosis. 10 cirrhotic patients were studied twice while in balance on a 10 meq Na, 100 meq K diet: control (C) and during 5 h of NI. $U_{Na}V$ was constant throughout C, ranging from 1 to 2 μeq/min. During NI, $U_{Na}V$ ↑ progressively from 2 ± 1 (SEM) during the prestudy hour to 100 ± 24 μeq/min during hour 5 of NI ($P < 0.005$), greatly exceeding the comparable value found in normals on an identical diet. The ↑ $U_{Na}V$ was accompanied by a 2- to 3-fold ↑ in U_KV ($P < 0.001$). In seven patients restudied during chronic spironolactone (SL) therapy (400 mg/day), SL alone resulted in a modest ↑ $U_{Na}V$ to 26 ± 3. In contrast, during NI + SL, $U_{Na}V$ ↑ from prestudy hour mean of 34 ± 8 to peak mean of 179 ± 21 ($P < 0.001$). Despite SL, the marked natriuresis was accompanied by an ↑ U_KV from prestudy mean of 33 ± 5 to mean peak of 79 ± 11 ($P < 0.005$), demonstrating enhanced distal delivery. These data demonstrate that the ↑ $U_{Na}V$ of cirrhosis is only partially attributable to ↑ Aldo. Furthermore, in contrast to an earlier report (1969. *J. Clin. Invest.* **48**: 975), the present study supports the older postulate that a diminished "effective" blood volume participates in mediating the decreased $U_{Na}V$ of cirrhosis.

85. Transient Loss of Lymphocyte 5′-Nucleotidase (5′N) Activity in Infectious Mononucleosis (IM). DOUGLAS FAIG,* MARYROSE CONKLYN,* FRANCO QUAGLIATA,* AND ROBERT SILBER, New York.

We have recently observed that lymphocytes from 18 out of 27 patients with chronic lymphocytic leukemia (CLL) have diminished or nondetectable membrane-associated 5′-nucleotidase (5′N) activity. This finding, which has remained constant for a period in excess of 1 yr in all patients treated over this time period, was unrelated to the ratio of T:B cells or PHA response. In a survey of lymphocytes from 103 patients with a wide variety of other diseases, normal 5′N activity was found in subjects with cardiopulmonary, metabolic, or dermatologic disorders, bacterial as well as viral infections, neoplasms, and nonneoplastic hematologic diseases. 5′N activity was markedly decreased (< 0.6 μmol/h per

10^8 cells) or nondetectable (< 0.1 μmol/h per 10^8 cells) in lymphocytes from 6 out of 8 patients with infectious mononucleosis (IM) at the time of diagnosis. Serial determinations of lymphocyte 5'N activity were performed during the course of the illness in five patients. Within 2–14 days the activity returned to a normal level (2.2 ± 0.95) despite persistent abnormal morphology, positive heterophile, and abnormal liver chemistries. The temporary loss of this surface enzyme activity in IM lymphocytes is a biochemical similarity to the finding in the majority of patients with CLL. In contrast to CLL, the decrease in 5'N in IM appears to be transient in nature. Two mechanisms which could explain this phenomenon are the proliferation of a population of 5'N negative lymphocytes or a virally induced membrane alteration. (Research supported by grants from NIH).

86. Impaired Water Handling in Chronic Obstructive Lung Disease. MARK O. FARBER,* ROBERT A. STRAWBRIDGE,* THOMAS P. BRIGHT,* FELICE MANFREDI,* AND GARY L. ROBERTSON,* Indianapolis, Ind. (introduced by Harvey Feigenbaum).

Impaired water excretion has been described in stable, nonedematous patients with chronic obstructive lung disease (COLD). To elucidate the mechanism involved, we measured basal GFR and RPF, and water, sodium, and solute excretion for 4 h after water loading (20 ml/kg orally or as 5% D/W intravenously) in two groups of 10 age-matched, hypoxic, stable, nonedematous COLD normocapneic and hypercapneic patients (P_{CO_2} greater or less than 45 mm Hg, respectively). In five patients of each group, additional measurements of plasma and urine osmolality and plasma vasopressin (1973. *J. Clin. Invest.* **52**: 2340) were made at 30-min intervals after oral water loading and the results compared to those obtained in 14 normal controls. Hypoxic(P_{O_2} 61 ± 2 mm Hg), normocapneic(pCO_2 39 ± 1 mm Hg) patients had normal GFR(114 ± 5 ml/min) and RPF(517 ± 32 ml/min) and excreted the load normally(101 ± 7% of oral or intravenous water per 4 h). This was associated with a normal rate of sodium excretion(0.6 ± 0.1 meq/kg per 4 h) and low-normal plasma vasopressin(1.9 ± 0.7 pg/ml) which suppressed appropriately with water loading. Hypercapneic(P_{CO_2} 62 ± 5), hypoxic(P_{O_2} 57 ± 2) patients had normal GFR (106 ± 7), low base line vasopressin(1.1 ± 0.2) which suppressed appropriately, and decreased ($P < 0.05$):4 h water excretion (63 ± 8%), 4 h sodium excretion (0.3 ± 0.1), and RPF(394 ± 31). Significant correlations were found between P_{CO_2} and either RPF, sodium excretion, or water excretion, and between water excretion and sodium excretion. These studies indicate that hypercapnia but not hypoxemia is responsible for the abnormal water retention in COLD, and that this results not from a defect in vasopressin secretion or metabolism, but from increased reabsorption of sodium by the renal tubules. This alteration in sodium excretion may be due to hypercapneic-induced increase in renal bicarbonate reabsorption and/or abnormal renal blood flow. (Supported in part by the Indiana Lung Association Grant 32-150-08.)

87. Adaptation of a Transplantable Hepatoma to Vitamin Deficiency. ARPAD G. FAZEKAS,* RITA CHAUDHURI,* AND RICHARD S. RIVLIN, New York.

To explore adaptive mechanisms which permit tumors to proliferate despite vitamin deprivation of the host, Novikoff hepatoma, a rapidly growing undifferentiated tumor, was transplanted in the peritoneal cavities of adult male riboflavin-deficient and nondeficient rats. From 5 to 19 days after tumor transplantation, each animal received subcutaneously 2.5 μCi/100 g of [2-^{14}C]riboflavin and was sacrificed 1 h later. Formation of the coenzyme flavin adenine dinucleotide ([^{14}C]FAD) from radioactive riboflavin was determined in tumor and liver by newly devised techniques of isotope dilution and ion-exchange column chromatography with DEAE Sephadex A-25. Tumor transplants in nondeficient rats exhibited brisk rates of [^{14}C]FAD synthesis: 5,268 ± 396 dpm/100 mg tumor compared to 12,323 ± 817 dpm/100 mg liver. Because FAD concentrations in tumor (1.79 ± 0.53 μg/g) were so much less than in liver (23.48 ± 0.47 μg/g), the rate of [^{14}C]FAD synthesis per microgram FAD in tumor was more than five times greater than in liver. In tumor from riboflavin-deficient rats, neither FAD concentrations (1.66 ± 0.11 μg/g) nor rates of [^{14}C]FAD synthesis (5,575 ± 744 dpm/100 mg) were altered, whereas hepatic FAD concentrations (8.11 ± 0.35 μg/g) were reduced to 1/3 of those in nondeficient rats. Radioactivity remaining as [^{14}C]riboflayin in tumor from deficient rats (485 ± 103 dpm/100 mg) was markedly less than that in tumor from nondeficient rats (1,278 ± 112 dpm/100 mg). In this model, tumor apparently maintains its high FAD concentrations despite dietary riboflavin restriction by at least two mechanisms: (*a*) maintaining its own high rate of FAD synthesis, and (*b*) utilizing nearly completely the dispensable supply of free riboflavin. These mechanisms may provide insights into tumor-induced cachexia in man. (Supported by grants from USPHS (CA-12126) and the Stella and Charles Guttman Foundation.)

88. Activation of the Complement System by the Properdin Pathway in Patients with Gram-Negative Bacteremia. DOUGLAS T. FEARON,* SHAUN RUDDY, WILLIAM R. MCCABE, AND PETER H. SCHUR, Boston, Mass.

Serum concentrations of the third component (C3) of complement are depressed in patients with gram-negative bacteremia and shock. Two complement activating pathways are recognized: the properdin pathway, consisting of properdin, factor B (C3 proactivator), and other factors that can be activated by bacterial lipopolysaccharides; and the classical activating pathway, composed of C1, C4, and C2, that is initiated by certain antigen-antibody complexes. Sera were collected from 23 patients with gram-negative bacteremia and no signs of shock (group I) and 19 with shock (group II). Convalescent sera were obtained from surviving patients. Serum concentrations of C3, properdin factor B, C1q, C1s, C4, C2, C5, C6, and C9 were measured by radial immunodiffusion with monospecific antisera. The mean concentrations of C3 and properdin in group II were significantly lower than those in group I or normals ($P < 0.005$), and factor B and C3 levels were highly correlated ($r = 0.74$, $P < 0.0005$). The mean concentrations of C1q, C1s, C4, and C2 did not differ in the three populations. The mean concentration of C5 in group II was depressed relative to group I ($P < 0.01$); C6 and C9 were moderately depressed. In the group II convalescent sera, the mean levels of C3 and properdin had returned to the normal range. Activation of the complement system by the properdin pathway, rather than the classical pathway, in patients with gram-negative bacteremia is indicated by depressions of properdin, the correlation of factor B and C3 levels, and the normal concentrations of the early acting classical components. Depressions of C3 and C5 may reflect cleavage of these components and release of biologically active products that may contribute to the shock syndrome. (Supported by NIH research grants.)

89. The Absence of Serologic Response to Hepatitis A in Patients with Posttransfusion Hepatitis Unrelated to Hepatitis B. STEPHEN M. FEINSTONE,* ALBERT Z. KAPIKIAN,* HARVEY J. ALTER,* PAUL V. HOLLAND,* AND ROBERT H. PURCELL,* Bethesda, Md. (introduced by Robert M. Chanock**).

We have recently visualized virus-like particles in the stool of patients with hepatitis A (HA) and have etiologically

related the particles to HA by immune electron microscopy (IEM). IEM can be utilized not only to detect antigenic particles but also to measure antibody to such antigens. Since nonhepatitis B (HB) posttransfusion hepatitis (PTH) presently accounts for 50–75% of PTH, we attempted to determine the role of hepatitis A in such cases of the disease. Using IEM, we have studied the serologic response of 17 patients who developed PTH which could not be related to HB either by the presence of HB antigen (HB Ag) or by the development of antibody to HB Ag as determined by sensitive radioimmunoassays. The patients were selected retrospectively from open heart surgery patients at NIH who have been followed for the development of PTH. The patients represented a spectrum of PTH with varying incubation periods and severity of clinical illness. Pretransfusion sera and 6 month postillness sera from these 17 patients were studied for antibody to the HA particle. None of the 17 patients developed a serologic rise in antibody to the hepatitis A antigen in their paired sera. We conclude that non-HB PTH is not related to HA.

90. Functional Implications of Changes in Left Ventricular Shape in Aortic Valve Disease. STEPHEN FISCHL,* MICHAEL HERMAN,* RICHARD GORLIN,** EDMUND SONNENBLICK, RICHARD HELFANT,* AND HOWARD HORN,* Boston, Mass.

Left ventricular (LV) morphology was studied by ventriculography in 12 normal subjects, 18 with pressure-load due to isolated aortic stenosis (AS), and 19 with volume-load secondary to isolated aortic regurgitation (AR). Mean end-diastolic volumes were 84, 113, and 179 ml/m², respectively, and ejection fractions 0.71, 0.69, and 0.60, respectively. Roundness or globularity of the LV was calculated as eccentricity at both end-diastole (e_{ed}) and end-systole (e_{es}). In normals, mean e_{ed} was 0.82 and mean e_{es} increased to 0.88, i.e., LV became more elongated. In AS, e_{ed} was normal (mean 0.83) regardless of EDV, EF, or clinical state. The e_{es} increased appropriately (mean 0.90) in all but two. In AR mean e_{ed} (0.70) was depressed regardless of EDV; however, lower e_{ed} accompanied lower EF. In AR with normal EF (mean 0.74), e_{ed} (0.74) rose to near normal e_{es} (0.85), while in AR with low EF (mean 0.46), e_{ed} (0.66) increased to subnormal e_{es} (0.75). AR with advanced heart failure was associated with severely depressed e_{ed} in six of eight patients, including two with normal EF. Thus, in concentric hypertrophy of chronic pressure load (AS), LV maintains normal elongated shape in diastole and systole even late in the disease. The chronic volume-loaded LV (AR) however, becomes rounded in diastole at an early stage of the disease. End-systolic shape in AR remains near normal in conjunction with adequate LV function. Deterioration of LV function, however, is associated with more rounded chamber at end-systole. These morphologic changes may indicate basic change in cardiac architecture. Such may objectively indicate when a given load has begun to alter structural characteristics of the heart. (Research supported by grant from HL 5-T1HL-05890-04.)

91. Molecular Genetics of Hemoglobinopathies: Study by Fingerprint Analysis of Normal and Thalassemic Globin Messenger RNA (mRNA). BERNARD G. FORGET, CHARLES A. MAROTTA,* SHERMAN M. WEISSMAN, RONALD P. MCCAFFREY,* INDER M. VERMA,* AND DAVID BALTIMORE,* Boston and Cambridge, Mass., and New Haven, Conn.

The primary structure of human globin mRNA has been analyzed in an attempt to study normal and abnormal gene expression in human erythroid cells. Globin mRNA isolated from peripheral blood reticulocytes was transcribed into complementary DNA (cDNA) by the RNA-dependent DNA polymerase of avian myeloblastosis virus. The cDNA was then transcribed into ^{32}P-labeled complementary RNA (cRNA) by *E. coli* RNA polymerase. The ^{32}P-labeled cRNA was digested with T_1 RNase and fingerprinted. Specific and reproducible fingerprint patterns were obtained. A number of oligonucleotide sequences were derived by secondary analysis of the separated RNA fragments and could be matched to globin amino acid (aa) sequences. The derived nucleotide sequences are consistent with the origin of known structurally abnormal globin chains by single base substitutions in codons of the mRNA. Analysis of α- and β-thalassemic cRNA revealed quantitative deficiencies of oligonucleotide sequences which match α- and β-globin chain aa sequences, respectively; this technique, therefore, confirms previous demonstrations, by mRNA-cDNA hybridization assays, of deficient chain-specific mRNA in α- and β-thalassemia. Oligonucleotide sequences were present in normal cRNA which do not match normal globin aa sequences, but do match aa sequences in the abnormally long α-chain segment of hemoglobin Constant Spring (HbCS); our findings are consistent with the previously proposed hypothesis of a chain termination codon mutation in HbCS, which allows translation of normally untranslated sequences in the mRNA. No sequences were found to match aa sequences in the abnormally long β-chain segment of Hb Tak, which, therefore, may have resulted from a Lepore type crossover with DNA not normally transcribed into globin mRNA. Fingerprint analysis of globin cRNA is a useful technique to study the molecular pathology of hemoglobinopathies. (Supported by grants from the NIH.)

92. A Comparison of the Interaction of Angiotensin II and a Competitive Inhibitor at Different Receptor Sites In Vivo. BARR H. FORMAN, ARTURO FERNANDEZ-CRUZ, JR., AND P. J. MULROW,** New Haven, Conn.

Blockade of the renin-angiotensin system by inhibitors of angiotensin II (AII) action may become a useful treatment in clinical medicine. We examined the interaction of AII and the competitive inhibitor 1-sar-8-ala angiotensin II (P113) at different receptor sites in vivo. In the dexamethasone-suppressed, nephrectomized dog, infusion of P113 up to 64 μg/min had no significant pressor action but blocked the pressor action of AII infusions. Infusion of P113 stimulated aldosterone secretion in four of six dogs with a mean percent increase for the six dogs of 755 ± 269 SE ($P < 0.025$). However, when AII was infused and a dose of P113 sufficient to completely block the pressor action of the A II was subsequently added to the infusion, the aldosterone response to AII was inhibited by 303% ($P < 0.025$). The aldosterone increase with AII alone was 895% and reduced to 592% when P113 was added. Infusions of P113 did not alter the ACTH-aldosterone dose response curve (ACTH 10–2,000 nm), n = 3, nor inhibit the aldosterone stimulation by KCl infusions. In intact anesthetized dogs, infusions of doses of P113 which significantly inhibited the pressor response and the lowered renal plasma flow produced by AII infusions did not block the inhibition of renin release, percent decrease PRA with AII alone 54%, AII + P113 40%, or P113 alone 80%. The difference between AII + P113 vs. P113 alone is significant ($P < 0.01$). In conclusion we propose: (*a*) adrenal receptor sites for AII are different from those of ACTH and potassium; (*b*) the potency of blockade of AII action by P113 varies at different receptor sites.

93. Hemodynamic Effects of Oral Isosorbide Dinitrate in Heart Failure. JOSEPH A. FRANCIOSA,* ESTEBAN MIKULIC,* ANASTACIA FABIE,* ERNESTO JOSE,* AND JAY N. COHN, Washington, D.C.

Efficacy of orally administered isosorbide dinitrate (ID) has been questioned. Recently vasodilators given intravenously or sublingually have been shown to lower left ventricular filling pressure (LVFP) and raise cardiac output in heart failure. In the present study oral ID was evaluated in patients with high LVFP due to chronic failure or acute myocardial infarction. After right heart catheterization, patients with LVFP > 14 mm Hg (taken as pulmonary artery diastolic or occluded pulmonary artery pressure) were randomized double-blind to receive either 20 mg ID or placebo (P). Supine heart rate (HR), arterial pressure (BP), and LVFP were monitored for 5 h. In 10 patients receiving ID, LVFP fell in 30 min from an average of 28 to 23 mm Hg ($P < 0.025$) and reached a peak reduction to 21 mm Hg (−25%, $P < 0.005$) at 90 min. Significant reduction persisted for 3.5 h. BP fell concomitantly with the LVFP. At 90 min systolic BP was reduced by 6.0% ($P < 0.05$) from 117 to 110 mm Hg and diastolic BP by 4.7% ($P < 0.01$) from 85 to 81 mm Hg. HR was unchanged (99–101 beats/min). No side effects were observed. In nine patients receiving P, control values were not significantly different from those given ID; and HR, BP, and LVFP failed to change significantly over the next 5 h. Thus oral ID produces a sustained reduction in LVFP, probably by reducing impedance to left ventricular ejection, while HR is unchanged. The drug in this or a larger dose merits further evaluation as chronic therapy for left ventricular failure.

94. A Granulocyte Factor That Rapidly Causes Discrete Permeability Changes in Some Gram-Negative Organisms. R. FRANSON,* S. BECKERDITE,* J. WEISS,* K. SCHMEIDLER,* C. MOONEY,* AND P. ELSBACH,** New York.

Rabbit granulocytes contain a fast-acting permeability-increasing factor (PI) that renders *E. coli* macromolecular synthesis sensitive to actinomycin D but does not cause leakage of the *E. coli* intracellular enzyme β-galactosidase. Despite rapid killing of *E. coli*, macromolecular synthesis persists in the absence of actinomycin D. PI is measured as the difference between [^{14}C]uracil or [^{14}C]leucine incorporation into microbial macromolecules in the presence and absence of actinomycin D. PI is active over a wide range of pH (6.5–9.0) and granulocyte to *E. coli* ratios (up to 1/200). PI binds to susceptible but not to resistant bacteria. Mg^{2+} and Ca^{2+} completely block both binding and permeability effect. Permeability change induced by purified PI is reversed by addition of Mg^{2+} or Ca^{2+} up to 20 min after initial interaction, but is irreversible after 1 min exposure to granulocyte homogenates. PI is totally sedimentable and acid extractable and has been purified 1000-fold on CM-Sephadex. Preparations of purified PI are devoid of lysozyme, myeloperoxidase, and protease activities but do contain phospholipases A of comparable purity. Whereas gram-negative organisms susceptible to purified PI (several *E. coli* strains and *S. typhimurium* G-30) are also effectively killed (2–4 μg kill 2.5×10^8 organisms), strains of *Serratia marcescens*, unaffected by PI, are not killed. However, permeability and cidal effects are dissociated in other strains of *S. typhimurium* and in *P. aeruginosa*. Although other unidentified factors in the partially purified granulocyte preparations may cause loss of viability of susceptible organisms, the close temporal association of cidal and permeability effects suggests that discrete structural changes, perhaps mediated by modification of phospholipids, are important in the fate of several species of gram-negative organisms. (USPHS Grant AM 05472.)

95. A New Biochemical and Morphologic Sequela of Vitamin B_{12} Deficiency in Animals and Man. EUGENE P. FRENKEL, AMAL MUKHERJEE,* CHARLES R. HACKENBROCK,* AND PAUL A. SRERE,* Dallas, Tex.

Previous studies documented that B_{12} deficiency results in increased activity and synthesis of the enzymes of fatty acid synthesis and increased hepatic triglycerides. To evaluate the effect of B_{12} deficiency on enzymes in another metabolic pathway, citrate synthase was studied. Assay of total and specific citrate synthase was performed in livers of control and B_{12}-deprived (with low serum and tissue B_{12} levels and a functional coenzyme defect) rats. Total activity in the control group was 10.7 ± 1.6 U/g and 22.2 ± 4.1 U/g in the B_{12}-deprived animals. Specific activity was 0.11 ± 0.02 U/mg protein in controls and 0.23 ± 0.05 in the B_{12}-deprived animals. This cytosol-synthesized enzyme which is located and functions within the mitochondria was compared to succinate cytochrome C reductase (SCCR), an enzyme both synthesized and functional within mitochondria. No significant change in SCCR activity was seen in B_{12} deprivation. Since mitochondrial cristae correlate with Krebs cycle enzyme activity, electron microscopy was performed and demonstrated mitochondria of normal volume, but with markedly increased cristae membranes and an increase in total mitochondria. Similar findings were seen in liver biopsies from patients with B_{12} deficiency. Chloromycetin, an inhibitor of mitochondrial protein synthesis, decreased SCCR activity 12% in controls and 26% in B_{12}-deprived animals. Citrate synthase activity decreased 24% in controls and 17% in the B_{12}-deprived animals. In summary, B_{12} deprivation resulted in a twofold increase in activity of citrate synthase, an enzyme whose activity is unchanged by dietary or hormonal manipulation. Concomitant evidence of a new morphologic alteration, that of increased mitochondrial cristae, was seen in B_{12} deficiency in animals and man.

96. DNA Release As a Measure of Microbial Killing by Leukocytes. ARTHUR M. FRIEDLANDER,* San Diego, Calif. (introduced by Abraham I. Braude**).

Leukocyte bactericidal assays are indirect and based upon inhibition of growth as measured by colony-forming units (CFU). The methodology is slow and insensitive and may give false evidence of killing due to clumping of organisms after phagocytosis. Workers using [^{32}P]-labeled bacteria have shown some degradation of DNA after phagocytosis. I have developed a killing assay using bacteria grown in [^{14}C]thymidine (TdR) to label the DNA. It is based upon the assumption that *release* of radioactive DNA from the organism can be taken as *direct* evidence of death, and extends to leukocytes earlier observations on serum killing proving that the release of DNA accurately measures bactericidal activity. A serum-resistant *Salmonella typhimurium* was labeled during logarithmic growth with [^{14}C]TdR giving 5×10^4-1×10^5 cpm/10^8 bacteria. Labeled and washed bacteria were incubated with human leukocytes in 10% serum giving a bacteria:leukocyte ratio of ~ 5:1. At various times after incubation, the release of radioactivity from bacteria was determined by the percent radioactivity filtered through 0.45 μm filters. At zero time, < 1% of the radioactivity in the bacteria-WBC mixture is filterable. After 4 h incubation, 42–45% of the radioactivity was filterable. Controls using 10% serum and boiled WBC released 1.6–6.6%. After 4 h, viability as measured by CFU was

reduced by > 90%. These data show that leukocytes release significant amounts of radioactivity from [^{14}C]TdR-labeled micro-organisms. This release of DNA gives independent evidence for bacterial killing. It is a quantitative killing assay for bacteria and should be useful for examining any microbe which can incorporate sufficient DNA precursor. (Supported by a grant from NIH.)

97. Specific Markers of Chronic Myelogenous Leukemia (CML) Cells (Ph_1 Chromosome), Thymic-Derived Cells (Terminal Transferase), and Type-C RNA Tumor Virus (Reverse Transcriptase) in Blastic Leukemia. ROBERT GALLO, JIT BHATTACHARYYA,* AND PAUL ANDERSON,* Bethesda and Baltimore, Md.

Lymphadenopathy occasionally complicates late chronic myelogenous leukemia (CML). Biopsies, cytogenetics, and histochemistry usually indicate undifferentiated blasts compatible with myeloblasts, but sometimes confused with lymphosarcoma. Here we report biochemical studies on one such case which showed markers of type-C virus and thymic cells. Marrow and blood smears, interpreted as typical CML, contained CML markers: Ph_1 chromosome and low leukocyte alkaline phosphatase. We purified type-C viral related reverse transcriptase (RT) and normal cellular DNA polymerases from these blood cells. We also found terminal transferase (TT) not found in other CML patients without lymphadenopathy. This enzyme, discovered by Bollum in calf thymus, may be thymic-specific marker since they failed to find TT in other tissues. McCaffrey et al. later found TT in cells of some patients with acute lymphoblastic leukemia, implying possible thymic origin of the disease. TT is easily distinguished from RT by: size, primer responses, requirement by RT (like all true polymerases) but not TT for template as well as primer (TT uses primer alone), and by specific immunological reagents, e.g., IgG prepared against simian type-C sarcoma virus RT-inhibited human leukemic RT but not TT nor normal cell polymerases. Purified TT possessed properties of thymic TT: incorporation of radiolabeled deoxymononucleoside triphosphate (dNTP) is inhibited by presence of other "cold" dNTP's; primer preference oligo dA > oligo dT, > oligo dC, > oligo dG with [^{3}H]dGTP alone as substrate; size, 46,000 daltons. These findings suggest either: (*a*) TT is not thymic specific or is normally thymic specific but can be derepressed in other cells by type-C virus information; (*b*) TT is thymic specific and involved cells of some CML's are thymic derived which with progression "turn off" recognizable T cell markers; or (*c*) CML and thymic-derived malignancies have propensity to co-associate.

98. Angiotensin Inhibition and the Volume-Vasoconstrictor Interaction in Hypertension. HARALAMBOS GAVRAS,* HANS R. BRUNNER,* JOHN H. LARAGH,** JEAN E. SEALEY,* AND IRENE GAVRAS,* New York.

The nonapeptide SQ 20881 which competitively inhibits enzymatic conversion of angiotensin I to angiotensin II was administered intravenously to 11 hypertensive patients (six renovascular, three malignant, and two essential). In all 11 patients, a single injection (2–4 mg/kg body weight) induced an immediate fall in blood pressure which lasted for up to 16 h. The duration of the response was dose dependent. Mean reduction in BP was from $\frac{191}{127} \pm \frac{6}{4}$ (SE) to $\frac{147}{99} \pm \frac{6}{4}$ mm Hg. During treatment, plasma renin activity increased from 10.1 ± 1.7 to 34.4 ± 9.4 ng/ml per h, expressing interruption of negative feedback control. However, plasma aldosterone fell from 27.3 ± 7.4 to 14.1 ± 6.0 ng/100 ml, indicating that angiotensin II is necessary to sustain aldosterone secretion. Five patients in whom BP was not normalized were then sodium depleted with diuretics for 2–4 days; this did not lower BP $\left(\text{mean} = \frac{181}{129} \pm \frac{3}{3} \text{ mm Hg}\right)$. However, readministration of the nonapeptide after sodium depletion normalized BP in all $\left(\text{mean} = \frac{128}{86} \pm \frac{3}{4}\right)$. We conclude the following. Blockade of angiotensin II-induced vasoconstriction can fail to normalize blood pressure because of an existing inappropriate volume expansion. Diuretics too can fail to normalize blood pressure, in this case because of the compensatory rise in renin which then maintains the hypertension by vasoconstriction. However, simultaneous curtailment of both vasoconstrictor and volume factors normalized blood pressure in all patients tested. Therefore, hypertension is maintained by vasoconstrictor or volume factors or by an inappropriate interaction of the two. These results suggest that most hypertensive states can be controlled by a single or dual pharmacologic approach which delimits the vasoconstrictor (i.e., angiotensin) and volume (i.e., sodium) factors. (Research supported by USPHS Grant HL-14148.)

99. Effects of Alterations in Plasma Free Fatty Acids (FFA) on Glucagon Secretion in Man. JOHN E. GERICH,* MAURICE LANGLOIS,* CLAUDIO NOACCO,* VICTOR SCHNEIDER,* JOHN H. KARAM,* AND PETER H. FORSHAM,** San Francisco, Calif.

Pancreatic glucagon regulates nutrient homeostasis in man. Although the influence of glucose on glucagon secretion is well characterized, the effects of the predominant fuel of man, FFA, have not been similarly evaluated. Therefore, the effects of alterations in plasma FFA on human pancreatic glucagon secretion were studied. Elevation of plasma FFA from 0.478 ± 0.036 to 0.712 ± 0.055 mM after heparin administration lowered plasma glucagon 50%, from 122 ± 15 to 59 ± 14 pg/ml ($P < 0.001$). Lowering of plasma FFA from 0.520 ± 0.046 to 0.252 ± 0.041 mM after nicotinic acid administration raised plasma glucagon from 113 ± 18 to 168 ± 12 pg/ml ($P < 0.005$). During both studies no change in plasma glucose or insulin levels occurred. To compare the relative effects of glucose and FFA, glucose was infused to elevate plasma glucose levels to the same degree that heparin had raised plasma FFA levels. This diminished plasma glucagon levels, despite the fact that plasma FFA fell, indicating that hyperglycemia exerted greater influence over glucagon secretion than hypolipidacidemia. Additionally, the effects of plasma FFA and glucose on glucagon responses to arginine were examined. Although diminution of plasma FFA by nicotinic acid did not augment glucagon responses, both heparin-induced elevation of plasma FFA ($P < 0.01$) and infusion of glucose ($P < 0.01$) diminished glucagon responses to arginine. Thus, alterations in plasma FFA within the physiologic range and of rather small magnitude affect glucagon secretion in man. Under certain conditions, the effects of glucose predominate over those of FFA. However, since the pancreatic alpha cell appears more sensitive to alterations in plasma FFA levels, FFA may nevertheless exert an important physiologic influence, especially in the postabsorptive state.

100. Prostaglandin A_1: Protective Effect in Cardiac Failure. M. F. GHANI,* St. Louis, Mo. (introduced by John R. Smith**).

The role of prostaglandin A_1 (PGA_1) in protecting against toxic cardiac failure was studied in canine heart-lung preparations (HLP). In 12 dogs cardiac failure was induced by adding 1 ml of 20% solution of chloral hydrate (CH) to the

perfusing blood at 5-min intervals until failure appeared. In the control HLP there was a decrease in the cardiac output (CO) by 27% after 1 ml CH, and by 71% after 2 ml. In seven HLP where 50 μg of PGA_1 was given 5 min before CH administration was started, there was no fall in the CO after 2 ml of CH, and a 72% decrease in CO was brought about by 8 ml of the substance. With progressive fall in the CO, dilatation of the heart was evident and lung congestion and edema occurred. In both types of experiments CH administration was continued to the point of total cessation of CO. To achieve this, a total of 2.8 ± 0.7 ml of CH was required in the control HLP as compared to 8.6 ± 0.6 ml of CH in the HLP treated with PGA_1 ($P < 0.001$). However, when the hearts were still beating but without any CO, treatment with 50 μg of PGA_1 did not reverse the cardiac enfeeblement. The ability of PGA_1 to protect against this experimental cardiac failure suggests that it may be of value in supporting the heart as a pump.

101. Cellular Origin of Colony-Stimulating Activity (CSA) in Leukemia. D. W. GOLDE* AND M. J. CLINE, Los Angeles, Calif.

Alterations of CSA levels in the serum and urine of patients with leukemia have been related to the pathogenesis of the disease process. The cellular sources of CSA in the various leukemias, however, have not been determined. We studied patients with acute myelogenous leukemia (AML), acute myelomonocytic leukemia (AMML), acute lymphocytic leukemia (ALL), chronic myelocytic leukemia (CML), and CML in blast crisis. Additionally, alveolar macrophages were obtained by broncho-pulmonary lavage from three patients with AML and longstanding granulocytopenia and monocytopenia. Bone marrow and peripheral blood cells were cultured in Leighton tubes and with the in vitro diffusion chamber (Marbrook). Conditioned media from these cultures were tested for CSA using a standard double layer agar technique with normal human marrow as the target cells. Purified peripheral blood monocytes, lymphocytes, and neutrophils served as controls. High levels of CSA were found in conditioned medium from cultures of normal monocytes and AMML and CML cells. Negligible activity was observed in cell cultures from patients with AML, ALL, and CML in blast crisis. Morphologically and functionally normal alveolar macrophages were retrieved from three patients with AML and severe monocytopenia. These pulmonary macrophages produced substantial CSA in vitro. These data suggest that residual normal tissue macrophages provide a source for CSA in leukemia and that neoplastic hematopoietic cells with the capacity for mononuclear cell differentiation can also produce CSA. CSA from tissue macrophages may provide the means whereby normal hematopoiesis is reestablished with the onset of remission.

102. Rat Thymocytes and the Mode of Action of Thyroid Hormones. IRA D. GOLDFINE,* CYRENA G. SIMONS,* EUGENE C. JORGENSON,* AND SIDNEY H. INGBAR, San Francisco, Calif.

Isolated rat thymocytes, previously used to study the action of insulin and prostaglandin E_1, were used as a model system to study thyroid hormone action. In vivo, cells from thyroidectomized animals showed a decrease in the transport of the nonmetabolizable amino acid cycloleucine (Cle) which was restored with replacement doses (2 μg/100 g) of L-thyroxine (T_4). In vitro, thyroid hormones were active at 1 μM. 3,5,3'-Triiodo-L-thyronine (T_3) at 10 μM caused a maximal increase in Cle uptake within 10 min. This effect was not inhibited by 100 μM cycloheximide or by lowering the incubation temperature to 22°C. In contrast, insulin and prostaglandin E_1 also increase amino acid transport in these cells, but their effects are delayed and are inhibited by cycloheximide and temperature reduction. Further, T_3 increased amino acid transport by inhibiting amino acid efflux; insulin and prostaglandin E_1 act by stimulating influx. Of the naturally occurring thyroid hormones, T_4 was 1/10 as active as T_3 in stimulating Cle uptake, and the effect of T_4 could not be entirely explained by conversion to T_3. 3-Monoiodo-L-tyrosine (MIT) and 3,5-diiodo-L-tyrosine (DIT) were without effect. Of the synthetic thyroid analogs, both 3,5-diiodo-3'-isopropyl-L-thyronine and 3,5-dimethyl-3'-isopropyl-L-thyronine (a halogen-free compound) were more potent than T_3. By the thymocyte model system, the following were concluded concerning thyroid hormone action: (*a*) thyroid hormones can influence cellular processes rapidly and directly without prior stimulation of protein synthesis; (*b*) T_4 may have intrinsic biological activity; (*c*) iodine atoms may not be essential for the biological activity of thyroid hormones. (Research supported by grants from the NIH.)

103. An Active Enterohepatic Circulation of 25-Hydroxy-Vitamin D_3 ($25OHD_3$) in Man. RALPH S. GOLDSMITH,* SARA B. ARNAUD,* PHILLIP W. LAMBERT,* AND V. L. W. GO,* Rochester, Minn. (introduced by Claude D. Arnaud).

In the course of studying $25OHD_3$ kinetics in patients with renal failure, we were surprised to find two rapidly exchanging pools of $25OHD_3$. Systematic examination in two normal volunteers disclosed the two pools to be plasma and the biliary and intestinal phase of an active enterohepatic circulation. After intravenous [^{3}H]$25OHD_3$, ^{3}H was measured directly in duodenal aspirates and feces, corrected for recovery of polyethylene glycol and chromic oxide, respectively. Specific activity of serial serum samples was calculated from the chromatographic peak of $25OHD_3$. Fecal recovery of the ^{3}H secreted into the duodenum indicated intestinal reabsorption of over 85%; this compares well with a value of 90% calculated by resolution of the compartmental model from the serum specific activity curve. Further evidence for a high reabsorption rate was the postprandial increase in serum specific activity after gallbladder contraction (confirmed by increased ^{3}H and bile acids in duodenal aspirates). Compartmental analysis of the serum specific activity disclosed that the two pools were of approximately equal size: plasma 64–123 μg, enterohepatic 85–100 μg. The initial, rapid component of the curve ($t_{1/2}$ = 2.3 h in both subjects) corresponded to the rapid delivery to, and equilibrium with, the enterohepatic circulation. The slow component ($t_{1/2}$ = 113–126 h) corresponded to the turnover of total $25OHD_3$. The rate of 25-hydroxylation of precursor vitamin D_3 was calculated to be 0.8–1.3 μg/h. These data suggest that a relatively small decrease in intestinal $25OHD_3$ reabsorption, such as might occur in some biliary and intestinal diseases, could lead to significantly increased losses of this important metabolite. (Research supported by grants from NIH.)

104. Diabetes Mellitus and Prediabetes: Decreased Replicative Capacity of Cultured Fibroblasts. SAMUEL GOLDSTEIN,* ELENA J. MOERMAN,* J. STUART SOELDNER,* RAY E. GLEASON,* AND DONALD M. BARNETT,* Hamilton, Canada, and Boston, Mass. (introduced by John W. Littlefield**).

We have previously shown that cultured fibroblasts from subjects with genetic prediabetes (P) have a decreased replicative capacity compared to normal controls (1969. *Proc. Natl. Acad. Sci. U.S.A.* 64: 155). In a new series, several skin

fragments were explanted from individual biopsies of 30 subjects with P, 26 with overt diabetes (D), and 25 normal controls (N). On the first day of outgrowth, fibroblasts appeared in a greater percentage of N fragments: 17.5 ± 2.8% (mean ± SEM) compared to P 14.7 ± 3.4 and D 6.1 ± 1.9 (N vs. D, $P < 0.01$) and with more vigor per N fragment: 0.047 ± 0.006 arbitrary units; P 0.035 ± 0.007, D 0.014 ± 0.004 (N vs. D, $P < 0.01$). Maximum percent outgrowth occurred earlier in N fragments: 13.3 ± 0.6 days, P 15.9 ± 0.6, D 17.8 ± 0.5 (N vs. P, $P < 0.02$; N vs. D, $P < 0.001$), and at the time of harvest N had more vigorous outgrowth per fragment: 0.531 ± 0.031, P 0.369 ± 0.033, D 0.336 ± 0.017 (N vs. P, $P < 0.01$; N vs. D, $P < 0.001$). After harvest, N fibroblasts grew more rapidly so that less time elapsed between each of the first five subcultures (range): 5.3 ± 0.4 days to 6.6 ± 0.3, P 6.1 ± 0.3 to 7.8 ± 0.3, D 7.0 ± 0.3 to 7.9 ± 0.3 (N vs. P and D, $P = 0.02$ or less), but subsequently, no significant differences were observed. Senescent slowing occurred at a higher passage level in N fibroblasts: 33.4 ± 2.8 mean population doublings, P 24.1 ± 2.6, D 23.5 ± 2.8 (N vs. P, $P < 0.01$, N vs. D, $P < 0.01$) and at a later time: 53.9 ± 5.0 days, P 32.1 ± 5.5, D 33.1 ± 5.8 (N vs. P, $P = 0.01$, N vs. D, $P < 0.02$). The total number of mean population doublings that occurred before loss of replicative capacity was N 52.9 ± 2.1, P 47.7 ± 1.9, D 47.5 ± 2.1 (N vs. P and D, $0.05 < P < 0.1$). These data confirm that the effect of the diabetic gene(s) is to decrease the replicative capacity of cultured fibroblasts, and in the case of P, before glucose intolerance is detectable. This system should be useful to explore the inheritance and molecular basis of D as well as the pathogenesis of the diabetic state and associated disorders. (Supported by grants from MRC Canada and USPHS.)

105. "Angiotensin Three," the Des¹-Heptapeptide That May Be the Intracellular Mediator of Some Angiotensin Actions. THEODORE GOODFRIEND,* Madison, Wis. (introduced by Robert Schilling**).

Labeled angiotensin II (the octapeptide) incubated with bovine adrenal slices is recovered bound to intracellular fractions, primarily the mitochondria, as the des¹-heptapeptide. Experiments by M. Peach (University of Virginia) show the des¹-heptapeptide to be more rapid and more potent than the octapeptide in stimulating aldosterone production by bovine adrenal cells (1973 *Fed. Proc.* **32**: 765). The estrus rat uterus responds more rapidly to the heptapeptide, and the response decays quickly. By contrast, the response to octapeptide frequently lags, then builds in intensity. Macromolecules in the plasma of some seriously ill patients resemble soluble angiotensin-binding fractions from target tissues. Both bind the des¹-heptapeptide 10 times more avidly than the octapeptide. A lipid extracted from bovine kidney cortex inhibits binding of angiotensin to target cells, response of the estrus uterus to angiotensin, and "conversion" of the octapeptide to the des¹-heptapeptide. It does not inhibit cleavage to the inactive des⁸-congener. Natural phospholipids can carry angiotensin into chloroform, a measure of their association. The most potent, cardiolipin, carries the des¹-heptapeptide 30 times better than octapeptide. "Scatchard" plots with subcellular fractions from target tissues show an "always-negative" slope with heptapeptide, but an initial up-swing with octapeptide, suggesting that two events are required for binding of the octapeptide, one for the heptapeptide. Radioimmunoassay data about angiotensin II must be qualified by the significant cross-reactivity of most antisera with heptapeptide, and the contamination of some labeled preparations with it. In sum, the observations suggest that the des¹-heptapeptide is more than a degradation product, and may mediate some intracellular or pathological actions of angiotensin. The term "angiotensin III" is suggested for it. (Supported by NIH.)

106. Phospholipase Activation and Enhanced Fatty Acid Elongation and Desaturation in the Response of the Toad Bladder to Aldosterone. DAVID B. P. GOODMAN,* MITZI WONG,* AND HOWARD RASMUSSEN,* Philadelphia, Pa.

Aldosterone stimulates sodium transport across the toad urinary bladder, and it enhances the effect of vasopressin upon water and urea permeability of this tissue. In exploring the possibility that these hormonal alterations of membrane function result from altered membrane lipid metabolism, we have previously shown that aldosterone stimulates lipid synthesis and increases the weight percentage of membrane long-chain polyunsaturated fatty acids (PUFA). The present studies help to delineate the mechanism of the observed hormone-induced alteration in membrane lipid composition. Within 30 min aldosterone increases the conversion of [1-¹⁴C]palmitate, [1-¹⁴C]stearate, and [1-¹⁴C]oleate, but not [1-¹⁴C]linoleate or [1-¹⁴C]linolenate to phospholipid. Determination of membrane phospholipid fatty acid specific activities by radio-gas chromatography indicates a specific increase (twofold) in oleate elongation and desaturation. Similar analysis of tissue treated for 4 h with aldosterone and pulsed for 30 min with [1-¹⁴C]fatty acids reveals further hormone-stimulated increases (fourfold) in oleate elongation and desaturation and a 2- to 16-fold increase in specific activity of membrane PUFA derived from [1-¹⁴C]oleate. To examine the effect of aldosterone on preexisting lipids, tissue has been preincubated overnight in [¹⁴C]acetate. Aldosterone augments endogenous phospholipase activity. After 30 min, hormone-treated tissue exhibited a 1.5- to 3.0-fold increase in the specific activity of various free fatty acid classes and a concomitant fall in phospholipid fatty acid specific activity. The toad urinary bladder responds to aldosterone with specific changes in membrane function. These changes in membrane function can now be correlated with specific changes in membrane lipid metabolism: phospholipase activation, enhanced oleate elongation and desaturation, and increased weight percentage of membrane PUFA. (Supported by NIH and ONR grants.)

107. Somatostatin, a Hypothalamic Inhibitor of the Endocrine Pancreas. C. J. GOODNER, J. W. ENSINCK, E. CHIDECKEL,* J. PALMER,* D. J. KOERKER,* W. RUCH,* AND C. GALE,* Seattle, Wash.

We recently reported that somatostatin, a peptide from the hypothalamus, named for its ability to inhibit release of growth hormone, also inhibits the secretion of insulin and glucagon. In overnight-fasted baboons, infusion for 30 min lowered plasma insulin and glucagon to less than 15% of basal levels. Somatostatin also inhibited arginine-stimulated secretion of insulin and glucagon. Secretory blockade was established promptly and dissipated quickly after stopping the infusion. Plasma glucose fell concurrently with somatostatin infusion as a result of decreased glucose production (demonstrated isotopically). We have postulated that this hypoglycemia is due to reduction of glucagon-mediated hepatic glucose production. In support of this hypothesis, simultaneous infusion of glucagon reversed somatostatin hypoglycemia. Insulin's role to restrain hepatic glucose production appeared to be less important since glucose fell despite marked lowering of insulin. In further studies, inhibition of basal insulin and glucagon secretion was sustained during 2-h infusions of somatostatin. Somatostatin also inhibited the insulin response to infusion of glucose or glucagon and the glucagon response to insulin

hypoglycemia. Inhibition was reversible since after infusion, the alpha and beta cells responded appropriately to prevailing signals. During insulin hypoglycemia, glucagon secretion partially escaped from blockade. Similar escape occurred during the second hour of somatostatin infusion alone in animals displaying more pronounced hypoglycemia after prolonged fasting. These data suggest that hypoglycemia and somatostatin may interact competitively at the alpha cells. Blockade of insulin secretion, however, appears to be little affected by the prevailing glucose concentration. In addition to establishing somatostatin's usefulness as a tool for investigation of the endocrine pancreas, our studies raise the possibility that somatostatin is part of a new glucoregulatory system. (Research supported by grants from NIH.)

108. Effect of Clotting on Factor VIII Structure and Function. NORMAN R. GORDON,* N. RAPHAEL SHULMAN,** AND CECEIL N. COLEMAN,* Bethesda, Md.

Factor VIII (F VIII) purified by gel filtration of cryoprecipitate has a molecular weight of one million or greater. Recent studies by several groups indicate that F VIII purified from normal plasma can be dissociated into high and low molecular weight components by high ionic strength or detergents. The low molecular weight component (LMWC) has procoagulant activity; the high molecular weight component (HMWC) does not. Rabbit and human anti-F VIII inhibit the coagulant activity of the LMWC. Immunoprecipitation or radioimmunoassay with rabbit anti-F VIII detects HMWC but not LMWC. After removal of the dissociating agent (0.25 M Ca^{++}) LMWC and HMWC apparently recombine to regenerate a high MW active F VIII. We have observed that physiologic recalcification (4–12 mM Ca^{++}) of citrated normal human cryoprecipitate, which results in spontaneous clotting at 37°C, yields two components of F VIII by gel filtration which are similar in size and procoagulant activity to those obtained by other dissociating agents. However, when physiologic amounts of calcium are removed the components do not recombine as they do after removal of 0.25 M Ca^{++}. The procoagulant activity of purified F VIII and LMWC are equally inhibited by the same amounts of human or rabbit anti-F VIII. The inactive HMWC appears to effectively neutralize both human and rabbit anti-F VIII. Thus, F VIII appears to be separated irreversibly by intrinsic clotting into high and low molecular weight components that are functionally distinct, yet antigenically related. In von Willebrand's disease, there is a deficiency of HMWC and a selective increase in LMWC after treatment; in classic hemophilia HMWC is identifiable. It is therefore possible that HMWC is the von Willebrand's factor that supports ristocetin-induced platelet aggregation, and/or an adsorbant of, rather than an integral part of F VIII.

109. Mechanism of the Oliguria in a Model of Nephrotoxic Acute Renal Failure (ARF) in the Dog. JEROME GOTTSCHALL,* RICHARD W. OSGOOD,* JAY H. STEIN,* AND THOMAS F. FERRIS, Columbus, Ohio.

Recent data has suggested that renal vasoconstriction is responsible for the oliguria of ARF in a number of experimental models including uranyl nitrate (UN) administration. Yet, others have found renal blood flow (RBF) to be increased after UN. To evaluate this model further, studies were performed before and after the intravenous administration of UN, 10 mg/kg. Renal blood flow (RBF) was measured with the radioactive microsphere method. RBF 6 h after UN fell in each of eight studies with a mean change from 204 to 121 ml/min ($P < 0.001$). At 48 h, the rise in BUN from 13 to 123 mg/100 ml ($P < 0.001$) was independent of the change in RBF which had, in fact, increased in 11 of 20 studies. In nine micropuncture studies, RBF at 48 h was unchanged or increased in seven with a mean change in these experiments from 139 to 191 ml/min ($P < 0.005$). Virtually all tubules were patent but not dilated. In these seven studies, proximal tubular nephron GFR was 68 nl/min and transit time of lissamine green through the proximal tubule was 21 s. No lissamine green was seen to appear in the distal tubule. In three of the studies there was no urine flow, while in the remaining four the calculated total GFR was 1 ml/min or less. We conclude that the oliguria of UN ARF is not dependent on renal vasoconstriction but is primarily due to leakage of filtrate and/or tubular obstruction beyond the proximal tubule.

110. Absence of Folic Acid Conjugase in Proximal Gastrointestinal Secretions in Man. DAVID Y. GRAHAM* AND HERMAN A. GODWIN,* Houston, Tex. (introduced by Harold Brown**).

Digestion of folic acid conjugates within the intestine releases monoglutamic folate. The site of hydrolysis, whether intraluminal, at the brush border, or within the intestinal cell, is undetermined (1971. *Gastroenterology.* **60:** 445). We have studied digestion and absorption of [^{3}H]pteroylheptaglutamic acid ([^{3}H]PteGlu$_7$) (1972. *J. Biol. Chem.* **247:** 2266) in a patient with an end-jejunostomy which provided a unique opportunity for obtaining quantitative collections of proximal gastrointestinal secretions. After oral administration of 0.6 μmol [^{3}H]PteGlu$_7$, 35.9% of the dose disappeared (as compared with 55.6% of 0.6 μmol [^{3}H]PGA). Progressively smaller quantities of unabsorbed radioactivity appeared in enterostomy fluid with time and fell below detectable levels by 4 h after ingestion. In the initial 30 min collection, 94.1% of the excreted radioactivity was unaltered [^{3}H]PteGlu$_7$ (0.16 μmol). In subsequent collections, an increasing proportion of radioactivity was [^{3}H]pteroylmonoglutamate and intermediate folate compounds. To determine if the observed hydrolysis was mediated by intraluminal enzyme, in vitro studies were performed by incubating [^{3}H]PteGlu$_7$ with jejunostomy fluid collected either in the basal state or successively after the ingestion of water, an elemental diet, and a Lundh test meal. No evidence of hydrolytic activity was detected in any specimen over a broad pH range (4.0–7.0), after prolonged incubation (2 h), or with increasing quantities of enterostomy fluid. Passage of fluid through a Millipore filter did not remove hypothetical enzyme binders or inhibitors. Other enzymes (trypsin, amylase, lipase, alkaline phosphatase) were present. Although in vivo studies showed products of enzymatic hydrolysis of [^{3}H]PteGlu$_7$ in gastrointestinal secretions, no evidence for free intraluminal hydrolytic enzyme has been demonstrated. Luminal appearance of hydrolytic products may represent reflux into the lumen of materials hydrolyzed intracellularly.

111. 25-OH-Vitamin D Metabolism in Anephric Patients. R. W. GRAY,* H. P. WEBER,* J. H. DOMINGUEZ,* AND J. LEMANN, JR., Milwaukee, Wis.

Animal studies have documented the importance of the kidney in the further metabolism of 25-OH-D. To verify this role of the kidney in man, we have compared serum total 25-OH-D concentrations by rat kidney binding assay as well as the metabolism and excretion of [^{3}H]26,27-25-OH-D$_3$ in six healthy adults and seven anephric patients. Serum total 25-OH-D levels averaged 26 ± 4 SE ng/ml in normals, and 19 ± 2 ng/ml in anephrics ($P < 0.05$). After injection of [^{3}H]26,27-25-OH-D$_3$, metabolite profiles in large

plasma samples showed that 25-OH-D_3 constituted more than 80% in normals and more than 90% in anephrics of the circulating label, up to 3 wk after dosing. All normal subjects formed 24,25-$(OH)_2$-D_3, and 4/7 anephrics formed small amounts of a similar metabolite. We could not detect 1,25-$(OH)_2$-D_3 in plasma of either group, yet all normals and anephrics made metabolites more polar than 1,25-$(OH)_2$-D_3. t½ of plasma radioactivity averaged 22 ± 1 days in normal subjects and 40 ± 2 days in anephrics ($P < 0.001$). Turnover of the plasma pool of 25-OH-D was estimated and averaged 2.8 ± 0.4 μg/day in normals and 1.0 ± 0.1 μg/day in anephrics ($P < 0.001$). By 6 days after injection of [^{3}H]26,27-25-OH-D_3, normal subjects excreted 10% of the dose in urine and 16% in feces, while anephrics excreted 15% in feces. Loss of radioactivity during hemodialysis could not be detected. Metabolite profiles in feces were similar in normals and showed metabolites more polar than 1,25-$(OH)_2$-D_3. These results confirm the importance of the kidney in the further metabolism of 25-OH-D in man. However, anephrics do make other as yet unidentified metabolites that could potentially have significant biological effects. (Supported by NIH RR-00058 and AM 15089.)

112. Combined Effects of Digitalis and Hypoxia on Myocardial Diastolic Stiffness. H. LEON GREENE* AND MYRON L. WEISFELDT,* Baltimore, Md. (introduced by Richard S. Ross**).

Effects of the interactions between digitalis and hypoxia on diastolic stiffness were examined in isolated cat right ventricular papillary muscles. Muscles were paced at 12/min and bathed at 29°C in Krebs-Ringer bicarbonate solution with 17 mM glucose, 2.6 meq/liter Ca^{++}, 1.5 μg/ml digoxin, and equilibrated with 95% O_2-5% CO_2. After 60 min of exposure to digoxin 26 muscles were made hypoxic (HDM) for 45 min with 95% N_2-5% CO_2. These muscles were compared with six muscles (DM) which were exposed to digoxin but not made hypoxic and four other muscles (HM) which were made hypoxic but not exposed to digoxin. There was no significant difference in resting tension between HDM before hypoxia (0.46 ± 0.05 g/mm²) and either DM (0.43 ± 0.08 g/mm²) or HM (0.50 ± 0.18 g/mm²), nor was there any difference in resting tension between HDM at the end of hypoxia (0.39 ± 0.04 g/mm²) and either DM (0.36 ± 0.08 g/mm²) or HM (0.38 ± 0.12 g/mm²) at comparable times. Resting tension rose 49% to 0.58 ± 0.05 g/mm² 5 min after reoxygenation in HDM, while resting tension was unchanged at the comparable time in DM (0.37 ± 0.08 g/mm², $P < 0.001$, HDM vs. DM) and HM (0.37 ± 0.12 g/mm², $P < 0.02$, HDM vs. HM). Delayed relaxation was also present during reoxygenation in HDM, but interruption of pacing did not alter resting tension. In nine HDM decreasing the calcium concentration in the bathing fluid from 2.6 meq/liter to 0.65 meq/liter just before reoxygenation attenuated the rise in resting tension. Acidosis (pH = 7.1) in another seven HDM reversed the rise in resting tension, whereas norepinephrine did not significantly affect the rise in resting tension in 10 other HDM. Thus the combination of digoxin and recovery from hypoxia is associated with increased diastolic stiffness which is not attributable to delayed relaxation. These studies suggest that this increase in diastolic stiffness is caused by increased calcium binding to the contractile element which can be prevented by lowering extracellular calcium or reversed by acidosis.

113. Serum β_2-Microglobulin in Homografted Patients. HOWARD M. GREY, RALPH T. KUBO,* THOMAS E. STARZL,* AND BO HUSBERG,* Denver, Colo.

β_2-Microglobulin (β_2m), a protein with amino acid sequence homology to immunoglobulins, has recently been shown to be a subunit of the HL-A antigens. It is present in elevated amounts in the urine and serum of patients with renal tubular disease and is elevated in patients with renal transplants. It has been assumed that the elevated levels are secondary to the tubular disease present in these patients; however, because of its association with HL-A, the present study was undertaken to evaluate whether β_2m was also a sensitive indicator of graft rejection rather than only of tubular disease. Patients were followed for kidney function and serum β_2m before and after renal transplantation. Three patients had no evidence of renal abnormality after transplantation, and their serum β_2m levels fell rapidly to normal within a week of transplantation and remained within normal range (1–3 μg/ml). In three other patients renal function studies indicated a graft rejection between 1 and 3 wk after transplantation. β_2m levels rose concomitantly with elevations in creatinine levels. In one patient renal function studies were normal at a time when β_2m was elevated, and shortly thereafter a clear-cut rejection phenomenon was observed. Four patients with liver grafts and normal renal function were also studied. Distinct elevations of β_2m occurred in two patients, one of whom had abnormal liver function studies. These data indicate that serum β_2m levels closely correspond in most cases to the status of renal allografts and in some cases may precede other evidence of graft rejection. The elevation observed in some liver transplants suggests it is raised on the basis of an immunologic attack on the HL-A antigens. (Research supported by NIH.)

114. The Pharmacology of Essential Tremor. JOHN H. GROWDON,* BHAGWAN T. SHAHANI,* AND ROBERT R. YOUNG,* Boston, Mass. (introduced by Mandel E. Cohen**).

This study attempts to localize the site of action of (*a*) oral propranolol, which was shown by Winkler and Young to diminish significantly the amplitude of essential tremor in 20 of 24 patients, and (*b*) ethanol, which previously was the only agent capable of suppressing such tremor. Traditionally, tremors have been considered to arise from rhythmical discharges within cerebral structures. Recently however, peripheral beta-adrenergic receptors (such as in the muscle spindle) have been implicated in the origin of essential tremor because a similar tremor localized to one arm of normal subjects can be produced immediately by intra-arterial (brachial) isoproterenol and blocked acutely by propranolol. In patients with essential tremor we have contrasted such local adrenergic effects with the systemic actions of propranolol and ethanol. This new information regarding site of drug activity should help resolve the controversy over the origin of essential tremor—whether central or peripheral. Isoproterenol, propranolol, and/or dilute ethanol were infused at different times through an indwelling brachial artery catheter in one arm while continuous accelerometer and EMG records were made of the tremor in both upper extremities of eight patients with essential tremor. On separate days, similar tremor tracings were made after oral ingestion of ethanol or propranolol. Both oral ethanol and propranolol significantly decreased tremor amplitude: ethanol within 15 min and propranolol after 1 or 2 days. In contrast, there was no immediate decrease in amplitude of essential tremor after the intra-arterial infusion of these drugs. It is therefore concluded that essential tremor has a central origin, not peripheral, and its effective treatment depends upon the actions of ethanol and propranolol on the central nervous system.

115. Role of Feeding in Regulation of Bile Acid Synthesis. SCOTT M. GRUNDY, La Jolla, Calif.

Bile acids regulate their own synthesis in the liver by feedback control. Continuous diversion of bile acids from the enterohepatic circulation (EHC) causes a marked increase in synthesis rates. However, bile acids are removed from the

EHC for several hours daily during fasting by storage in the gallbladder without apparent increase in synthesis. Therefore, we examined whether feeding is required with interruption of the EHC to enhance bile acid production. Two studies were done in six subjects. They were intubated with a 3-lumen tube for measurement of hepatic secretion rates of bile acids and were simultaneously given radioactive bile acids. In study I, secretion rates were measured during constant infusion of liquid formula for 12 h without interrupting the EHC; specific activities of bile acids were constant throughout this period indicating minimal new synthesis; immediately thereafter, bile acids were withdrawn for 12 h with continued feeding. A quantity of bile acids equivalent to the entire pool was removed; specific activities fell indicating increase in synthesis; despite removal of large amounts of bile acids, hepatic secretion rates were maintained near normal. Study II was identical except that in the second 12 h period, when bile acids were withdrawn, feeding was discontinued. (Bile acids were diverted from the gallbladder for duodenal collection either by repeated induced contractions or because of previous cholecystectomy.) During this fasting period removal of bile acids did not increase synthesis. Thus, feeding is essential for stimulation of bile acid synthesis that is induced by interruption of the EHC, and the response of the liver to feeding may be crucial for determining synthesis rates and pool sizes of bile acids. (Research supported by grants from NIH and the VA.)

116. The Role of the Kidney in Maintaining Ionized Calcium Levels in the Acute Uremic State. LINDA HALSTEAD,* WILLIAM SHIEBER,* JANET CANTERBURY,* ERIC REISS, AND LOUIS AVIOLI, Miami, Fla.

Adult mongrel dogs were subjected to either total nephrectomy (TN) or bilateral ureteral ligation (UL) and circulating ionized calcium (ICa) inorganic phosphate (PO_4^{-3}), pH, and immunoreactive parathyroid hormone (iPTH) measured sequentially for the subsequent 24 h period. Whereas no change in ICa obtained in the UL dogs during the 24-h experimental period (4.25 ± 0.30 to 4.27 ± 0.50 mg/100 ml), a significant fall in ICa was observed in the TN animals during this time (4.51 ± 0.31 to 3.94 ± 0.30 mg/100 ml). Changes in plasma PO_4^{-3} were similar in both UL (6.0 ± 0.4 to 8.6 ± 0.3 mg/100 ml) and TN (5.6 ± 0.3 to 8.2 ± 0.7 mg/100 ml) groups; pH values were essentially unchanged at 7.31 – 7.34 in each group for the entire 24-h sampling period. These findings were associated with a 29–130% and a 430–900% increase in iPTH in UL and TN dogs, respectively. The data support the hypothesis that, in the acute uremic state, renal mass is essential for the maintenance of normal ICa levels and that changes in PO_4^{-3} and pH play an insignificant role in this regard. (Research supported by NIH Grant AM 11674 and NIH Contract 70-2219.)

117. Delayed DNA Chain Growth in Bloom's Syndrome. ROGER HAND* AND JAMES GERMAN,** Montreal, Canada, and New York.

Bloom's syndrome is characterized clinically by growth retardation, sun-sensitive facial telangiectasia, and a predisposition to cancer. Its autosomal recessive transmission reflects defectiveness in some one enzyme. Characteristic chromosome instability is demonstrable: in addition to various breaks and rearrangements, symmetrical chromatid exchanges between homologous chromosomes, apparently at homologous sites, occur more frequently than in normal cells. The nature of the homologous exchanges indicates that they occur in paired chromosome regions during or after DNA replication. The cytogenetic observations suggest that semiconservative DNA replication might be altered, either in multifocal initiation of chain synthesis or in chain propagation. The second component, chain propagation, was examined directly in dermal fibroblasts by [^{3}H]thymidine pulse-labeling and DNA-fiber autoradiography, a technique which permits examination of events on individual replication units (segments 50 μm long). The rate of chain propagation in cells from Bloom's syndrome was compared with that in normal persons, and also in Fanconi's anemia, another condition with increased chromosome instability, but in which asymmetrical, nonhomologous chromatid exchanges are commoner than symmetrical, homologous ones. 14 determinations from five Bloom's syndrome lines showed a mean rate of chain growth of 0.47 ± 0.024 μm/min (range: 0.31–0.58). 24 determinations from nine normal lines showed a rate of 0.64 ± 0.014 μm/min (22 of 24 > 0.55). 10 determinations from three Fanconi's anemia lines showed a rate of 0.65 ± 0.019 μm/min (10 of 10 > 0.55). Although limited by the recognized heterogeneity of diploid fibroblasts, the data indicate reduced rates of DNA chain growth in Bloom's syndrome. The enzymatic defect responsible for the reduction remains to be identified; decreased DNA polymerase activity is a possible explanation. (Supported by MRC of Canada Grant 5143 and NIH Grant HD 04134.)

118. Homocystine-Induced Arteriosclerosis. L. A. HARKER, R. ROSS, S. J. SLICHTER, AND R. C. SCOTT, Seattle, Wash.

The mechanism underlying the development of arteriosclerosis and arterial thrombosis in homocystinuria has been studied in patients and homocystinemic baboons. Kinetic studies of [^{51}Cr]platelets, [^{131}I]fibrinogen, and [^{125}I]plasminogen in four patients demonstrated a threefold selective increase in platelet consumption with the reduction in platelet survival directly related to the log[homocystine] (mean survival and turnover 4.3 ± 0.6 days and 96,000 platelets/μl per day compared with normal of 9.5 ± 0.6 days and 35,000 platelets/μl per day, respectively). Platelet consumption was interrupted by either dipyridamole therapy (survival 8.2 ± 0.7 days) or pyridoxine-induced clearing of the homocystinemia. When comparable homocystinemia (~0.2 μm/ml) was maintained by continuous infusion into 18 chaired baboons, platelet consumption and arterial thrombosis were predictably reproduced. Similar to the homocystinurics, the shortening of platelet survival was proportional to the log[homocystine] (survival average 2.1 ± 0.4 days compared with normal of 5.5 ± 0.2 days). After 1 wk of homocystinemia, direct examination of aorta and iliac arteries fixed by pressure infusion in vivo with 0.3% $AgNO_3$ stain and glutaraldehyde, respectively, in six animals demonstrated patchy de-endothelialization of 12% ± 3 of the vessel surface coupled with circulating endothelial cells. In six animals examined 6 wk after continuous homocystine infusion, focal intimal lesions consisting of proliferating myointimal cells 15–20 cells deep were regularly found. The addition of antiplatelet agents (dipyridamole 10 mg/kg per day or sudoxican 5 mg/kg per day) during the period of homocystine infusion in another six animals prevented both platelet consumption and arterial intimal lesions. It is concluded that the pathogenesis of homocystine-related arteriosclerosis results from a platelet-dependent proliferation of arterial smooth muscle cells that follows chronic endothelial injury; interruption of the associated platelet consumption prevents the intimal lesions. These results are supported by the in vitro demonstration of a platelet-derived stimulator of myointimal cell growth in culture.

119. A Structurally Abnormal $\overline{C1}$ Inhibitor Isolated from Two Patients with Hereditary Angioneurotic Edema (HANE). PETER C. HARPEL AND NEIL R. COOPER,* La Jolla, Calif.

$\overline{C1}$ inhibitor preparations were isolated from sera of a mother and daughter with the variant form of hereditary angioneurotic edema (HANE) and their properties compared

to those of normal $C\bar{1}$ inhibitor. In these sera the concentration of $C\bar{1}$ inhibitor antigen was 50% of normal and its mobility on immunoelectrophoresis was similar to that of normal, yet the sera failed to inhibit $C\bar{1}s$-induced inactivation of C4 or the hydrolysis of the ester substrate, ALMe. Isolation was accomplished by polyethylene glycol precipitation, ion-exchange, and molecular sieve chromatography. Analysis of the covalent structure of each $C\bar{1}$ inhibitor by SDS-polyacrylamide-gel electrophoresis showed that the inhibitors derived from HANE sera possessed a higher apparent molecular weight than did normal $C\bar{1}$ inhibitor. The apparent molecular weight of the single polypeptide chain of both abnormal $C\bar{1}$ inhibitors was 109,000, in contrast to that of normal inhibitor which had mol wt 105,000. Cleavage products resulting from incubation of the $C\bar{1}$ inhibitors with plasmin and trypsin demonstrated similar molecular weight differences. Treatment of HANE $C\bar{1}$ inhibitors with plasmin produced a chain of mol wt 99,500, whereas normal inhibitor yielded a derivative chain of mol wt 96,000. The trypsin-abnormal inhibitor reaction yielded two derivative chains with molecular weights of 99,500 and 90,000; the molecular weights of the trypsin-derived chains from normal inhibitor were 96,000 and 87,000. The HANE $C\bar{1}$ inhibitor and the trypsin-treated normal inhibitor failed to form a covalent complex with the light chain of $C\bar{1}s$ in contrast to normal $C\bar{1}$ inhibitor or its plasmin derivative which formed such a complex. These studies suggest that the observed alteration in covalent structure may be responsible for the biochemical defect associated with nonfunctional $C\bar{1}$ inhibitor.

120. Demonstration of Cellular Hypersensitivity to Normal Kidney Tissue in Rats with Chronic Pyelonephritis. HERBERT J. HARWICK,* GEORGE M. KALMANSON,* AND LUCIEN B. GUZE, Los Angeles, Calif.

Histologic changes of chronic pyelonephritis produced by intravenous inoculation of *Streptococcus faecalis* persist despite eradication of micro-organisms by antibiotic therapy. This suggests that a mechanism other than the direct action of the micro-organism may be playing a pathophysiological role. As pertibular infiltration of mononuclear cells is a predominant histopathologic change, cellular hypersensitivity might be involved. To explore this possibility we used in vitro inhibition of peritoneal macrophage migration as a test system. Rats infected for 25–29 wk and age-matched, broth-injected control rats were tested simultaneously. Significant inhibition of macrophage migration was seen in the presence of 40 μg protein/ml of an antigen prepared by homogenization and suspension of normal rat kidney; no inhibition of migration of cells of control rats was seen. Antigens prepared from normal rat liver and muscle had no effect on migration of cells from either infected or control rats. Significant migration-inhibition of cells from infected rats was also seen in the presence of 3 μg protein/ml of microbial cell wall and cell membrane antigens prepared by mechanical disruption. No effect of these latter antigens was seen in control animals. These data indicate that cellular hypersensitivity to microbial and renal antigens occurs in pyelonephritis. The role of this finding in the pathogenesis of renal damage requires further evaluation. (Research supported by grants from NIH and VA.)

121. Effect of Sodium Cyanate on Enzymes of Glycogen Metabolism In Vivo and In Vitro. MICHAEL HAUT,* PHILLIP TOSKES,* CATHERINE MCQUILKIN,* GEORGE SMITH,* AND MARCEL E. CONRAD, Washington, D.C.

Glycogen accumulation in livers of rats administered sodium cyanate (NaCNO) was demonstrated in our laboratory (Toskes et al. 1973. *J. Clin. Invest.* **52**: 85a) and confirmed by Scott et al. (1974. *Clin. Res.* **22**: 26a). Therefore, the activities of various hepatic enzymes involved in glycogen synthesis and degradation were measured in rats receiving NaCNO, 200 mg/kg orally, or 100 mg/kg intraperitoneally for 10 days. In orally dosed rats, significant decreases were seen in the activities of glucose-6-phosphatase (G6Pase) (67% control, $P < 0.001$) and glucose-6-phosphate dehydrogenase (G6PD) (80% control, $P < 0.05$), but not in phosphorylase, UDPG-pyrophosphorylase (UDPG-PP), glycogen synthetase, phosphoglucomutase (PGM), or debranching enzyme. In intraperitoneally dosed rats, which receive a higher effective dose of cyanate (five times that from similar oral dose), significant decreases were observed in the activities of G6Pase (57% control, $P < 0.001$), G6PD (64% control, $P < 0.005$), phosphorylase (60% control, $P < 0.05$), and UDPG-PP (72% control, $P < 0.025$), but not in glycogen synthetase, PGM, or debrancher. In vitro studies on purified enzymes showed strong inhibition of G6Pase and G6PD; in both cases this was competitive with glucose-6-phosphate. Cyanate inhibition of phosphorylase and UDPG-PP was less potent and was noncompetitive. At high concentrations, cyanate inhibited PGM. Our data suggest that: (*a*) in vivo administration of cyanate affects glycogen metabolism by direct effect on the enzymes; (*b*) in vivo inhibition of enzymes by cyanate (G6Pase and G6PD > phosphorylase and UDPG-PP) corresponds to in vitro sensitivities of the enzymes to cyanate; and (*c*) cyanate interacts with the glucose-6-phosphate binding site at the active center of the G6Pase and G6PD enzymes.

122. Carbohydrate and Protein Analysis of Carcinoembryonic Antigen. BERNARD J. HAVERBACK** AND BARBARA J. DYCE,* Los Angeles, Calif.

Carcinoembryonic antigen was purified from dissected hepatic colonic metastases by perchloric acid precipitation, negative pressure ultrafiltration, Sepharose 4B and A 0.5 M column chromatography, and isoelectric focusing. Between isoelectric points 1.6 and 4.7, six different carcinoembryonic antigen peaks were demonstrated by radioimmunoassay and the peak between pH 4.3 and 4.45 was analyzed for amino acid residues and sugar moieties. The amino acid residues per 100,000 daltons are as follows: Lys 28.9, His 22.3, Arg 21.3, Asp 114.8, Thr 99.4, Ser 102.2, Glu 85.1, Pro 84.1, Gly 53.3, Ala 66.7, Cys/2 15.3, Val 57.7, Met 2.6, Ile 38.4, Leu 76.1, Tyr 31.0, Phe 25.3, Trp not done, glucosamine 70.5, galactosamine 12.6, unknown 2.6. Sugar moieties were analyzed by gas chromatography and mass spectrometry and terminal sugar residues and intercarbohydrate linkages were determined by methylation analysis (Hakomori). The following sugars in percent to total sugar were identified: fucose 14.2, mannose 3.5, galactose 21.1, glucose 3.9, galactosamine 7.5, glucosamine 38.3, sialic acid 11.3. Partially O-methylated Sugars identified are as follows: 2,3,4-fucose 10.2, 2,3,4,6-galactose 8.5, 2,4,6-galactose 45.0, 2,4-galactose 3.2, 3,4,6-GlcNMeAc 3.1, 3,6-GlcNMeAc 9.2, 4,6-GlcNMeAc 5.7, 6-GlcNMeAc 15.1. (Numbers indicate the position of O-methyl group.)

123. The Role of HLA Compatibility in Platelet Transfusions from Unrelated Donors. JEANE P. HESTER,* KENNETH B. MCCREDIE,* ROGER ROSSEN,* BENJAMIN LICHTIGER,* AND EMIL J. FREIREICH, Houston, Tex.

45 thrombocytopenic (< 20,000/mm^3) acute leukemia patients received a total of 665 platelet transfusions of ACD platelet concentrate packs, collected by plateletpheresis from normal unrelated donors. Platelet counts were done before and 1 h and 18 h posttransfusion and repeated daily until retransfusion. Median transfusions were 15 per patient. 148 transfusions were based on HLA antigen (Ag) similarity; 77 from donors sharing at least two Ag with recipients and 71 with one Ag

shared. 517 transfusions were nonantigen matched (random donors). Median pretransfusion platelet counts in the three groups were similar (13,000–16,000/mm³). Median platelet recovery (post-pre count × BSA/no. Units *tx*) in the random and one antigen shared groups showed identical values at 1 h (4.0×10^3) and 24 h (1.0×10^3), and in the 2 Ag-matched group was 10.0×10^3 at 1 h, and 6.0×10^3 at 24 h. Platelet recovery increases in the two antigen group with no reduction after multiple transfusions. Transfusions from random donors resulted in decreased recovery after four or more transfusions suggesting isoimmunization. In noninfected recipients, two Ag-compatible transfusions resulted in platelet counts $\geq 20{,}000/mm^3$ in 2/3 of transfusions at 48 h and 1/3 at 72 h posttransfusion. Platelet counts after random transfusions were $\geq 20{,}000/mm^3$ in 1/3 at 24 h and in none at 72 h. Infection lowered platelet recovery about 50% and increased transfusion requirements in all patient groups. Transfusions from unrelated donors sharing at least two HLA antigens with recipients are superior to transfusions from random donors, especially in the "immunized" thrombocytopenic patient. The necessity for two to seven transfusions per week per patient will require HLA typing of a pool of unrelated donors sufficiently large to identify two Ag compatibility. (Research supported by grants from NIH and ACS.)

124. Cyclic Nucleotide Modulation of Human Neutrophil Granulocyte Response to Chemotactic Stimulation. HARRY R. HILL,* RICHARD D. ESTENSEN,* NELSON D. GOLDBERG,* NANCY A. HOGAN,* AND PAUL G. QUIE,** Minneapolis, Minn.

The cyclic nucleotides have been shown to be involved in the regulation of a number of diverse biological functions such as smooth and cardiac muscle contraction, carbohydrate metabolism, and cell proliferation. In many systems, increase in the intracellular concentration of cyclic 3′5′-guanosine monophosphate (GMP) is associated with the mediation of effects opposite to those that occur in association with increases in cellular cyclic 3′5′-adenosine monophosphate (AMP) concentration. The proposal that a number of cellular processes are modulated through opposing actions of the two cyclic nucleotides has been termed the "Yin Yang" hypothesis of biological control. To determine if cyclic nucleotides also modulate neutrophil function, we examined cyclic GMP and cyclic AMP and agents known to increase intracellular concentrations of these compounds for their effect on human neutrophil chemotactic responsiveness. When neutrophils were incubated with 10^{-5}–10^{-9}M cyclic GMP, there was a 200–400% enhancement of the chemotactic response to a bacterial chemotactic factor. Similarly, agents which increase intracellular concentrations of cyclic GMP in a number of cell-types, including imidazole, phorbol myristate acetate, phenylephine, and prostaglandin $F_{2\alpha}$, stimulated neutrophil chemotactic responsiveness. Cholinergic agents acetylcholine and carbamylcholine also enhanced the chemotactic response and this effect could be partially blocked with atropine. In contrast, cyclic AMP and adrenergic agents epinephrine, norepinephrine, and isoproterenol effectively inhibited chemotactic responses. Aminophylline, cholera toxin, histamine, and prostaglandin E_1 and E_2 were also potent inhibitors of chemotactic responsiveness. The regulation of human neutrophil responses to chemotactic stimulation appears to be modulated in a reciprocal fashion by cyclic GMP and cyclic AMP. Cyclic nucleotides and pharmacologic agents affecting the cellular concentration of these compounds may influence host response to infection. (Supported by research funds from NIH.)

125. A Mutant Form of Adenosine Deaminase in Severe Combined Immunodeficiency. ROCHELLE HIRSCHHORN,* VERA LEVYTSKA, AND ROBERTSON PARKMAN, New York and Boston, Mass. (introduced by Jamshid Javid).

Adenosine deaminase (ADA), a polymorphic enzyme, is deficient in one form of inherited severe combined immunodeficiency (SCID). Although this enzyme normally exists in several forms, that is, a "red cell" and various "tissue" isozymes, we have determined that these various forms are all the products of the same structural gene and are all deficient in SCID. We have succeeded in converting the red cell isozyme to the various tissue forms by incubating with tissue specific conversion factors. Despite the general deficiency of ADA in the tissues of such patients, we have found residual enzyme activity (7.84 ± 2.3 vs. 22.5 ± 3.9 nmol inosine/mg protein per min) in fibroblasts derived from a patient with this disorder. This residual enzyme moves faster anodally after electrophoresis in starch gel than the normal tissue enzyme of fibroblasts but has a molecular weight resembling tissue enzymes (approximately 220,000), that is considerably larger than the red cell enzyme (approximately 35,000). Its Km is similar to that of the normal enzyme (5.0×10^{-5}M vs. 7.1×10^{-5}M), but it shows greater heat stability. We have shown that the residual enzyme is not derived from the serum of the culture medium since it remains present during culture in horse serum which contains no ADA and is electrophoretically different from that found in calf serum. We therefore consider this to be a structurally mutant enzyme whose activity may be sufficient to allow normal metabolism of many tissues but too low to permit the rapid growth and differentiation of lymphoid tissue, thereby resulting in SCID.

126. Insulin and Epidermal Growth Factor: Human Fibroblast Receptors Related to DNA Synthesis and Amino Acid Transport. MORLEY D. HOLLENBERG* AND PEDRO CUATRECASAS, Baltimore, Md.

The biological activities and specific cell binding of both insulin and epidermal growth factor (EGF) have been simultaneously measured in intact cultured human skin fibroblast monolayers. In comparison with EGF, insulin is a poor stimulant of DNA synthesis. When EGF is added in amounts insufficient to stimulate DNA synthesis, the action of insulin is augmented and a dose-response relationship for insulin-mediated DNA synthesis can be measured. The apparent Km for insulin-stimulated DNA synthesis is 10^{-9} M; that for EGF is 7×10^{-11} M. Both insulin and EGF increase the uptake of α-aminoisobutyric acid (AIB). For this stimulation, the apparent Km of insulin is 10^{-9} M; that of EGF is 10^{-10} M. Both peptides lower the apparent Km of the transport system for AIB. Stimulation of AIB uptake requires a 40 min preincubation period with either peptide; cycloheximide, if added before but not after the preincubation period, abolishes peptide-mediated stimulation. If maximal stimulation of AIB uptake is achieved with either peptide alone, no further effect is observed on adding a second peptide. This result contrasts with the ability of EGF to stimulate further DNA synthesis in cells maximally stimulated by insulin. Measurements of the specific binding of ^{125}I derivatives of both peptides to intact replicate cell monolayers reveal differences in the numbers and affinities of cell receptors for insulin and EGF. There are approximately 4×10^3 insulin-binding sites per cell, with an apparent $K_D = 10^{-9}$ M; for EGF, there are correspondingly 4×10^4 sites per cell with $K_D = 3 \times 10^{-10}$ M. (Research supported by grants from ACS, NIH, and Canadian MRC.)

127. Cyanate and Thiocyanate Stimulation of cAMP-Responsive Erythrocyte Membrane Protein Phosphokinase. CHRISTOPHER HOLROYDE,* BERNADETTE WOMER,* AND THOMAS G. GABUZDA,* Philadelphia, Pa. (introduced by Allan J. Erslev**).

Protein phosphokinases (PPK) are believed to mediate the intracellular effects of cAMP and represent, therefore, a metabolite amplification mechanism potentially available for pharmacologic manipulation. The recent clinical administration of cyanates prompted an investigation into possible effects on the cyclic nucleotide system. PPK was prepared from erythrocyte membranes by the method of Dodge et al. followed by freeze-thawing to expose enzyme activity. Assay of phosphokinase was according to the method of Tao, which measures the transfer of γ P^{32} from ATP to a variety of protein acceptors in the presence of Mg^{++}. Inorganic cyanate and thiocyanate stimulated PPK approximately 50–100% in a manner which depended both on time and concentration. Linear dependence on concentrations (0–75 mM) was observed for basal and cAMP-responsive activity. The degree of stimulation depended also on the protein acceptor, being maximal with protamine (the normally preferred substrate), intermediate with histones and an endogenous membrane protein, and negligible with casein. Chloride, which is handled by tissue in a manner analogous to thiocyanate, was without effect while cyanide caused inhibition. Cyanate increased reaction rates for both basal and cAMP-responsive activity, but did not change the pH or Mg^{++} optima. Chelation of inhibiting cations with 1 mM EGTA did not affect the degree of cyanate-induced PPK stimulation, the mechanism of which remains unknown. In view of the known protein-binding properties of cyanates and the ubiquitous nature of PPK, cyanate-induced PPK stimulation may have potential clinical importance. (Research supported by grant from NIH.)

128. In Vitro Effects of Various Agents on B and T Lymphocyte Markers and Function. SHELDON HOROWITZ* AND RICHARD HONG, Madison, Wis.

We are studying the in vitro effects of various agents (thymosin, cyclic AMP, cyclic GMP, endotoxin, uridine) on B and T cell markers and function in peripheral blood lymphocytes (PBL) from normal individuals and patients with immunodeficiencies and malignancies. Surface Ig and Fc receptors were used as B cell markers and spontaneous rosette formation (RFC) as a T cell marker. "Null" cells were defined as mononuclear cells that did not have B or T cell markers and were not monocytes. PBL from normal individuals (n = 10) have less than 5% null cells and showed no change in the numbers of B and T cells or monocytes after exposure to these five agents. There was no effect of the agents on PBL from patients with acute lymphatic leukemia, although large numbers of null cells were present. One patient with the DiGeorge syndrome who had 20% null cells and low RFC (38%) developed normal numbers of T cells (56%) after incubation of PBL with thymosin or cAMP. A girl with an isolated T cell defect and a possible abnormality of pyrimidine metabolism had a significant increase of T cells after exposure to uridine, thymosin, or cAMP. PBL from a boy with no B or T cell function but normal numbers of cells with B and T cell markers were not affected by these agents. Fractions obtained from separation of normal PBL on BSA gradients behaved differently with regard to the induction of B and T cell markers and mitogen responsiveness after exposure to these agents. These data show varying responses to different agents among lymphoid subpopulations and in disease states. (Supported by grants from NIH.)

129. Biologic Activities of a Lymphocyte Regulatory Immunoglobulin (LRG). DAVID A. HOROWITZ* AND JOHN B. COUSAR,* Charlottesville, Va. (introduced by Edward W. Hook).

Previous studies on cellular immunity in patients with primary intracranial tumors (PIT) or systemic lupus erythematosus (SLE) indicated the need for separating intrinsic lymphocyte defects from modifying factors in serum. Lymphocyte function was evaluated in each of these diseases with the mixed lymphocyte culture (MLC) and phytohemagglutinin (PHA) technique. Serum from both groups decreased blastogenesis of autologous lymphocytes. This abnormality was corrected by replacing patient serum with normal serum. IgG isolated from patients with PIT and SLE had suppressor activity equal to that of whole serum and was named lymphocyte regulatory immunoglobulin (LRG). To determine a possible biological role for LRG, we evaluated delayed hypersensitivity to common antigens in 18 patients with early, untreated SLE and correlated with levels of humoral inhibitor. Subjects were divided into two groups on the basis of clinical and laboratory criteria of activity. Cellular anergy was found in very active (but only rarely in mildly active) patients. Small lymphocyte counts in each group were comparable. Sera from active patients contained marked suppressor activity and decreased PHA values of autologous lymphocytes below the normal range. Sera from mildly active patients had significant, but less inhibitory effects. Suppressive effects on allogeneic lymphocytes paralleled those on autologous cells. No cytotoxic effects were noted in cell cultures. 6 of 10 sera with marked suppressor activity, however, also had lymphocytotoxic antibody measured with the microdroplet technique using rabbit complement. 2.5 mg/ml of IgG isolated from anergic patients inhibited lymphocyte function. Kinetic studies indicated that LRG had no effect on the initial binding of PHA to lymphocytes, but suppressed the proliferative response to mitogen. The correlation of increased LRG with disease activity suggests that elevated levels are a consequence of chronic antigenic stimulation and result in cellular dysfunction clinically recognized as impaired delayed hypersensitivity. (Research supported by grants from NIH and the John A. Hartford Foundation.)

130. Renal Metabolism of Parathyroid Hormone (PTH) and Its Fragments. K. HRUSKA,* R. KOPELMAN,* W. E. RUTHERFORD,* S. KLAHR, AND E. SLATOPOLSKY,* St. Louis, Mo.

The kidney plays an important role in the metabolism of peptide hormones. These studies were designed to evaluate the renal contribution to the metabolism of PTH and the 1-34 amino acid fragment of the hormone. Studies were performed in five normal and four uremic dogs after administration of bovine PTH (b-PTH) or synthetic 1-34 (S1-34). b-PTH and S1-34 were determined by radioimmunoassay. S1-34 was measured with an antiserum obtained from a chicken immunized with S1-34 and specific for the amino terminal of PTH. Total metabolic clearance rates (MCR), determined by standard methods, averaged 85 ml/min for b-PTH and 366 ml/min for S1-34. Uremia decreased total MCR to 34 ml/min for b-PTH and to 52 ml/min for S1-34. Normal renal extraction was 18% of the delivered b-PTH. Renal clearance (extraction × RPF) of PTH was 56 ml/min or 67% of the total MCR in normal dogs. In uremia, renal clearance of b-PTH fell to 11 ml/min, accounting for most of the decrease in total MCR. Renal extraction of S1-34 averaged 39%, and renal clearance was 126 ml/min or 36% of the total MCR. Uremia decreased renal clearance of S1-34 to 29 ml/min. These studies indicate that the kidney plays an important role in

the metabolism of parathyroid hormone and its fragments. The kidney catabolizes a large proportion of both intact PTH and S1-34. Uremia decreases the total MCR of PTH and S1-34 by decreasing their renal clearance. No evidence for increased extrarenal metabolism of b-PTH or S1-34 was found in uremia. These results suggest that decreased renal metabolism of b-PTH and S1-34 may be in part responsible for the elevated concentrations of the hormone and its fragments seen in chronic renal disease. (Supported by NIH Grant AM09976.)

131. Chemical Structure of Human Aorta Collagen: Relationship to Platelet Aggregation. ELDRIDGE HUTCHESON* AND ANDREW KANG,* Memphis, Tenn. (introduced by Gene H. Stollerman**).

Since the discovery that specific glycopeptide fragment of skin collagen induces human platelet aggregation, questions concerning the chemical structure of collagen present in the arterial wall have become more cogent. In the present investigation the nature of collagens in human aorta was thus characterized after solubilization with pepsin. Aortae, obtained at necropsy, were cleaned of fatty and adventitial tissues, homogenized, and digested with purified pepsin in 0.5 M acetic acid at 4°C for 24 h. The pepsin-solubilized collagen was then purified by repeated NaCl precipitations at neutral and acid pH by previously described methods. Fractionation of the extracted collagen on a calibrated column of agarose 1.5 M equilibrated with 1 M $CaCl_2$ yielded two polypeptide fractions, β and α. Carboxymethyl-cellulose chromatography of the α fraction showed that it contained both the α_1 and α_2 chains. Their amino acid compositions were indistinguishable from those of the corresponding chains of human skin collagen (type I). Treatment of the β fraction with dithiothreitol and rechromatography on agarose in the presence of the reducing agent demonstrated that 25–50% of β was converted to α. On carboxymethyl-cellulose chromatography, it eluted at the position of α_1 as a homogeneous peak. Its amino acid composition was clearly different from other α-chains so far characterized. These results clearly indicate that in addition to common collagen type I, human aorta contains a distinct species of collagen previously not described. Contrary to previous reports, both the pepsin-solubilized aorta collagen and denatured α-chains are capable of inducing platelet aggregation. These data suggest that covalent structures of aorta collagen may play a role in human platelet aggregation. (Supported by grants from NIH and VA.)

132. An Unusual Inherited Form of Male Pseudohermaphroditism. A Model of 5α-Reductase Deficiency in Man. JULIANNE IMPERATO-MCGINLEY,* LUIS GUERRERO,* TEO GAUTIER,* AND RALPH E. PETERSON,** New York and Santo Domingo.

We have investigated 12 families with 22 pseudohermaphrodites from a village in the Dominican Republic. The affected (46 XY), unlike testicular feminization, are born with ambiguous genitalia, and masculinize at puberty without breast enlargement. They have normal testes histologically, no Mullerian structures, complete Wolffian differentiation, small phallus, bifid scrotum, urogenital sinus with perineal hypospadias, and blind vaginal pouch. At puberty, they show male habitus with excellent muscular development, voice change, enlargement of phallus, and semen, but prostate is small and beard scanty. Plasma testosterone is normal; however, 5α-dihydrotestosterone is less than 2% of plasma testosterone (normal 10%). During constant infusion of radioactive testosterone, less than 1% is converted to dihydrotestosterone (normal 4–7%). The urinary 5β/5α androstane 17-ketosteroid (etiocholanolone/androsterone) ratio is markedly elevated. After glucuronidase hydrolysis, 7–14/1 (normal 0.4–2.0/1)—after glucuronidase and hot acid hydrolysis, 4.5–8.0/1 (normal 0.4–1.3/1). Urinary 5β/5α androstane (3α/3β hydroxy) 17β-hydroxysteroid ratio, 6.5–10/1 (normal 1.2–2.2/1). The ratio of the above urinary 5β/5α metabolites derived from infused radioactive testosterone is similar. Analysis of pedigrees reveal inheritance as autosomal recessive. The clinical abnormality is expressed in men—one woman studied shows the same biochemical defect. Carriers show modest increase in urinary 5β/5α ratio. We postulate this condition as the distinct clinical entity of steroid 5α-reductase deficiency which may delineate the roles of testosterone and dihydrotestosterone in sexual development. At critical period in utero masculinization of external genitalia may be dihydrotestosterone-dependent, but Wolffian differentiation testosterone-dependent. The events at puberty may be mainly testosterone-dependent, with exception of facial hair and prostate growth, which may be dihydrotestosterone dependent.

133. Hormonal Regulation of the Intrarenal Distribution of Blood Flow. HAROLD D. ITSKOVITZ* AND JOHN C. MCGIFF,** Milwaukee, Wis.

Total renal blood flow (RBF) and its zonal distribution are affected by the level of activity of the renal prostaglandin (PG) and renin-angiotensin systems. Blood flows were calculated for the outer and inner cortical halves by the radioactive microsphere method. Blood levels of a PGE compound ("PGE") were determined by bioassay and radioimmunoassay. In isolated canine kidneys, fractional and absolute blood flow increased to the inner cortex as concentrations of renin substrate (RS) decreased from 206 to 54 ng/ml plasma and PGE increased from 0.04 to 1.42 ng/ml blood during 5 h. Thus, the fractional distribution of blood flow (outer:inner cortex) changed from a control of 77:23 during the first 90 min to 65:35 after 3 h and 61:39 after 5 h. These changes were primarily the result of increased blood flow to the inner cortex which could be reversed by either: (*a*) increasing the activity of the renin-angiotensin system through replenishment of RS by tetradecapeptide (2.5–15 μg/min), or (*b*) decreasing the activity of the renal PG system by inhibiting PG synthesis with indomethacin. The latter resulted in a greater than 70% reduction in PGE; i.e., from 1.12 to 0.28 ng/ml blood ($P < 0.05$). The effect of tetradecapeptide RS on the distribution of RBF could have been due to formation of either angiotensin I or II. Doses of angiotensin I and II were infused which reduced RBF by 10–20%; i.e., similar to the decreased in RBF produced by sodium depletion. Angiotensin I, as did RS, selectively decreased inner cortical blood flow from 50 to 34 ml/min ($P < 0.005$), despite doses of SQ 20881, which maximally inhibits angiotensin I conversion intrarenally. In contrast, angiotensin II decreased outer cortical blood flow from 130 to 106 ml/min ($P < 0.005$) and variably affected inner cortical blood flow. However, after inhibition of PG synthesis, angiotensin II consistently decreased blood flow to both inner and outer cortex. These results indicate a function for angiotensin I as a local hormone and support the proposal that angiotensin I and PGE_2 are opposing intrarenal hormones which regulate blood flow to the inner cortex and medulla and thereby affect distribution of RBF.

134. Lithium Therapy for Neutropenias. ELIZABETH JACOB* AND VICTOR HERBERT,** New York.

Manic-depressive subjects treated with $LiCO_3$ tend to develop granulocytosis and elevated unsaturated B_{12} binding capacity

(UBBC); $LiCO_3$ also appears to enhance granulocyte colony growth in vitro. This background suggested the use of similar oral doses of $LiCO_3$ (enough to sustain a serum Li level of 0.5–1.5 meq/liter) in treatment of granulopenias. Initial results were as follows. A 46 yr old alcoholic with cirrhosis, congestive splenomegaly, leukocyte count (WBC) regularly <2000 (63–71% neutrophils) and platelets regularly <50,000, with serum UBBC = 535 pg/ml (of which 225 pg/ml was granulocyte related [G-R]), on day 6 of $LiCO_3$ had no rise in WBC, but on day 12 WBC = 2120, followed by a gradual rise to 3600 on day 34 and 6000 on day 55, at which time serum UBBC = 675 pg/ml (228 pg/ml G-R) but platelets had not risen. It should be noted that such a patient could be considered to put Li therapy to maximum test because of the probability that the dominant cause of his granulopenia was increased destruction rather than decreased production. A 69 yr old black man with apparent congenital neutropenia, WBC regularly ≦4000 (neutrophils 63–70%), and serum UBBC = 614 pg/ml (of which 207 pg/ml was G-R), on day 5 of $LiCO_3$ had no rise in WBC but serum UBBC = 979 pg/ml (315 pg/ml was G-R), on day 11 WBC = 4000, on day 14 WBC = 11,200 (neutrophils 90%) with UBBC = 1179 pg/ml (503 pg/ml G-R), and on day 15 WBC = 6250 (neutrophils 88%). A patient with carcinoma of the bronchus was given $LiCO_3$ to determine if it would prevent the granulopenia expected with his third course of cytoxan therapy (he had granulopenia during each prior course). He developed granulocytosis rather than granulopenia, but probable intercurrent pulmonary infection at the present time precludes attribution of his white cell rise to Li therapy. These pilot results are sufficiently encouraging to suggest that more extensive studies should be carried out in the various chronic and cyclic granulopenias to further evaluate $LiCO_3$ therapy. (Supported by USPHS Grant AM15163, VA Medical Investigatorship, and Career Scientist Award I-683 of the Health Research Council of NYC.)

135. Synthesis of von Willebrand Factor by Cultured Human Endothelial Cells. ERIC A. JAFFE,* LEON W. HOYER, AND RALPH L. NACHMAN, New York and Farmington, Conn.

Previous studies from this laboratory have demonstrated that cultured human endothelial cells synthesize and secrete a protein(s) which has factor VIII antigen ($VIII_{AGN}$) but which lacks factor VIII clot-promoting activity (1973. *J. Clin. Invest.* 52:2757). We have now identified von Willebrand factor ($VIII_{VWF}$) activity in media from cultured human endothelial cells. This material supported ristocetin-induced aggregation of washed normal human platelets and corrected the defect in platelet adhesiveness of blood from patients with von Willebrand's disease. The $VIII_{VWF}$ from cultured endothelial cells appears in the void volume when chromatographed on Sepharose 4B, resists adsorption by $Al(OH)_3$ and is stable at −20°C. It is thus similar to the $VIII_{VWF}$ and $VIII_{AGN}$ of human plasma and the $VIII_{AGN}$ synthesized by cultured endothelial cells. Antibodies were prepared in rabbits to endothelial cell factor VIII (void volume chromatographic fractions containing $VIII_{VWF}$ and $VIII_{AGN}$). This antibody reduced the platelet retention of normal blood in glass bead columns to levels seen in von Willebrand's disease. This effect was neutralized by absorption with concentrated human factor VIII. On immunodiffusion, rabbit anti-endothelial cell VIII and two other rabbit antibodies to purified human factor VIII formed a line of identity against human factor VIII. Rabbit anti-endothelial cell VIII had a minimal effect on plasma factor VIII clot-promoting activity. Since cultured human endothelial cells synthesize $VIII_{AGN}$ and release $VIII_{AGN}$ and $VIII_{VWF}$ into the culture medium and since these activities have very similar physicochemical and immunologic properties, we suggest that endothelial cell $VIII_{AGN}$ and $VIII_{VWF}$ are synthesized and released by the cell either as one molecule or as separate subunits of a closely related macromolecular complex.

136. Unique Characteristics of the Mechanism for Reabsorption of Filtered Versus Secreted Urate. PAUL JENKINS* AND RICHARD E. RIESELBACH, Madison, Wis.

Recent studies indicate that the magnitude of urate secretion may approach that of filtration; other data suggest substantial postsecretory reabsorption. Thus, new models for renal urate handling have been proposed; some involve cumulative reabsorption of filtered and secreted urate by the same mechanism, at a site distal to secretion. If this were the case, simulation of a normal reabsorptive load in the absence of secretion by increasing the filtered load of urate in conjunction with pharmacologically induced secretory suppression should result in a normal rate of urate excretion. This experimental design was achieved in six normal men by administering pyrazinamide and yeast RNA for several days, with a resultant plasma urate (P_{ur}) of 13.5 ± 0.9 mg/100 ml; then, clearance studies were performed under pyrazinamide suppression. Although mean filtered urate was elevated to 17.4 mg/min, fractional excretion (FE_{urate}) was only 0.51 ± 0.06%. Thus, mean urate reabsorption (T_{ur}) was 17.3 mg/min, reaching 21.9 mg/min in one subject. Similar studies on these subjects during normouricemia (P_{ur} 6.26 ± 0.6 mg/100 ml) revealed no difference in FE_{urate} (0.71 ± 0.07%; $P > 0.1$), while filtered urate was 8.6 and T_{urate} 8.5 mg/min. During hyperuricemia, urate excretion was negligibly increased (0.005 mg/min). Thus, the reabsorptive mechanism for filtered urate is uniquely characterized by its high capacity and affinity. If this same mechanism were operative for secreted urate, even if the magnitude of secretion equaled filtration in man, urate excretion would be substantially less than normally observed. Therefore, in health, filtered urate appears to be reabsorbed more avidly than secreted urate; reabsorption of secreted urate probably plays a major role in modulating final urate excretion. (Research supported by NIH Grant AM 15512-03.)

137. Increased Urea Production Due to Positive K Balance During the Administration of K-Retaining Diuretics. D. E. KAMM* AND B. PAL,* Rochester, N.Y. (introduced by John Jaenike).

These experiments examined whether K retention contributes to the azotemia found during the administration of K-retaining diuretics. Rats, previously depleted of 0.9–1.1 meq of Na/100 g with furosemide, were placed in metabolic cages and given either 10% dextrose and water containing KCl, 7.5 meq/kg per day (controls-C), or the same solution plus aldactone (12 mg/kg per day) and triamterene (15 mg/kg per day) (diuretic-D). Urea-N excretion (mg/100 g per day) progressively increased in D: day 1, C 40.0 ± 2.4, D 45.1 ± 2.6 ($P < 0.2$); day 2, C 39.7 ± 2.1, D 50.4 ± 3.9 ($P < 0.02$); day 3, C 38.4 ± 2.0, D 51.2 ± 4.5 ($P < 0.02$). These results combined with increased plasma urea-N (mg/100 ml) observed on day 3 (C 14.5 ± 0.56; D 57.0 ± 5.2, $P < 0.01$) indicate enhanced urea production in D. D induced positive K balance as indicated by increased plasma K (meq/liter) (C 5.33 ± 0.38; D 6.96 ± 0.46, $P < 0.02$) and muscle K (μeq/g dry wt) (C 451 ± 5.1; D 496 ± 9.1, $P < 0.01$). Measured K balance over 3 days was also more positive in D, albeit insignificantly. D also had more negative Na balance (C −0.076 ± 0.002; D −1.25 ± 0.04 (meq/100 g per 3 days) and reduced clearances (ml/100 g per min) of creatinine (C 0.96 ± 0.03; D 0.44 ± 0.04, $P < 0.01$) and urea (C 0.187 ± 0.01; D 0.064

± 0.005, $P < 0.01$). Although Na depletion contributed to the azotemia by decreasing urea clearance, it cannot explain the enhanced urea production since similar negative Na balance (−1.16 ± 0.05) after furosemide failed to increase urea production unless enough potassium was also given to produce hyperkalemia. We conclude that K-retaining diuretics induce azotemia by a combination of reduced urea clearance due to Na depletion and enhanced urea production due to K retention. (Research supported in part by NIH Grant AM 11023.)

138. Absence of α-Globin mRNA in Homozygous α-Thalassemia. YUET WAI KAN,* DAVID TODD,* JANICE HOLLAND,* AND ANDREE DOZY,* San Francisco, Calif., and Hong Kong (introduced by Thomas B. Bradley).

In homozygous α-thalassemia (hydrops fetalis), the predominant hemoglobin is hemoglobin Barts (γ_4), with small amounts of hemoglobin H (β_4) and hemoglobin Portland ($\zeta_2\gamma_2$). No α-globin chain is synthesized. This may be due to the total lack of α-globin mRNA; however, some nonfunctional α-globin mRNA may be present. In this study, we investigate these possibilities in an infant born with this disorder. Messenger RNA was prepared from the peripheral blood of the hydropic infant and assayed in a cell-free system derived from the wheat embryo. Only γ- and β-chains were produced, and no α-chain was synthesized. In this system α-chain was translated normally when mRNA from nonthalassemic blood was added. Thus, no functional α-globin mRNA was present in the blood of this hydrops. The presence of a nonfunctional α-globin mRNA was investigated by hybridization with cyclic (c) DNA's copied from purified rabbit α- and β-globin mRNA, both of which were demonstrated to be 90% pure by translation. Against the β-globin cDNA, the rate and extent of hybridization were similar to those of the nonthalassemic. In contrast, the α-globin cDNA was hybridized by the hydrops' mRNA to less than 10% of the normal even at high CRT values. This small amount of hybridization was most likely due to the 10% contamination of the α-globin probe by β-globin cDNA. Hence, we conclude that in homozygous α-thalassemia α-globin mRNA is absent. This may be the result of a defect in transcription or of deletion of the α-globin chain structural loci. (Research supported by grants from the NIH and the John A. Hartford Foundation.)

139. Evaluation of Physicians in Family Practice. DAVID KAPLAN,* MAX WEINER,* AND CHARLES M. PLOTZ,* Brooklyn, N.Y. (introduced by Paul Dreizen).

24 family practitioners were observed in their offices, each for a 2 h period, while seeing a total of 219 patients. The physicians were graded by one observer (scores from 1 to 5 for increasing levels of adequacy or appropriateness) in seven areas of medical skills: history, physical examination, use of diagnostic studies, treatment, follow-up, and use of consultants. In addition, the medical sophistication required to manage each patient and whether the patient actually required a physician's services were also judged. An overall effectiveness rating was devised and calculated for each physician—the product of his skills and the requirement for the use of those skills (maximum score = 30). Three physicians with effectiveness ratings of 19, 22, and 30 were revisited by a second observer not associated with this study who rated them 17, 22, and 28, respectively. The physicians fell into three distinct groups: (*a*) three who were giving excellent care to people in need of physician's services, with ratings of 26, 27, and 29; (*b*) 10 who were generally practicing at an adequate level with scores of 22–24, and (*c*) 11 physicians who were either seeing people with only a questionable need for a physician's services or were managing significant illness inappropriately, with ratings of 16–20. Two relationships were noted: (*a*) the more competent physicians were more likely to see patients with significant illness ($P = 0.02$), and (*b*) the 17 graduates of American medical schools functioned at a more effective level than did the seven graduates of foreign or osteopathic schools ($P = 0.01$). This report is offered as a practical methodology for the evaluation of health care delivery and as an alternative to the traditional method of chart review.

140. Studies of Normal Human Lymphocytes Having Both B and T Membrane Receptors. MANUEL E. KAPLAN AND CONNIE CLARK,* Minneapolis, Minn.

It is generally accepted that human thymic-dependent lymphocytes (T) have the capacity to form nonimmune rosettes (RFC) with unsensitized sheep erythrocytes (SRBC) but carry little, if any, surface immunoglobulin (SIg); conversely, thymic-independent lymphocytes (B) have readily detectable SIg but do not form rosettes. Bentwich et al. (1973. *Clin. Immunol. Immunopathol.* **1**: 511) recently reported that 2–4% of normal peripheral blood lymphocytes (PBL) exhibited characteristics of both B and T cells. Our studies support their findings and suggest that the dual-marker (RFC^+-SIg^+) cells represent a substantial proportion of the SIg^+ population and are largely confined to lymphocytes with SIgG. PBL of >95% purity and viability were isolated from 16 normal donors by Ficoll-Hypaque centrifugation and exposure to colloidal iron. Lymphocytes were stained with polyvalent, fluorescein-conjugated (FC) antiserum to human immunoglobulins (GMA) and then rosetted with sulfhydryl-treated SRBC (Kaplan and Clark. 1973. *Clin. Res.* **21**: 877). Cell preparations were examined by phase and fluorescence microscopy. Results: RFC^+, 77.8 ± 5.5; % PBL, SIg^+, 16.7 ± 5.0; $RFC^+ - SIg^+$, 4.9 ± 2.9. Of the total PBL population, 4.9% were both SIg^+ and RFC^+; of the total SIg^+ population, 13–68% (mean 29%) formed rosettes. The studies were repeated in four PBL donors with monospecific, FC antisera to μ, γ, and α. Essentially all dual-marker lymphocytes had SIgG. Of the total $SIgG^+$ lymphocytes, 41–81% (mean 65.6%) formed rosettes. These findings demonstrate a significant population of lymphocytes with both B and T membrane receptors in normal individuals. Although the dual-marker cells appear to be almost exclusively $SIgG^+$, this observation must be confirmed on PBL isolated by other methods. At present the biologic role of these cells is unknown. (Research supported by Grants AM13717 from the USPHS and Grant 01/4828 from the VA.)

141. Toad Bladder Sodium Transport and Intracellular Water and Electrolyte Content in Relation to Osmotic Variation. MICHAEL A. KAPLAN* AND JACQUES J. BOURGOIGNIE, Bronx, N.Y.

The toad urinary bladder transports sodium and water transepithelially and simultaneously must control its own cell volume composition. It also is exposed to solutions of different osmolalities at its two borders. Both structually and functionally it resembles the mammalian collecting duct. Sodium transport (SCC) in the intact bladder was unaffected by changes in mucosal osmolality induced by changes in sodium chloride concentration of the bathing medium. However, when serosal osmolality was increased progressively, SCC decreased pari passu; and, when osmolality was decreased, SCC rose reciprocally. At steady state isolated epithelial cells in the same solutions were changed in volume to some degree. With a wide range of hypo- and hypertonic solutions intracellular sodium content was increased and decreased, re-

spectively, and the changes occurred in parallel with the change in cell volume ($r = +0.99$). Over the entire range intracellular sodium concentration remained constant. Intracellular potassium content, on the other hand, did not change while its concentration varied directly with the osmolality of the external medium ($r = +0.99$). Thus, the cells showed evidence of volume regulation. Sodium permeability appeared to change directly with osmolality and transepithelial sodium transport tended to respond to changes in intracellular sodium content. The overall adjustment of intracellular tonicity was accomplished primarily by a change in intracellular potassium concentration while holding intracellular sodium concentration relatively constant.

142. On the Characterization of a Natriuretic Hormone. MICHAEL A. KAPLAN,* JACQUES J. BOURGOIGNIE, AND NEAL S. BRICKER,** Bronx, N.Y.

For several years we have been searching for a natriuretic hormone. The search may be narrowing. Uremic subjects were used because nephron reduction is attended by an ever-increasing natriuresis per nephron. An inhibitor of sodium transport was first found in uremic serum, then in uremic urine. It disappears if fractional excretion of sodium is low (e.g., in nephrotic uremics). It is natriuretic in the rat and inhibits short circuit-current, increases intracellular sodium content, and decreases pyruvate oxidation in the toad bladder. It is not lipid soluble. It appears in a low molecular weight fraction with gel filtration, and by ultrafiltration, its molecular weight may be less than 1,000. It appears to be a peptide in that all the foregoing effects are blocked by leucine aminopeptidase. It is also inactivated by acid hydrolysis and boiling at pH > 10.5. It is stable at room temperature. The active gel filtration fraction contains at least seven peptide peaks with identical patterns for serum and urine. All but two of these peaks have been shown to have no natriuretic activity. The two remaining peaks are different in character: one is highly acidic and the other basic. Neither peak is vasopressin. Separation and characterization of the last two peaks is in progress to see whether the long-elusive natriuretic hormone has been captured.

143. Importance of an α-Methyl Mannoside Residue in the Thrombin Molecule. MARGARET KARPATKIN* AND SIMON KARPATKIN, New York.

The functional significance of carbohydrate groups on the fibrinogen and thrombin molecules was investigated. Concanavalin A (Con A), a carbohydrate ligand with specificity for α-methyl mannoside (α-MM), prolonged the clotting of purified human fibrinogen by purified bovine thrombin from 16 to 40 s (final concentration of Con A 0.5 mg/ml). Prolongation was prevented by α-MM (0.7 mg/ml) but not by other sugars tested at similar concentrations (α-methyl glucoside, 3-*O*-methyl glucoside, β-methyl xylopyranoside, β-methyl glucoside). In a system with an apparent clotting time Km for fibrinogen of 0.5 mg/ml, inhibition was competitive with a Ki of 0.3 mg/ml for Con A. Fibrinopeptide release by thrombin was measured by a sensitive fluorescent assay employing fluorescamine which binds to NH_2-terminal amino groups (Udenfriend et al. 1972. *Science (Wash. D.C.).* **178:** 871). Peptide release was inhibited 100% by Con A (0.3 mg/ml) 5 min after the addition of thrombin and 80% after 15 min. Inhibition was prevented by α-MM (1.0 mg/ml). Con A did not inhibit polymerization of purified fibrin monomer prepared by the method of Donelly et al. (1955. *Arch. Biochem. Biophys.* **56:** 369). An indirect system employing purified fibrinogen and hirudin confirmed inhibition of peptide release and normal polymerization. Con A (0.10 mg/ml) inhibited hydrolysis of protamine sulphate by thrombin as determined by the method of Brown et al. (1973. *Biochem. Biophys. Res. Commun.* **53:** 75); this was also prevented by α-MM. Con A did not inhibit clotting of fibrinogen by reptilase, which releases fibrinopeptide A only, or by *Ancistrodon contortrix contortrix,* which releases peptide B initially followed by a small amount of A (Herzig et al. 1970. *J. Lab. Clin. Med.* **76:** 451). These data demonstrate that Con A binds to the thrombin molecule obscuring its active site. It is suggested that an α-MM residue may be at the active site of the thrombin molecule. (Supported by NHLI HL-13336.)

144. Studies on the Specificity of Antiplatelet Antibodies in Autoimmune Disease, and on Circulating Immunoglobulin Levels in Idiopathic and in Autoimmune Thrombocytopenic Purpura (ATP). SIMON KARPATKIN AND GREGORY W. SISKIND, New York.

We have previously described a platelet factor 3 (PF-3) immunoinjury technique for detection of antiplatelet antibody in 65% of patients with idiopathic thrombocytopenic purpura (ITP). In the present studies the specificity of antiplatelet antibody and immunoglobulin levels were investigated. Sera from 17 patients with antiplatelet antibody were examined in the PF-3 test with platelets from 10 different donors. Although similar results were generally obtained with platelets from different donors, it was clear that significant differences were sometimes noted when the same sample was tested with platelets from different donors. Platelets from different donors appeared to vary in their sensitivity to antiplatelet antibody. In addition, the pattern of reactivity indicated that antiplatelet antibodies from different patients vary in detailed specificity. In addition, platelets from five patients with ATP in remission were tested against their previously stored serum. Three of the five demonstrated significantly higher titers in the PF-3 test when their own platelets were used as compared with platelets from an unrelated donor. Circulating immunoglobulin levels (IgG, IgM, and IgA) were normal in patients having detectable antiplatelet antibody. However, at least 8 out of 34 ITP patients (without detectable antiplatelet antibody) had elevated immunoglobulin levels. Furthermore, at least five out of six ITP patients with elevated IgG levels and six out of eight with elevated IgM levels had unusually severe disease which was relatively refractory to steroids and/or splenectomy. It is possible that this elevated immunoglobulin, "refractory" group might represent a different disease. (NHLI, HE-13336.)

145. Does Na-K-ATPase Modulate Acute Changes in Renal Tubular Na^+ and K^+ Transport? ADRIAN I. KATZ* AND MARSHALL D. LINDHEIMER,* Chicago, Ill. (introduced by Leif B. Sorensen).

To elucidate the role of Na-K-ATPase in modulating rapid changes in renal Na^+ reabsorption and K^+ secretion, its properties and specific activity were measured (A) in rat and guinea pig outer medulla after acute Na^+ loading (0.58 ml/min isotonic saline for 3 h) and (B) in the whole kidney of rats after acute K^+ loading (0.02 ml/min 1 M KCl for 3 h). Controls in both studies received 0.02 ml/min isotonic saline. In (A), whole homogenate Na-K-ATPase specific activity (μmol Pi/mg protein per h) was moderately higher after Na^+ loading in both species (guinea pig: 36.3 ± 2.0 vs. 28.4 ± 1.6, $P < 0.01$; rat: 52.9 ± 3.0 vs. 46.4 ± 1.6, $P = 0.05$). However, microsomal Na-K-ATPase after Na^+ loading was similar to control activity in both guinea pigs (66.5 ± 4.7 vs. 60.3 ± 4.7) and rats (98.0 ± 4.2 vs. 92.5 ± 4.8). In vitro [^{3}H]ouabain binding to guinea pig microsomes was also similar in saline-loaded and control animals (130 ± 8 vs. 113 ± 6

pmol/mg protein, $P \geq 0.1$). Apparent K_m of the microsomal enzyme with Na^+ as substrate was 16.3 mmol/liter in saline-loaded rats and 17.0 mmol/liter in controls. After K^+ loading (B) plasma K^+ increased to 8.3 meq/liter and K^+ excretion averaged 11.54 ± 0.33 μeq/min compared with 0.74 ± 0.10 μeq/min in controls. Despite the 15-fold increase in K^+ excretion, Na-K-ATPase specific activity was not different in the two groups either when measured in whole homogenates (28.9 ± 1.0 vs. 28.3 ± 0.4) or in microsomes (109.8 ± 2.8 vs. 108.7 ± 3.7). Apparent K_m of the microsomal enzyme with K^+ as substrate was 0.5 mmol/liter, also identical in both groups. These results do not support a role for Na-K-ATPase in acute changes in Na^+ and K^+ tubular transport. However, enzyme concentrations in vivo might sufficiently exceed basal requirements so that large increments in cation transport could occur by increasing saturation of unused active sites.

146. Cell-Mediated Immunity During Influenza. CAROL A. KAUFFMAN,* JAMES S. TAN,* CALVIN C. LINNEMANN, JR.,* GILBERT M. SCHIFF, AND JOHN P. PHAIR,* Cincinnati, Ohio.

Cell-mediated immunity (CMI) was studied in 15 persons with naturally acquired influenza A/England (Flu Eng) infection and in 5 persons with experimentally induced influenza A/Hong Kong (Flu HK) infection. Skin tests (ST) with four antigens and phytohemagglutinin-(PHA-) induced lymphocyte DNA synthesis were assessed in persons with Flu Eng. All patients had negative ST and depressed PHA responses. Nonstimulated cultures showed normal or decreased background DNA synthesis. Three of these persons had a noncytotoxic plasma inhibitor that depressed the response of normal lymphocytes to PHA. Of five persons with moderately severe Flu Eng, only one had negative ST and two had diminished PHA stimulation. No person with mild influenza had abnormalities of CMI. CMI returned to normal in all surviving patients after convalescence. PHA stimulation, assessed in persons with mild experimentally induced Flu HK, was normal. Addition of Flu Eng and Flu HK viruses to cultures of lymphocytes from normal donors did not result in cytotoxicity nor was PHA stimulation significantly depressed. Background counts, in contrast to cultures obtained from infected patients, were elevated, resulting in a decreased stimulation index (PHA stimulated/non stimulated). Although clinically mild influenza does not result in a deficit in CMI, severe influenza is associated with ST anergy and PHA unresponsiveness. In vitro studies did not reveal a direct viral effect on PHA-induced DNA synthesis. (Research supported by grant from NIH.)

147. Evidence for Electrogenic Transport Processes in the Two Different Types of Proximal Straight Tubules. S. KAWAMURA,* M. IMAI,* AND J. P. KOKKO, Dallas, Tex.

Electrophysiological studies were conducted in the rabbit in vitro perfused proximal straight tubules obtained from superficial (SF) and juxtamedullary (JM) regions in order to examine the nature of sodium and chloride transport. Perfusing with an isosmolal artificial solution simulating ultrafiltrate of rabbit serum used for bath, the transtubular potential difference (PD) was -2.1 ± 0.1 mV in the SF tubules and -1.8 ± 0.1 mV in the JM tubules, $P > 0.5$. Addition of 10^{-5} M ouabain to the bath decreased the PD to zero in both segments. Isosmolal replacement of glucose and alanine in this perfusate did not decrease the PD significantly in either segment. When this perfusate was changed to isosmolal fluid simulating fluid present in the end proximal convoluted tubule ($NaHCO_3$; glucose and alanine replaced by NaCl), the PD was $+1.6 \pm 0.2$ mV in the SF tubules and -1.3 ± 0.3 mV in the JM tubules, $P < 0.001$. The relative electrochemical permeability of chloride to sodium (P_{Cl}/P_{Na}) was calculated as 1.9 in SF nephrons and 0.6 in JM nephrons. Isosmolal replacement of NaCl by Na-cyclamate in the perfusate and the bath caused an increased luminal negativity in both segments. With similar substitution of NaCl by choline Cl the PD became slightly positive, $+0.6 \pm 0.1$ mV in SF and $+1.3 \pm 0.1$ mV in JM tubules. In summary: (*a*) proximal straight tubules obtained from SF and JM regions have different passive permeability characteristics: SF, $P_{Na} < P_{Cl}$; JM, $P_{Na} > P_{Cl}$; (*b*) sodium is necessary for the generation of negative PD; and (*c*) the luminal PD becomes more negative with complete substitution of Cl^- by cyclamate. In conclusion our studies suggest that the active transport mechanism in the proximal straight tubule is primarily an electrogenic sodium pump, and therefore, these segments are fundamentally different from the proximal convoluted tubule. (Research supported by grants from NIH.)

148. Gonadotropin-Receptor Interactions in Rat Testis: Kinetic and Equilibrium Binding Analysis. JEAN-MARIE KETELSLEGERS,* GARRY D. KNOTT,* AND KEVIN J. CATT, Bethesda, Md.

The binding of hCG to specific gonadotropin receptors in the rat testis was studied with [^{125}I]hCG and the data analyzed by an interactive nonlinear curve-fitting program (MLAB). The data obtained for kinetic and equilibrium binding were fitted to mathematical models based on a bimolecular reaction and the equilibrium (K_a) and rate constants of the hCG-receptor reaction were determined. Equilibrium data were analyzed by an equation which expressed bound hormone as a function of total hormone concentration, obtained by solving the second-order rate equation for equilibrium conditions ($K_a = 2.5 \times 10^{10}$ M^{-1}). Kinetic data fitted the differential equation for a second-order chemical reaction at 24°C and 37°C and over a wide range of hormone concentrations. The association rate constant (K_1) was $2.9 \times 10^7 M^{-1}$ min^{-1} at 24°C, and 6.5×10^7 M^{-1} min^{-1} at 37°C, and was independent of the reactant concentrations. Dissociation of bound hCG followed first-order kinetics at 24°C, with a rate constant (K_2) of 5.1×10^{-4} min^{-1}; at 37°C, dissociation followed a double exponential process with rate constants of 3.9×10^{-3} min^{-1} and 3.5×10^{-12} min^{-1}. Rate constants were also derived from kinetic studies at 24°C by taking in account progressive degradation of the free hormone during incubation in vitro. Receptor degradation was found to be relatively slow at 24°C, and it did not significantly influence the analysis. No significant effect of hormone degradation on the derivation of K_1 during short-term incubation could be demonstrated. These results indicated that the interaction of hCG with testis receptors can be described and analyzed in terms of a simple reversible bimolecular reaction between the hormone and a homogeneous population of independent binding sites.

149. Alteration of Glycoprotein-Associated Antigens in Colonic Neoplasm. YOUNG S. KIM, JOSE PERDOMO, AND RICHARD ISAACS, San Francisco, Calif.

Partial or complete loss of ABH group-specific isoantigens has been reported in primary gastrointestinal tumors. The carbohydrate moieties of glycolipids and glycoproteins are important determinants of immunologic specificity and are the structures in which blood group determinants of mammalian cells reside. In the present study, we sought to measure the level of blood group and other carbohydrate-associated antigens of membrane glycoproteins from colonic carcinoma and adjacent normal tissue and to quantitate enzymes required for the synthesis and degradation of these antigens. Tissues were

obtained at surgery and membrane glycopeptides were prepared by pronase digestion of the particulate fraction of tissue homogenates. By using antisera and various blood group specific lectins, the levels of the blood group antigens associated with membrane glycopeptides from cancer tissue and adjacent normal tissue were compared. The level of the blood group A antigen was significantly lower in the cancer tissue. The cancer tissue also had markedly less membrane-bound *N*-acetylgalactosamine, a part of the blood group A determinant. The levels of six glycosyltransferases were measured. The specific activities of the *N*-acetylgalactosaminyltransferase (the enzyme responsible for the formation of the blood group A determinant), a galactosyltransferase, and the two fucosyltransferases were significantly lower in cancer tissues. The levels of the sialyltransferase and another galactosyltransferase were similar in both tissues. Six glycosidases, including an *N*-acetylgalactosaminidase which destroys blood group A activity, were similar in normal and cancer tissues. The results of this study indicate that there is a marked diminution in the level of the blood group A determinant in glycoproteins of colonic carcinoma. The data strongly suggest that this alteration is due in part to a deficiency of glycosyltransferases responsible for the synthesis of the blood group A determinant.

150. Cell Membrane Asymmetry and the Antidiuretic Action of Vasopressin. ROLF KINNE, LINDA SHLATZ, EVA KINNE-SAFFRAN, AND IRVING L. SCHWARTZ,** Frankfurt, Germany and New York.

To elucidate the molecular events underlying hormonal regulation of transepithelial transport processes, it is necessary to resolve the plasma membrane of the polarized epithelial cell into its functionally different apical (luminal) and basal (contraluminal) components. In a previous study of the rat kidney cortex we prepared plasma membranes from proximal tubular epithelial cells by differential centrifugation and then resolved this preparation into highly purified luminal and contraluminal membrane fractions by free-flow electrophoresis. The luminal membrane fraction (brush border) was characterized by its morphologic appearance and by the enrichment of alkaline phosphatase and bicarbonate-activated ATPase. The contraluminal membranes were also identified morphologically and by their content of Na^+-K^+-activated ATPase and Ca^{++}-activated ATPase. We are now reporting the application of similar methodology to the papillae of the bovine kidney. A plasma membrane preparation from papillary collecting duct epithelial cells was resolved into luminal and contraluminal fractions identified by bicarbonate-activated ATPase and Ca^{++}-activated ATPase, respectively, as marker enzymes. The luminal membrane fraction contained a cAMP-dependent self-phosphorylating system consisting of a membrane-bound protein kinase and its membrane-bound substrate. This endogenous protein kinase was not found in the contraluminal membrane fraction which, however, did contain all of the ADH-sensitive adenylate cyclase activity. These findings support the following model for ADH action on its renal target cell: (*a*) ADH-receptor interaction and activation of adenylate cyclase in the basal cell membrane, (*b*) generation of cAMP and its translocation to the apical cell membrane, (*c*) activation of apical membrane-bound protein kinase, and (*d*) phosphorylation of the apical membrane causing an increase in its permeability. The approach employed in this study for resolution of the plasma membrane of a transporting epithelial cell into its basal and apical components should find application in many other efforts to elucidate the biochemical basis of the effects of hormones, drugs, and disease states on water and solute transport processes. (Supported by USPHS Grant AM 10080 and the Life Sciences Foundation Inc.)

151. Tocopherol As a Specific Component of Adenyl Cyclase Activity and Steroidogenesis in Isolated Adrenal Cell System. ABBAS E. KITABCHI, ANNE NATHANS,* PRANEE JAMES,* AND LOUISE KITCHELL,* Memphis, Tenn.

Although adrenal glands contain high concentrations of tocopherol, ascorbic acid, and polyunsaturated lipids, the role of these components in steroidogenesis in the adrenal has not been fully elucidated. Recently, we reported on the diminution of the ACTH-induced steroidogenesis in vitamin E-deficient adrenal cell in the presence of 0.5 mM ascorbic acid. This inhibition was associated with an increase in lipid peroxidation. Addition of tocopherol in vivo or in vitro reversed the inhibition with a concurrent decrease in lipid peroxidation. ACTH is believed to be mediated through stimulation of the membrane-bound enzyme adenyl cyclase which contains phospholipid; therefore, the effect of tocopherol on the activity of adenyl cyclase was studied by measuring the formation of cyclic AMP from [^{14}C] adenine with varying concentration of ACTH (100–10,000 μU of ACTH/ml). In vitamin E deficiency in the presence of ascorbic acid, ACTH-induced adenyl cyclase was inhibited with concomitant stimulation of lipid peroxidation. Addition of tocopherol to the isolated adrenal cell reversed the inhibition of adenyl cyclase activity and returned steroidogenesis to nearly control level. In the presence of Ca^{++} (10 mM) ACTH-induced adenyl cyclase inhibition was reversed with concomitant reduction of lipid peroxidation. We suggest that tocopherol is an integral component of the adrenal cell membrane to ensure optimal adenyl cyclase activity. (Research supported by grants from NIH and Veterans Administration.)

152. Glucagon Binding and Adenylate Cyclase: Evidence for a Dissociable Receptor Site. IRWIN KLEIN,* GERALD S. LEVEY, AND MARY ANN FLETCHER,* Miami, Fla.

The initial interaction of glucagon with its various target tissues, including heart, liver, and pancreas, is thought to be binding to a cell surface receptor with subsequent activation of membrane-bound adenylate cyclase. Little is known about the nature of the interaction between the receptor and adenylate cyclase which results in activation of the enzyme. Moreover, it is unclear whether the receptor is a discrete molecule or a functional part of the catalytic subunit of adenylate cyclase. We utilized a solubilized preparation of cat myocardium to study the interaction of glucagon with its receptor and adenylate cyclase. Solubilized adenylate cyclase has a molecular weight of about 100,000–200,000. When the solubilized preparation was chromatographed on Sephadex G-100, [^{125}I]glucagon binding activity and fluoride-stimulatable adenylate cyclase activity appeared in the elution fractions excluded from the gel. Prior incubation of the binding peak with glucagon shifted its elution pattern on Sephadex G-100 to a smaller molecular weight fraction of approximately 24,000–28,000 (determined by SDS-gel electrophoresis) and produced a dissociation of the receptor from catalytic adenylate cyclase activity, which continued to be excluded from the gel and represents the larger molecular weight component (>100,000) of the enzyme. The [^{125}I]glucagon-receptor complex had a strong positive charge as manifested by its adsorption to the Sephadex G-100 gel and subsequent elution at a column volume consistent with the salt peak. After neutralization of the charge-gel interaction by chromatography in 0.025 N NaOH, the [^{125}I]glucagon-receptor complex eluted at a more appropriate column volume for its apparent molecular weight. These findings are consistent with a dissociable receptor site for glucagon and may provide a mechanism for activation-inactivation of adenylate cyclase after hormone binding as well as a potential method for purification of the glucagon receptor. (Supported by NIH Grants HL13715-03 and AM16763-01.)

153. Elevation of Creatinphosphokinase (CPK) Activity by Intravenous D-Fructose. J. P. KNOCHEL,* K. UYEDA,* AND T. FULLER,* Dallas, Tex. (introduced by Donald W. Seldin**).

Recent observations from this laboratory have shown that alcoholics who demonstrate abnormally increased serum creatinphosphokinase (CPK) activity have a low skeletal muscle resting membrane potential (Em) and intracellular edema (1974. *Clin. Res.* 21: 49). Hypophosphatemia and hypokalemia occur commonly in these patients and tend to be especially marked after glucose. Even in the absence of hypokalemia, CPK may rise sharply as serum phosphorus (Pi) falls. In certain tissues, intravenous D-fructose has been shown to cause intracellular trapping of Pi, depletion of ATP, and alteration of cellular structure. Thus, the possibility was considered that unavailability of inorganic phosphorus (Pi) might be implicated in acute alcoholic myopathy. Using CPK activity in serum as an index of muscle injury, D-fructose, 0.5 g/kg body wt, was infused into 12 normal dogs over a period of 210 min. To determine if its effect is potentiated by a preexistent myopathy with a low Em, identical studies were conducted in 6 K-deficient dogs (16–19% depleted). In normal dogs, venous CPK before fructose was 59 ± 12 (SEM) IU/ml. By 165 min, although three of the nine values rose, the overall change was not significant. However, 24 h after the infusion, CPK had risen in 10 of 12 dogs ($P < 0.01$) to 1,166 ± 376 IU/ml (range 40–4,240). In four of these dogs, CPK was measured simultaneously in coronary sinus and arterial blood at 24 h. Respective paired values were 2,020: 1,770, 460:290, 60:40, and 200:210 IU/ml. There was no CPK activity in spinal fluid. In K-deficient dogs, CPK in arterial blood rose from 62 ± 29 to 243 ± 50 IU/ml at 165 min ($P < 0.01$) and to 2855 ± 376 IU at 24 h (range 530–7300; $P < 0.001$). The values at 24 h for each group were not significantly different. Control studies with pentobarbital alone, glucose, or saline had no effect on CPK. We conclude the following. (*a*) Intravenous fructose in the dog is followed by a major rise of CPK. (*b*) The origin of CPK is probably muscle. These preliminary studies suggest that the heart may be at least a partial source of CPK. (*c*) Potassium deficiency may accelerate the rise of CPK activity. (Research supported by VA and Grant AM 16194 from NIH.)

154. Serum Complement Components in Glomerulonephritis. J. D. KNOSTMAN,* P. H. SCHUR, D. T. FEARON,* C. B. CARPENTER, S. RUDDY, AND J. P. MERRILL,** Boston, Mass.

Varying patterns of serum complement components in glomerulonephritis (GN) have suggested two different mechanisms of activation. Depression of the early reacting components indicate activation of the classical pathway, while low levels of terminal components, properdin, and properdin factor B (pfB) with normal early components indicate alternate pathway activation. Clq, Cls, C4, C2, C3, C5, C6, C9, properdin, and pfB concentrations were measured by radial immunodiffusion using monospecific antibody on serial specimens in 53 patients with GN and 35 with systemic lupus (SLE). All components were normal in most patients with Nil disease (4), focal sclerosing GN (5), membranous GN (12), rapidly progressive GN (3), and chronic GN (16). In SLE, Clq, C4, and C3 were frequently depressed but they did not directly correlate. C2, C6, and C9 were normal. C5, properdin, and pfB were infrequently depressed and did not significantly correlate with other components. 12 of 12 patients with membranoproliferative GN (MPGN) had normal early components and low C3. 8 of these 12 had persistent and profoundly depressed C3 (13 ± 5 mg/100 ml; $n = 91–198$) associated with decreases in C5 (47 ± 27 μg/ml; $n = 65–184$) and properdin (67 ± 7%; $n = 72–140$). Four with moderately low C3 (60 ± 13 mg/100 ml) had normal C5 (145 ± 29 μg/ml) and highly variable properdin levels. The pfB was similar in both groups (77%; $n = 60–140$). This study shows that some but not all early components are depressed in SLE, but not in MPGN. Profound depression of C3 in MPGN patients also includes evidence of alternate pathway activation, whereas moderately depressed levels might be secondary to a synthetic defect. (Supported by NIH grants.)

155. Serum Gastrin-Calcium Correlations during Calcium and Secretin Infusions in the Zollinger-Ellison Syndrome. BYRON E. KOLTS,* CHARLES A. HERBST,* AND JAMES E. MCGUIGAN, Gainesville, Fla.

Intravenous calcium infusion induces marked increases in serum gastrin in patients with the Zollinger-Ellison syndrome (ZES). Paradoxical increases in serum gastrin and in serum calcium with secretion infusion have been noted recently in ZES patients; it has been suggested that gastrin increases may be secondary to secretin-induced changes in serum calcium. Serum gastrin and calcium in response to GIH secretin and to calcium infusions were examined and compared in four ZES patients and three controls. Calcium was given by 3 h intravenous infusion (5 mg Ca/kg per h), and secretin by 1 h intravenous infusion (9 U/kg per h), and by single dose injection (1 U/kg). Gastrin was measured by radioimmunoassay and calcium by atomic absorption in sera obtained before and at 30-min intervals during calcium infusion, and before and at 15-min intervals after secretin. Basal serum gastrin (range 450–16,400 pg/ml) increased after both doses of secretin in each ZES patient (range of increase 320–6,450 pg/ml), in contrast to decreases in controls. Infusion of 9 U/kg secretin increased serum calcium (mean increase 0.9 ± 0.14 SEM mg/100 ml): secretin 1 U/kg produced no serum calcium change. Calcium infusion in ZES patients induced marked increases in serum gastrin (range of increase 510–15,100 pg/ml). With calcium infusion in ZES patients there was a significant ($P < 0.001$) positive correlation ($r = 0.87$) between serum gastrin and calcium. Although secretin infusion (9 U/kg per h) produced increases in serum gastrin and calcium, there was not a significant ($P > 0.10$) correlation ($r = 0.25$) between serum gastrin and calcium. These results indicate that paradoxical increases in serum gastrin after secretin administration in ZES patients are not mediated solely by changes in serum calcium. (Research supported by NIH Research Grant AM 13711.)

156. Circadian Rhythms in Urinary Cyclic Adenosine-3′,5′-Monophosphate, Creatinine, Phosphorus, and 17-Hydroxycorticosteroids in Man. LOWELL KOPP,* TU LIN,* AND JOSEPH TUCCI,* Providence, R. I. (introduced by Paul Calabresi).

The increasing significance of cyclic-AMP (cAMP) in the clinical setting and recent conflicting reports of the occurrence of a circadian rhythm in its urinary excretion prompted us to explore this matter further. Analysis of sequential 4-h urine specimens over 24 h from 16 normal subjects suggested that if there were a circadian rhythm in cAMP excretion, it was of relatively low amplitude. Further studies were performed with 4-h collections obtained from three normal subjects (two men, one woman) for 34, 22, and 16 days, respectively. Specimens were analyzed for their cAMP, creatinine, phosphorus, and 17-hydroxycorticosteroid (17-OHCS) content. Each showed a significant circadian rhythm ($P < 0.005$ by analysis of variance) and the patterns of excretion were similar in all three subjects. Both cAMP and creatinine levels varied approximately ± 11–17% from mean values with maximum and minimum excretion at 4–8 p.m. and 4–8 a.m., respectively.

Phosphorus excretion was maximal at 4–12 midnight and minimal at 8–12 noon with changes of approximately ±30% of the average 24-h excretion. Peak 17-OHCS excretion occurred from 8 a.m. to 4 p.m. with changes of ±30–60% of the average excretion while the nadir occurred from 12 midnight to 4 a.m. Both phosphorus and 17-OHCS excretion showed distinctly different time-dependent patterns compared with cAMP and creatinine. Both cAMP and creatinine excretion patterns were very similar to changes in glomerular filtration rate and effective renal plasma flow reported by Wesson, suggesting that the observed circadian rhythm in cAMP may be related more to changes in renal function than to changing levels of circulating hormones such as parathyroid hormone. (Supported by NIH Grants GMS16538 and CA 13943.)

157. Mechanisms of Hormone Action: Role of Specific Protein Kinase Translocation. STANLEY G. KORENMAN, REGGIE H. STEVENS,* LINDA S. WELLS,* AND LESLIE A. CARPENTER,* Iowa City, Iowa.

Myometrial contractility was inhibited by increases of cAMP by adenyl cyclase activation with isoproterenol or phosphodiesterase inhibitors. It was stimulated by oxytocin and prostaglandins E_1, E_2, and $F_{2\alpha}$, which inhibited the cAMP increment due to β-adrenergic action. We have demonstrated that cAMP generation activated myometrial cAMP-dependent protein kinase (RC) activity through binding to and dissociation of the cAMP receptor (R) from the catalytic (C) subunit. The proportion of C increased as expected, but total kinase activity was substantially reduced. The lost activity was recovered from the 20,000 *g* supernate and from the microsomal pellet by extraction with Triton X-100. R and C activities were studied in rat myometrial cytosol and Triton X-100 extracts of microsomes after purification by pH 5 and $(NH_4)_2SO_4$ precipitation and chromatography on DEAE-cellulose. In the cytosol two distinct peaks containing R and C activity and an R peak were found. The microsomal fraction contained only a single RC, indicating that only one of the two enzyme forms was microsome associated. The cellular components of the 20,000–145,000 *g* pellet were separated by sucrose density gradient ultracentrifugation. Substantial R and C activities were associated with two fractions identified as cell membrane by 5′-nucleotidase and Na/K ATPase activity, and as sarcoplasmic vesicle by Ca uptake activity and electron microscopy. There was little activity found in the endoplasmic reticulum. Both cell membrane and sarcoplasmic vesicle kinase were increased substantially by isoproterenol pretreatment. We conclude that hormones which regulate myometrial contraction through adenyl cyclase activation do so through translocation of a specific RC to a membranous substrate(s). The resultant membrane phosphorylation may be a principal consequence of cAMP-dependent hormone action.

158. Effect of Ca^{++} on Plasma Renin Activity (PRA). THEODORE KOTCHEN,* KIMBALL MAULL,* DOUGLAS REES,* AND ROBERT LUKE,* Lexington, Ky. (introduced by J. W. Hollingsworth**).

An elevation of filtered Ca^{++} increases Na^+ delivery to the macula densa. To evaluate the effect of Ca^{++} on renin release, PRA was measured after acute and chronic Ca^{++} administration. 1% $CaCl_2$ was infused (0.5 cm^3/min) into one renal artery of 10 anesthetized dogs. Ca^{++} excretion and EF_{Na^+} from the infused kidney were elevated ($P < 0.001$) during three successive 15-min infusion periods. Creatinine clearance, systemic arterial pressure, and renal blood flow (flow meter) did not change. Compared to control (45.4 ng/ml per h ± 8.4 SE), renal venous PRA was suppressed ($P < 0.005$) to 20.0 ng/ml per h ± 4.6, 15.8 ng/ml per h ± 4.1, and 12.8 ng/ml per h ± 2.7 after an infusion of Ca^{++} for 15, 30, and 45 min, respectively. 15 and 30 min postinfusion, PRA did not differ from control ($P > 0.2$). Chronic Ca^{++} loading was achieved in Sprague Dawley rats by replacing drinking water with 1% $CaCl_2$ for 17 days. At sacrifice (blunt trauma), serum Ca^{++}, Na^+, K^+, and BUN of controls ($n = 10$) did not differ from Ca^{++}-loaded rats ($n = 10$). Ca^{++} excretion (467 μeq/24 h ± 51) was elevated ($P < 0.001$) compared to controls (85 μeq/24 h ± 12). PRA (8.6 ng/ml per h ± 1.4) and renal renin content of Ca^{++}-loaded rats did not differ from controls ($P > 0.7$). To determine if Ca^{++} suppresses the renin response to Na^+ deprivation, six pair-fed control and six animals receiving 1% $CaCl_2$ consumed a Na^+-free diet for 7 days. On day 7, Na^+ excretion and PRA of Ca^{++}-loaded rats were greater than control values of 10.8 μeq/24 h ± 3.5 ($P < 0.05$) and 30.0 ng/ml per h ± 2.3 ($P < 0.001$). Thus, acute but not chronic Ca^{++} administration suppresses PRA. Acute $CaCl_2$ infusion may inhibit renin release by increasing the Na^+ load to the macula densa.

159. Discordant Responses of Human Thyrotropin (TSH) and Its Free Alpha and Beta Subunits to Thyrotropin-Releasing Hormone (TRH) and Thyroid Hormone. I. A. KOURIDES,* B. D. WEINTRAUB,* E. C. RIDGWAY,* AND F. MALOOF,** Boston, Mass., and Bethesda, Md.

We have studied serum concentrations of TSH, TSH-β, and the common alpha subunit of the glycoprotein hormones utilizing specific radioimmunoassays. TSH-β was not detectable (<0.5 ng/ml) in 28 normals before or after TRH; alpha was <0.5–2 ng/ml in 21 of them and 1–5 ng/ml in the 7 postmenopausal women and did not change after TRH. In 14 hypothyroid patients given TRH, mean TSH-β increased from 1.8 ng/ml to a peak of 3.9 ($P < 0.001$); in five, alpha increased from 2.4 ng/ml to a peak of 3.8 ($P < 0.05$) and in the nine postmenopausal women from 4.9 ng/ml to 7.3 ($P < 0.02$). In nine hypothyroid women treated with small doses of T_4 (25 or 50 μg four times daily) or T_3 (12.5–25 μg four times daily), there was a decrease in mean basal alpha (4.8 ng/ml to 3.4, $P < 0.05$) and peak alpha (7.5 ng/ml to 5.6, $P < 0.005$) after TRH, as well as a decrease in basal and peak TSH; however, neither basal nor peak TSH-β changed at these doses. At higher doses of T_4 (100–300 μg four times daily), basal and peak TSH-β after TRH decreased and then suppressed to undetectable levels (<0.5 ng/ml), as did basal and peak TSH (< 0.5 μU/ml). Although alpha response to TRH was inhibited, alpha remained detectable and was somewhat higher in postmenopausal women. In three hypothyroid patients given an intravenous bolus of labeled TSH, there was no dissociation into subunits for at least 3 h, indicating that the increment in serum subunits after TRH represented secretion from the pituitary. Free alpha and TSH-β, in addition to TSH, are secreted in hypothyroidism and demonstrate discordant responses to TRH and thyroid hormone. TSH-β, the subunit unique to TSH, appears to be fully responsive to the same positive and negative feedback as TSH, whereas the common alpha subunit appears less responsive. Since the alpha component unresponsive to thyroid hormone is increased in postmenopausal women, this portion may reflect alpha subunits related to gonadotropin biosynthesis. (Research supported by Am. Phil. Soc. 1–9 C.S. 36, and USPHS CA 12749 and AM 05195.)

160. A Comparison of the Effects of Adrenocorticotropin (ACTH) and Cholera Toxin on Adrenal Steroid and Cyclic Adenosine Monophosphate (cAMP) Synthesis. JEROME KOWAL, TOSHIKAZU SAITO,* AND S. SRINIVASAN,* Cleveland, Ohio.

Cholera toxin (CT) can stimulate adenyl cyclase activity in many hormone-responsive tissues. In monolayer cultures of mouse adrenal cortex tumor cells, it irreversibly stimulates cyclic-AMP and steroid biosynthesis in a manner different from that of adrenocorticotropin (ACTH). ACTH (100 μU/ml) produces a maximal rate of steroidogenesis detectable within several minutes, associated with rapid increases in intracellular cAMP levels and extracellular release of cAMP. cAMP is increased further at higher concentrations of ACTH. CT-induced increases in steroidogenesis are preceded by a concentration-dependent lag ranging from 30 min at 1 μg/ml CT to 2 h at 100 pg/ml. In ACTH-stimulated cells, intracellular cAMP peaks at 20 min and declines to basal levels; in CT-stimulated cells, cAMP is not increased during the lag, increases slowly, and remains persistently elevated. The increase in cAMP levels is unaltered by exposure to cycloheximide during the lag period. In ACTH-stimulated cells, maintenance of maximal steroidogenesis during longer incubations requires higher ACTH concentrations (e.g., 1 mU/ml for 2 h; 100 mU/ml for 21 h). The maximal rate of steroidogenesis ultimately declines. In contrast, after the lag, CT induces steady-state rates of steroidogenesis varying with CT concentration for at least 21 h. Detection of responses to CT concentrations less than 100 pg/ml is facilitated by incubating for longer periods. ACTH and CT introduced into the same culture have additive effects on cAMP metabolism. CT activity is inhibited by prior incubation with approximately equimolar concentrations of GM_1-ganglioside, whereas ACTH activity is unaffected. ACTH and CT appear to interact with different membrane receptors with resultant differences in the sequence of molecular events leading to increased cAMP synthesis and steroidogenesis. (Research supported by a grant from the NIH.)

161. Thiazides, Potent Inhibitors of Distal Tubular Chloride Reabsorption. ROBERT KUNAU, JR.,* Minneapolis, Minn. (introduced by Louis Tobian, Jr.**).

Thiazide diuretics are potent chloruretic agents. Although they inhibit carbonic anhydrase, it is unlikely that this effect is related to its ability to induce a chloruresis, since inhibition of renal carbonic anhydrase decreases chloride reabsorption in the proximal convolution without enhancing chloride excretion. To identify the characteristics of the chloruresis of chlorothiazide and the changes in chloride reabsorption after chlorothiazide administration which may be attributable to carbonic anhydrase inhibition, the following groups were studied. The first group of rats (I) received the carbonic anhydrase inhibitor, benzolamide (2 mg/kg). The second group of rats (II) received chlorothiazide (15 mg/kg). Both groups were studied with the recollection micropuncture technique, the drugs being administered to hydropenic rats after a control period. The percent of the filtered chloride load present, before and after the two drugs (before/after), (*a*) at the end of the proximal convolution, (*b*) early and (*c*) late distal convoluted tubule, and (*d*) in the urine, is listed in sequence. Group I (*a*), 52.5%/70.0%, $P < 0.001$; (*b*), 4.7%/6.6%, $P < 0.05$; (*c*), 1.2%/1.4%, $P > 0.2$; (*d*), 0.29%/0.27%, $P > 0.7$. Group II (*a*), 55.4%/67.6%, $P < 0.001$; (*b*), 5.8%/7.7%, $P < 0.1 > 0.05$; (*c*), 1.2%/6.6%, $P < 0.001$; (*d*), 0.37%/3.5%, $P < 0.001$. Chlorothiazide, like benzolamide, significantly decreased chloride reabsorption in the proximal convolution, presumably related to its ability to inhibit carbonic anhydrase. The ability of chlorothiazide to induce a chloruresis was not primarily related to the enhanced chloride delivery from the proximal convolution but rather due, at least in part, to marked inhibition of chloride reabsorption in the distal tubule. (Supported by USPHS Grant HL-13821.)

162. Angiotensin-Converting Enzyme from Rat Lung: Subunit Structure and a Possible Second Converting Enzyme. JOSEPH J. LANZILLO* AND BARRY L. FANBURG, Boston, Mass.

Angiotensin I is converted to vasoactive angiotensin II in the lung by enzymatic hydrolysis of the C-terminal dipeptide, histidyl leucine. Angiotensin-converting enzyme (CE) was isolated from subcellular particles obtained by centrifugation of rat lung homogenate at 775–54,000 *g*. The pellet was extracted with phosphate buffer, pH 8.3, dialyzed against acetate buffer, pH 4.9, redialyzed against phosphate, and chromatographed on DEAE-cellulose and Sephadex G-200. Two enzymatically active fractions were obtained from the Sephadex column, a major peak with a mol wt of ~ 270,000 and a minor peak with a mol wt of ~ 100,000. Both fractions were homogeneous by electrophoresis on 8% polyacrylamide gels, but the 270,000 fraction separated into two bands on 10% gels or on 4–24% gradient gels. These two bands had molecular weights of 180,000 and 120,000 on 5% SDS-polyacrylamide gels. Only the 180,000 mol wt band was enzymatically active. The 100,000 Sephadex G-200 fraction migrated on SDS-polyacrylamide as a single band of mol wt 100,000 and could not be produced from the 270,000 mol wt fraction with sulfhydryl reducing agents, urea, high ionic strength media, or detergent. Both the 270,00 and 100,000 fractions showed an absolute requirement of Cl^- for CE activity; both were inhibited by EDTA, angiotensin II, and bradykinin potentiating factor nonapeptide (BPP_{9a}). Rabbit serum antibody prepared against the 270,000 mol wt fraction inhibited activity of both the 270,000 and 100,000 mol wt fractions. We conclude that either two very similar angiotensin CE's exist in rat lung or that the 100,000 mol wt fraction is enzymatically cleaved from the 270,000 one during preparation. The relationship between the 120,000 and 180,000 mol wt subunits of the 270,000 fraction remains uncertain. (Supported by NIH Grant HL 14,456.)

163. Alterations in the Enterohepatic Circulation of Bile Acids Indicated by Postprandial Serum Bile Acid Concentrations. NICHOLAS F. LARUSSO,* MELVYN G. KORMAN,* NEVILLE E. HOFFMAN,* AND ALAN F. HOFMANN, Rochester, Minn.

We developed a radioimmunoassay for serum bile acids (SBA) with greater sensitivity than conventional chemical techniques. Using this assay, we observed a significant postprandial elevation of SBA in healthy subjects. To extend this observation, we measured SBA after an overnight fast (base line) and frequently during 24 h in 19 patients ingesting three equicaloric liquid meals. Eight healthy subjects, five patients after cholecystectomy, and six patients with documented severe bile acid malabsorption after ileal resection were studied. All had normal liver function tests. A distinct pattern of SBA concentrations was observed in each group. In healthy subjects, a large peak occurred 2 h after each meal and levels returned to base line by 4 h; this pattern was related to meals because levels remained at base line in four subjects undergoing a 22 h fast. In cholecystectomized patients, SBA

rose to lower levels after the first meal ($P < 0.01$) and then remained elevated throughout the day, gradually returning to base line approximately 10 h after the evening meal. In patients with bile acid malabsorption, the postprandial rise was small compared to that of healthy subjects ($P < 0.01$) after the first meal, barely detectable after the midday meal, and absent after the evening meal. We conclude that these different patterns of SBA reflect the presence or absence of both normal gallbladder contraction and efficient ileal absorption of bile acids. Our results employing a radioimmunoassay indicate that SBA determinations, previously limited solely to detecting impaired hepatic function, can now be used to investigate the enterohepatic circulation of bile acids. Also, postprandial SBA concentrations may provide a simple method for detecting bile acid malabsorption and abnormal gallbladder contraction. (Research supported by NIH Grant AM 16770.)

164. Selective Depletion of Brain Dopamine (DA) After Experimental Strokes. MICHAEL LAVYNE,* MICHAEL MOSKOWITZ,* FRANCES LARIN,* NICHOLAS ZERVAS,* AND RICHARD WURTMAN, Cambridge, Mass.

Experimental ligation of a middle cerebral artery in monkeys or a common carotid artery in gerbils causes a rapid, major, and selective depletion of ipsilateral brain dopamine (DA); DA levels fall within 24 h to 43% of control values (contralateral hemisphere or sham-operated animals). Brain norepinephrine does not decline significantly. Major depletions are observed in all three brain regions receiving dopaminergic inputs, i.e., the basal ganglia, hypothalamus, and limbic projections. Lesioned animals exhibit ipsilateral turning behavior similar to that observed after transection of the nigro-neostriatal tract; the turning pattern is correlated with the extent of DA depletion. In gerbils lesioned after intraventricular administration of [^{3}H]DA, it was found that the depletion largely reflected loss of unchanged DA from neuronal stores. We postulate that (*a*) the loss of large amounts of DA from ischemic neurons may amplify the local tissue damage that follows ischemic cerebrovascular accidents; and (*b*) the selective loss of dopaminergic neurons may account for some of the findings observed in patients who survive strokes. These postulated acute and chronic changes may be amenable to pharmacologic therapies.

165. Beta Adrenergic "Isoreceptors" and Adenylate Cyclase. ROBERT J. LEFKOWITZ,* Durham, N. C. (introduced by A. Wallace).

Closely related though distinct forms of enzymes in different tissues are called isoenzymes. Similarly, two types of beta adrenergic receptors (BAR) designated β_1 (cardiac contractility, lipolysis) and β_2 (glycogenolysis in skeletal muscle and liver, relaxation of smooth muscle) have been delineated by physiological experiments. Isoproterenol is a potent agonist and propranolol a potent antagonist at all BAR. β_1 and β_2 (BAR) are distinguished by (*a*) greater effectiveness of norepinephrine at β_1 vs. β_2; (*b*) selective agonists, e.g., soterenol (β_2); and (*c*) selective antagonists, e.g., butoxamine (β_2) and practolol (β_1). Since many beta adrenergic responses may be mediated by activation of adenylate cyclase (AC), we characterized AC-coupled BAR in membranes from five canine and rat tissues; myocardium, paraovarian fat, diaphragmatic skeletal muscle, liver, and lung. AC was measured by following conversion of [^{32}P]ATP to [^{32}P]cAMP. Ability of four agonists (isoproterenol, norepinephrine, soterenol, and dobutamine) to stimulate AC and of four antagonists (propranolol, DCI, butoxamine, and practolol) to block stimulation by isoproterenol were studied. Enzyme stimulation in heart and adipose membranes was mediated by a β_1 receptor: isoproterenol was only four times more potent than norepinephrine; soterenol was very weak; practolol was 5–15 times more potent than butoxamine. Conversely, AC-coupled BAR in skeletal muscle, liver, and lung were β_2: isoproterenol was 75–100 times more potent than norepinephrine and soterenol was very potent (1/3–1/4 isoproterenol); butoxamine was 10 times more potent than practolol as an antagonist. In all tissues, propranolol and DCI were the most potent antagonists and dobutamine was a weak agonist. The existence of two pharmacologically distinct AC-coupled β-adrenergic "isoreceptors" which parallel the specificity of physiological adrenergic responses, strongly supports the contention that AC is the mediator of β-adrenergic effects.

166. The Fungicidal Proteins of Mammalian Neutrophils. ROBERT I. LEHRER, KATHRYN I. MITCHELL,* AND RANDALL B. HAKE,* San Francisco, Calif.

Two general microbicidal mechanisms exist in mammalian neutrophils. One involves myeloperoxidase and H_2O_2. The other, most clearly demonstrated in rabbit and guinea pig heterophils, is attributable to the effects of lysosomal cationic proteins. We compared the fungicidal components of neutrophils from humans, guinea pigs, and rabbits. Human neutrophils were purified from normal blood by the method of Böyum. Rabbit and guinea pig heterophils were obtained from sterile peritoneal exudates. Purified granulocytes were homogenized in 0.34 M sucrose, and a lysosome-rich fraction was prepared by centrifugation at 27,000 *g*. In each species tested, this fraction contained components that killed *C. parapsilosis*. To identify the active principles, fractions were extracted with 0.01 M citric acid and subjected to micropreparative polyacrylamide electrophoresis. We confirmed the presence of a group of highly cationic proteins in the granules of rabbit and guinea pig heterophils. They were present in high relative concentration, stained metachromatically with eosin, lacked esterase activity, and most killed *C. parapsilosis* in vitro. They constituted the predominant fungicidal proteins of these cells. Human neutrophil granules possessed a family of cationic proteins whose mobility approximated that of lysozyme. These did not stain metachromatically with eosin, could hydrolyze the ester bond of naphthol ASD acetate, and were absent in rodent leukocytes and human mononuclear phagocytes. At pH 5.0, in a concentration of 10–20 μg/ml, a microgram of these proteins killed 10^4 yeast cells. Human granules contained additional components with fungicidal activity. Our studies establish the nature of a second fungicidal mechanism in human neutrophils and demonstrate important differences between the granulocytes of man and of two species widely used in experimentation. (Research supported by grants from NIH and the University of California.)

167. Detection of Hypokinesis by a Quantitative Analysis of Left Ventricular Cineangiograms. RICHARD F. LEIGHTON,* SHARON M. WILT, AND RICHARD P. LEWIS,* Columbus, Ohio (introduced by Charles A. Doan**).

The detection of hypokinetic segments is an important part of the analysis of left ventricular cineangiograms in patients with heart disease. A quantitative method (QNM) is proposed for detecting hypokinetic segments. It involves superimposition of left ventricular silhouettes traced from cineangiograms taken in the 30° right anterior oblique position at the onset and end of ejection. The method corrects for (*a*) thoracic cage or diaphragmatic motion, (*b*) movement of the aortic valve toward the apex, and (*c*) rotation of the apex. Values for the percent of systolic motion of seven endocardial segments have been established in 20 patients with normal left ventricular

function. Use of the QNM has been compared to a qualitative method (QLM) of inspecting cineangiograms, resulting in differences in definition of hypokinetic segments in 13 of 16 patients with coronary heart disease. Most differences were found in the apical segments of both anterior and inferior walls. When the QNM was used, only one hypokinetic segment was found which did not correspond to an obstructive coronary lesion while six such hypokinetic segments were defined using the QLM. In four patients segments thought to be hypokinetic (QLM) appeared to be akinetic (QNM). In six patients with cardiomyopathy, thought to have diffusely hypokinetic segments (QLM), all seven left ventricular segments were hypokinetic in only three patients (QNM). The use of a QNM appears to be essential to the proper interpretation of left ventricular wall motion and particularly to the detection of hypokinetic segments. (This project was supported in part by NIH Training Grant 5 TO1 HL05968 from the National Heart and Lung Institute.)

168. Antiviral Synergy by 9-β-D-Arabinofuranosyladenine (ara-A) and Human Interferon in Combination Versus Herpes Simplex Virus, Type 1 (HSV-1). A. MARTIN LERNER** AND ELIZABETH J. BAILEY,* Detroit, Mich.

Methods have been developed to specifically measure minimal inhibitory concentrations (MIC's) of antiviral substances, and, after administrations to patients, their concentrations in body fluids. Knowledge of these pharmacokinetics must precede formulations of adequate controlled clinical trials which are necessary for proof of efficacy of any antiviral substance in man. MIC's for the Ket. strain of HSV-1, isolated from the brain of a fatal case of encephalitis, versus ara-A, 9-β-D-arabinofuranosylhypoxanthine (ara-Hx), 5-iodo-2'-deoxyuridine (IDU), and human leukocyte-derived interferon (IF) were: 22.7, 34.1, 1.1, and 181.8 μg/ml, respectively. Measures of levels in body fluids of man for each of the drugs have shown that therapeutic and toxic doses approach unity, severely limiting application. In an effort to increase potential antiviral activity without augmenting toxicity, combinations of two antiviral agents versus HSV-1 in the same in vitro assay were done. We define "antiviral addition" as 50% reduction in plaque-forming units (pfu) of HSV-1 by fractions of MIC's of antiviral drugs totaling greater than ½ MIC, but less than 1 MIC. On the other hand, "synergy" is present if a similar decrease in pfu is produced by combinations of drugs totaling ½ MIC or less. Ara-A and IDU are not additive. Ara-A and ara-Hx are additive [½ MIC, ara-A + ¼ MIC, ara-Hx = 1 MIC]. Ara-a + IF are truly synergistic [¼ MIC, ara-A + ⅛ MIC, IF = 1 MIC]. These data require that ara-A and IF be given in combination to man in controlled clinical trials with patients afflicted by life-threatening infections with HSV-1. (Research supported by a research grant from the NIH, a training grant from the NIH, and a grant for general support in infectious diseases from the Skillman Foundation.)

169. Pederine, a New Method of Cell Fusion. MAURA R. LEVINE,* JOSEPH DANCIS,* MARIO PAVAN,* AND RODY P. COX,** New York.

Studies of cellular differentiation, analysis of human linkage groups, assignment of genes to specific chromosomes, and investigation of structural gene regulation are now possible through cell hybridization. The prerequisite in formation of interspecific cell hybrids involves fusion of two or more cell membranes, a relatively rare, spontaneous event in tissue culture. The resultant heterokaryons may undergo nuclear fusion, forming a cell hybrid. Unfortunately, presently available techniques (Sendai virus or lysolecithin) used for experimental induction of cell fusion in culture are cumbersome and the results are erratic. We have found that pederine, a naturally occurring crystalline product obtained from beetles, will reproducibly produce 40–60% fused cells in cultured human diploid skin fibroblasts. Pederine, a known inhibitor of protein synthesis in eucaryotic organisms, is apparently nontoxic when used under appropriate conditions. By varying the concentration of pederine from 0.1 to 8.0 ng/ml while maintaining a constant incubation period for 24 h, we found the effective dose ranged from 0.5 to 2.0 ng/ml for maximum fusion of 40–60%. Higher concentrations were toxic under the conditions of the experiment and caused detachment of cells from the monolayer. Kinetic studies of fusion indicated that incubation with pederine for 12–24 h was required to achieve a maximum effect. After pederine removal from cultures, the unfused cells are apparently unaffected as they proliferated normally. Pederine produces cell fusion at concentrations 1,000-fold less than other chemical agents presently used. Pederine should prove useful in research into mechanisms of membrane fusion as well as research in which cell fusion is used as an investigative tool. (Supported by NIH.)

170. Source of Gastric Juice Cyclic Adenosine Monophosphate (cAMP) in Pernicious Anemia. ROBERT A. LEVINE,* Syracuse, N.Y. (introduced by William J. Williams**).

Histamine increases adenylate cyclase in mammalian fundic but not antral mucosa (1973. *Am. J. Physiol.* **225**: 1359). This laboratory demonstrated that histamine and betazole increased cAMP production in human gastric juice. In order to evaluate the source of extracellular cAMP and the influence of acid and endogenous gastrin on its activity, studies were performed in six patients with documented pernicious anemia (PA), hypergastrinemia (580 ± 94 pg/ml, mean ± SE), and histologically proven fundic mucosal atrophy; in nine patients with peptic ulcer (PU) and normal serum gastrin (170 ± 21 pg/ml); and in one patient with Zollinger-Ellison disease (ZE) and hypergastrinemia (1,540 pg/ml). Fasting gastric samples collected every 15 min before and after standard maximal betazole stimulation (1.5 mg/kg) were analyzed for volume, pH, titratable acidity, sodium, and radioimmunoassayable cAMP. Sodium output was considered an index of nonparietal or interstitial fluid entering the stomach lumen. Basal cAMP (pmoles/ml) and sodium (mEq/liter) concentrations were, respectively, similar in PA (41 ± 12, 66 ± 11), PU (46 ± 5, 68 ± 9), and ZE (44,69) and failed to correlate with gastric pH or serum, gastrin level. After betazole stimulation cAMP and sodium *concentrations* remained unaltered in PA but decreased, respectively, ½ and 3-fold in both PU and ZE ($P < 0.05$). Concomitantly there was a 3- and 7-fold rise, respectively, in cAMP and acid *outputs* ($P < 0.001$), proportional to the increased volume. cAMP was also found in gastric juice from canine antral pouches (34 ± 12 pmol/ml). The presence of cAMP in unstimulated gastric juice in both PA and antral pouches suggests a nonparietal origin, probably derived from nonparietal secretions or transmucosal exudation. The data indicate that cAMP is not modulated by intraluminal pH or circulating gastrin. Secretagogue-induced cAMP production appears to be proportional to parietal volume flow.

171. The Effect of Vasopressin on the Movement of Drugs and Uric Acid. SHERMAN D. LEVINE,* NICHOLAS FRANKI,* AND RICHARD M. HAYS,** New York.

In addition to accelerating water flow, vasopressin increases the permeability of the collecting duct and toad bladder to urea and other amides. Recent studies indicate that the amides cross the toad bladder by facilitated diffusion, exhibiting satura-

tion kinetics, mutual inhibition between amides, and inhibition by phloretin. We wish to report an effect of vasopressin on the imides, a class of compounds including uric acid and many commonly used drugs. Drugs possessing an amide structure were studied as well. Toad bladder sacs were tied to glass bungs, minimizing tracer leakage across the bladder edge. With this technique, isotopically labeled imides (uric acid, phenobarbital, glutethimide, fluorouracil, and diphenylhydantoin) showed an approximately twofold increase in permeability after vasopressin, compared to paired controls without hormone. Amide-containing drugs (tetracycline, chloramphenicol, and INH) were similarly accelerated by vasopressin. While the imide structure (a nitrogen adjacent to a carbonyl in the ring) confers a close chemical relationship to the amides, the imides do not appear to utilize the amide pathway; they are not blocked by phloretin or competing amides. They therefore move by simple diffusion, or a transport pathway not yet characterized. We would conclude that (*a*) the movement of uric acid and many drugs is accelerated by vasopressin in the toad bladder, and, by analogy, in the collecting duct; (*b*) this may be a factor of importance in problems such as drug overdose and delivery of antibiotics to the renal medulla, where the patient's state of hydration may affect reabsorption; and (*c*) the effect of vasopressin extends to a broader range of molecules than has heretofore been recognized. (Research supported by grants from NIH.)

172. Membrane Changes During Induction of Erythroid Differentiation of Friend Virus-Infected Cells. J. LEVY, D. SINGER, R. A. RIFKIND, AND P. A. MARKS,** New York.

Induction of Friend virus-transformed mouse cells (FVC) to differentiate to erythroid cells is a model for studying mechanisms of cell differentiation. We report on the relationship of membrane changes to induction of hemoglobin synthesis by FVC. Less than 1% of FVC in culture differentiate spontaneously to erythroid cells. Addition of 2% dimethyl sulfoxide (DMSO) to FVC induces over 70% of the cells into erythroid differentiation. Membrane alterations, manifest as a decrease in concanavalin A (Con A) agglutinability occur as early antecedents of detectable hemoglobin synthesis. FVC grown in culture with 2% DMSO beginning hemoglobinization (benzidine reaction or ^{14}C incorporation into purified globin) by 72–96 h and maximum induction (> 70% of the cells contain hemoglobin) by 120–144 h. Loss of Con A agglutinability is related in time to the irreversible commitment to erythroid cell differentiation. FVC grown with 2% DMSO for 24 h lose Con A agglutinability. Such cells transferred to fresh medium without DMSO will synthesize hemoglobin at 72–96 h. By contrast, cells transferred to fresh medium with DMSO before Con A agglutinability is lost show no hemoglobinization even after 144 h of culture. Thus, DMSO induction of FVC to differentiate involves an alteration in cell membrane as an antecedent of hemoglobin synthesis. Change in cell membrane (Con A receptor sites) appears to be an early event in the induction of differentiation.

173. Neural Regulation of Circulatory and Respiratory Responses to Tissue Hypermetabolism. CHANG-SENG LIANG* AND WILLIAM B. HOOD, JR., Boston, Mass.

Both cardiac output and pulmonary ventilation increase during muscular exercise and after administration of 2,4-dinitrophenol (DNP), presumably because of increased tissue metabolism and oxygen demand (1973. *J. Clin. Invest.* **52**: 2283). These effects may be produced either by stimulation of metabolism-sensitive receptors in hypermetabolic tissue or by release of humoral stimuli into the general circulation from such tissue. These experiments were designed to explore the first of these hypotheses. Cross-circulation techniques utilizing femoral-to-femoral or femoral-to-aorta anastomoses were employed to perfuse either a hindlimb or the lower half-body of a chloralose-anesthetized dog from a second dog. The perfused area of the first dog (neural dog) was separated completely from its parent body except for nerve connections (i.e., femoral and sciatic nerves in hindlimb and spinal cord in lower half-body perfusion). Sufficient DNP was infused into the arterial side of the perfusion circuit to triple oxygen consumption and to increase lactate production in the perfusion region. Cardiac output and mean systemic arterial pressure increased in the neural dog after DNP infusion; neither heart rate nor pulmonary wedge pressure changed significantly. Minute ventilation and arterial pH also increased, while arterial P_{CO_2} decreased. The increases in cardiac output and minute ventilation correlated significantly with the decrease in oxygen saturation and with the increases in lactate concentration and lactate/pyruvate ratio in the venous efflux from the perfused area. Oxygen consumption of the neural dog increased slightly, probably because of increased cardiac and ventilatory work. All of these changes in the neural dog were reversed when the nerve connections between the perfused area and its parent body were severed. Unlike DNP, normal saline similarly administered increased neither ventilation nor cardiac output. These findings indicate that there are receptors sensitive to metabolic changes in tissues and that neural transmission is an important afferent link in regulating the cardiopulmonary responses to increased tissue metabolism. (Research supported by Massachusetts Heart Association Grant 1192 and NIH Grants HL 14646 and HE 07299.)

174. Alcoholic Fatty Liver As a Precursor of Hepatitis and Cirrhosis. CHARLES S. LIEBER, LAWRENCE FEINMAN,* LEONORE M. DECARLI,* AND EMANUEL RUBIN,* New York.

Alcoholics develop fatty liver, hepatitis, and cirrhosis, but the relationship of fatty liver to the later stages is controversial. This question was studied in 32 baboons pair-fed up to 4 yr with adequate diets containing 18% of total calories as protein and 36–50% either as ethanol or isocaloric carbohydrate. All animals maintained body weight. Liver morphology in the 16 controls was normal, whereas sequential surgical biopsies revealed steatosis in all animals given 36% calories as ethanol. When ethanol was increased to 50% by its incorporation in a liquid diet, nine baboons again had a simple fatty liver, with an average triglyceride of 44.0 ± 5.84 mg/g vs. 14.4 ± 2.2 in the corresponding controls ($P < 0.01$). Five animals developed in addition to the steatosis alcoholic hepatitis characterized by inflammation, central sclerosis, and hyaline bodies of Mallory. Two animals treated for 4 yr had cirrhosis. Thus hepatitis and cirrhosis developed despite an adequate diet, and these lesions were associated with an increase in liver triglycerides (147 ± 34.8 mg/g vs. 11.7 ± 1.8 in controls; $P < 0.01$) much more severe than the rise observed in connection with a simple fatty liver (44.0 ± 5.84; $P < 0.01$). These results suggest that the degree of steatosis may reflect the severity of liver injury. Moreover, this marked steatosis occurred before the development of the cirrhosis and already at the fatty liver state, collagen content was significantly increased with enhanced activity of peptidylproline hydroxylase, a key enzyme involved in the initial steps of collagen formation. These findings support the concept that alcoholic fatty liver is a precursor of more severe liver lesions and that the intensity of steatosis reflects the degree of metabolic injury which can culminate in hepatitis and cirrhosis. (Supported by Veterans Administration, NIAAA, and NIAMDD.)

175. An Intrarenal Effect of Blood pH on the Release of Renin. MEYER D. LIFSCHITZ* AND LAURENCE E. EARLEY, San Antonio, Tex.

Since increases in plasma potassium may suppress renin secretion by the kidney, the possibility was considered that changes in extracellular pH also could affect the release of renin, possibly as a consequence of the reciprocal relationship between intracellular H^+ and potassium. Accordingly, renin secretory rate (RSR) was measured before, during, and after the infusion of HCl into one renal artery of nine anesthetized dogs. During HCl infusion the pH of renal venous blood decreased to 7.28 ± 0.10 ($P < 0.01$) compared to values of 7.38 ± 0.09 and 7.36 ± 0.09 before and after infusion. RSR during control averaged 320 ± 107 U (ng angiotensin I per min) and decreased on the average of 50 ± 21% ($P < 0.05$) during infusion of HCl. After infusion of HCl RSR returned to 282 ± 100 U, a value not significantly different from the initial control. There was no change in RSR in contralateral kidneys. There were no consistent changes in glomerular filtration rate, renal plasma flow, arterial pressure, or renal vascular resistance. Potassium concentration in renal venous plasma did not change significantly during infusion of HCl (2.7 ± 0.3 to 2.8 ± 0.4, meq/liter). Ipsilateral sodium excretion increased from 93 ± 20 to 139 ± 28 μeq/min during infusion of HCl ($P < 0.05$), but remained at 134 ± 46 μeq/min after HCl. These studies indicate that a small increase in extracellular H^+ can result in an immediate suppression of renin secretion through intrarenal pathways. This effect was not accompanied by changes in plasma potassium but was associated with increased excretion of sodium. The latter could reflect a change in the concentration or load of sodium at the macula densa. (Supported by a grant from the NIH.)

176. Zinc Binding in Serum and Urine of Cirrhotic, Nephrotic, and Normal Subjects. ROBERT D. LINDEMAN* AND DONALD J. BAXTER,* Oklahoma City, Okla. (introduced by Solomon Papper**).

Serum zinc levels are decreased and urinary zinc excretions are increased in cirrhotic and nephrotic patients compared with normal subjects. This study was designed to investigate differences in these patient populations by observing patterns of binding of added zinc^{65} using column chromatography and polyacrylamide-gel electrophoresis. The mean serum zinc concentrations in seven cirrhotic, ten nephrotic, and five normal subjects studied were 54, 71, and 98 μg/100 cm^3, respectively. The mean urinary zinc excretions were 2033, 1033, and 610 μg/24 h. Using Sephadex G-200 gel column chromatography, most of the radiozinc added to serum was found in the albumin peak in normal subjects (78%). The peak concentrations of ^{65}Zn appeared in a slightly lower molecular weight fraction than did the peak concentrations of albumin. The percent of total radiozinc found in this peak was lower and more variable in nephrotic (mean 38%) and cirrhotic (mean 66%) patients. Most of the remaining ^{65}Zn was found over a wide range of lower molecular weight fractions. In normal and cirrhotic patients, essentially all urinary zinc was found in low molecular weight (< 1,000) fractions. In nephrotics, 9% was found in the albumin fraction, the remainder in low molecular weight fractions. Using polyacrylamide-gel electrophoresis, the highest zinc^{65} concentrations in serum and urine migrated with the prealbumin (mol wt 63,000) fraction explaining the failure of the zinc-65 and albumin peaks to correspond identically. The low serum zinc concentrations in cirrhotic and nephrotic patients compared to normal subjects appear to be explained, at least in part, by differences in the binding characteristics of zinc.

177. New Rapid Measurement of Cholesterol Synthesis in Man by Isotope Kinetics of Squalene. GEORGE C. K. LIU,* PAUL H. SCHREIBMAN,* PAUL SAMUEL,* AND E. H. AHRENS, JR.,** New York. (introduced by Robert H. Palmer).

We have developed a new method for measurement of daily cholesterol synthesis in man that has distinct advantages over current kinetic and balance methods. This out-patient procedure does not require controlled diets, stool collections, or the metabolic steady state; a 1 day infusion period at the hospital is followed by blood sampling (5 ml) at the third, fourth, and fifth weeks. Synthesis is measured irrespective of absorption and flux; results are available in less than 6 wk; the test can be repeated at frequent intervals to evaluate different regimens. We administer a single dose of [^{14}C]-mevalonic acid (MVA) and [^{3}H]cholesterol (CH) intravenously, then measure [^{14}C]squalene (SQE), [^{14}C]CH and [^{3}H]CH plasma specific activities (SA). SA curves of [^{14}C]CH and ^{3}H-CH (expressed as percent dose per gram CH) determine the percent conversion of [^{14}C]MVA to [^{14}C]CH, and thus the dose of [^{14}C]SQE. Multiplication of the reciprocal of the area under the [^{14}C]SQE SA curve by the dose of [^{14}C]SQE determines the CH synthesis rate. Three basic assumptions are made: (*a*) SQE is an obligatory precursor of CH; (*b*) input of biosynthesized SQE equals CH synthesis; and (*c*) equilibrium between plasma SQE and metabolically active SQE in tissues is rapid. [^{14}C]SQE reached its peak in plasma in 90 min; decay was first order, $t_{½max}$ 3.13–3.57 h; total exchangeable mass of SQE was 236–323 mg. [^{14}C]CH reached its peak in 5½ h, but [^{14}C]CH and [^{3}H]CH decay curves did not become parallel until 2 wk. In four studies of three patients, CH synthesis rates based on SQE-CH (1.0–1.5 g/day) compared favorably to those obtained simultaneously by balance methods. (Research supported by Grants HL-06222 and FR-00102 from NIH.)

178. Natriuresis Due to Chloride Depletion. R. G. LUKE,* B. T. KHANH,* R. D. SCHMIDT,* AND J. H. GALLA,* Lexington, Ky. (introduced by William S. Jordan**).

250- to 300-g rats were fed a low salt diet for 10 days, and peritoneal dialysis (PD) was performed in two groups; control (CON) against NaCl 150 + $KHCO_3$ 4 meq/liter, and chloride depletion (CLD) against $NaHCO_3$ 150 + $KHCO_3$ 4 meq/liter. DOCA and ADH were given and volume was restored by plasma (1 ml per 1% change in Hct after PD; CON were given 3.4 ± 1 ml [mean ± SEM] and CLD 3.8 ± 1 ml). PNa was then 135 ± 1.3 in CON and 134 ± 1.8 meq/liter in CLD, PCl 106 ± 1 and 86 ± 2 meq/liter ($P < 0.001$). Na balance during PD was (CON) + 198 ± 52 and (CLD) − 40 ± 78 μeq ($P < 0.01$). 5% mannitol was infused at 5.8 ml/h for 1 h and at 11.6 ml for a second hour and two 30 min urine collections made at each rate. GFR was measured by [^{14}C]inulin. Significant natriuresis (both absolute and as FENa%) was seen in all collection periods without any difference in GFR, osmolar excretion, or urinary pH. End PNa was (CON) 115 ± 3 and (CLD) 115 ± 2 meq/liter and end hematocrit 38 ± 3 and 37 ± 2%. In other studies in unanesthetized rats significant natriuresis was also seen for 24 h after PD in CLD as compared to CON. In both studies U_{max} was lower in CLD. Thus natriuresis was unexplained by osmotic diuresis, sodium balance, GFR, volume expansion, bicarbonate diuresis, or variation in mineralocorticoid hormones. It is postulated that chloride depletion imposes limitations on sodium transport in the loop of Henle such that the distal nephron is unable to conserve sodium appropriately. (Research supported by Grant AM 13859 from NIH.)

179. Colon Cancer: Diagnostic and Prognostic Use of Combined Immunological Testing. BENJAMIN B. LURIE,* DAVID M. BULL,* NORMAN ZAMCHECK,* ANTONY M. STEWARD,* AND RICHARD A. HELMS,* Boston, Mass. (introduced by Charles S. Davidson**).

Current methods for assessing human tumor immunity correlate imperfectly with clinical status. To determine whether combined studies would increase diagnostic and prognostic accuracy, we simultaneously measured circulating carcinoembryonic antigen (CEA), tumor antigen-induced inhibition of mononuclear cell migration (IMM), and cutaneous reactivity to the recall antigens PPD, streptokinase-streptodornase, and mumps in 16 colon cancer patients before and after surgery. The preoperative immunological profile was correlated with the surgical and histopathological findings and compared with age- and sex-matched controls. Preoperatively, 10 of 14 cancer patients studied had elevated CEA levels (> 2.5 ng/ml); each of 12 patients tested showed > 20% tumor antigen-induced IMM; and only 1 of 11 reacted preoperatively to two or more recall skin test antigens. Among controls, none showed elevated CEA or tumor antigen-induced IMM and 20 of 24 reacted strongly to two or more recall skin test antigens. Patients were divided into two groups based on surgical and histopathological findings: seven potentially cured and nine with disseminated cancer. Potential surgical cure (average follow-up 13 months) was accompanied by normal CEA in four of the seven, negative IMM in seven of seven, and increased cutaneous reactivity to recall antigens in six of seven. Disseminated cancer, in contrast, was associated with elevated CEA in nine of nine, negative IMM in nine of nine, and absent cutaneous responses to recall antigens in six of nine. Negative IMM and depressed cutaneous reactivity to recall antigens suggest broad impairment of cell-mediated immunity in disseminated cancer. The combined use of three immunological assessments was thus diagnostically and prognostically more useful than any single method. (Supported by grants from NIH and ACS.)

180. Conversion of Triglycerides into CO_2 by Cancer Patients. KENNETH LYLES,* LUDWIG ULLRICH,* MICHAEL CONDREY,* AND GIOVANNI COSTA,* Richmond, Va. (introduced by G. Watson James III).

Significant fat loss induced in nonanorexic Swiss mice by nonmetastasizing solid Krebs-2 carcinoma has been reported by our laboratory (1962. *Cancer Res.* 22: 1081–83). The relevance of this observation to man was supported by the recognition that total fat determined in muscle biopsies from 30 patients undergoing primary resection of breast or colon cancer was 50% lower than in 35 patients with a variety of nonneoplastic diseases. We then administered intraperitoneally to normal and Krebs-2-bearing mice 5μCi [^{14}C]tripalmitine and determined specific activity of respiratory CO_2. The presence of a tumor depressed peak CO_2 specific activity by a factor of at least 10, as early as 24 h after transplantation, allowing unambiguous identification of each tumor-bearing mouse. 50μCi [^{14}C]tripalmitine were then administered orally to (*a*) 33 normal volunteers; (*b*) 45 patients with known cancers; (*c*) 12 patients with nonneoplastic diseases; and (*d*) 22 patients with diagnosis initially unknown. The values of specific activity for respiratory CO_2 at 16 h after administration of the label were used to prospectively identify cancer and noncancer patients. 35/45 patients of group (*b*) fell above the 95% confidence limits of the normal group. 8/12 patients of group *c* fell within normal limits, three were false positives; 16/22 patients of group *d* were correctly identified. There were two false positives and four false negatives. Overall, 59/79 patients were correctly identified as to presence or absence of cancer, an overall accuracy of 75%. Improved accuracy can be expected by administering the label intravenously. The biochemical mechanism for our observation is as yet unknown.

181. The Effect of Thrombin on Protein Phosphorylation in Human Platelets. ROGER M. LYONS* AND PHILLIP W. MAJERUS, St. Louis, Mo.

The action of thrombin, an inducer of platelet aggregation and the release reaction, has been postulated to be mediated through a decrease in platelet cyclic adenosine monophosphate (cAMP) levels. In other tissues, cAMP has been shown to alter cellular function by controlling the activity of protein kinases and thus protein phosphorylation (i.e., decreased cAMP yields decreased kinase activity). In cell-free systems, protein kinases have shown little substrate specificity and therefore the in vivo substrates have been difficult to identify. Thus, we studied protein phosphorylation using intact platelets. We incubated washed platelets with $^{32}PO_4$ (0.5 mCi/ml) for 1 h to label the intracellular pools of ATP (mean SA = 0.1 mCi/μmol). When the proteins from platelets so treated were fractionated by sodium dodecyl sulfate polyacrylamide-gel electrophoresis, nine distinct phosphoprotein peaks were observed. After incubation of $^{32}PO_4$-loaded platelets with thrombin (0.2–1 U/ml), there was a rapid ($t_{1/2}$ = 10 s) 4- to 5-fold increased incorporation of $^{32}PO_4$ into a single protein of mol wt 40,000 (peak VII). There were 3000–4000 cpm $^{32}PO_4$ incorporated into peak VII/10^8 platelets. The radioactivity in peak VII was bound as a phosphomonoester to serine and threonine residues of the protein as demonstrated by hydrolysis of $^{32}PO_4$ by *E. coli* phosphomonoesterase or NaOH, by digestion of the protein by pronase, and by identification of phosphoserine and phosphothreonine in partial acid hydrolysates of the protein. Phosphorylation of peak VII after addition of thrombin was inhibited by prior addition of either prostaglandin E_1 (1 μg/ml) or dibutyryl cAMP (1 mM) to the platelets. These results suggest that phosphorylation of peak VII, although paradoxical to the cAMP hypothesis, may play a role in platelet aggregation and the release reaction. (Supported by NIH and ACS.)

182. Amplification Assay for Colony-Stimulating Activity in Human Serum. JAMES MABRY,* JOAN BULL,* AND PAUL CARBONE, Bethesda, Md.

Human monocytes are a source of stimulating activity for in vitro growth of granulocyte-monocyte colonies; in preliminary experiments, we observed a marked stimulation of colony growth when normal human serum was added to feeder layers. To study how normal serum factors modify feeder layer-stimulating activity, we prepared monocyte-rich feeder layers from one normal human bone marrow and five peripheral blood samples and added to the agar layer either human serum or, as control, fetal calf serum, before overlaying mouse bone marrow cells in methyl cellulose. At 4–6 × 10^5 cells per plate, feeder cells stimulated only 0–50 mouse colonies per 10^5 mouse cells plated. Human serum in agar produced only 0–10 colonies per 10^5 cells per 0.1 cm^3 serum. In all experiments, addition of serum to the monocyte-rich feeder layer resulted in more colonies (range 30–128 colonies per 10^5 cells) than expected from simple addition of effects. Incubation of feeder layers for 7 days, followed by freeze-thawing, and then addition of serum showed similar amplification of colonies. Supernatant of culture medium incubated with 5 × 10^5 monocytes per cc for 7 days produced 40–60 colonies per 10^5 cells in excess of addition when later mixed with 0.1 cm serum in an agar underlayer. Dilute conditioned medium from various human sources (embryo kidney cells,

blood leukocytes, bone marrow) in the presence of 0.1 cm^3 test normal serum produced a 4- to 10-fold amplification of serum stimulating activity. The amplification was abolished at high concentrations of added conditioned media. We interpret the evidence to indicate that amplification of low levels of stimulating activity present in normal serum results from combination of subthreshold activity present in both serum and feeder layers or conditioned medium. This effect provides the basis of a sensitive assay for stimulating activity in normal serum.

183. Reevaluation of In Vitro Cellular Immunity Using Purified Human T and B Cells: Some Unexpected Findings. RICHARD P. MAC DERMOTT,* ROSS E. ROCKLIN,* LEONARD CHESS,* JOHN R. DAVID, AND STUART F. SCHLOSSMAN, Boston, Mass.

Using a column immunoabsorbent technique, we have previously shown that human peripheral lymphocytes can be quantitatively separated into highly purified populations of T and B cells. Both the T and B cell populations proliferate in response to mitogens (PHA, Con A, pokeweed), but only T cells proliferate in response to specific antigens. In the present studies, we have further explored the cellular basis of antigen activation with respect to production of mitogenic factor and migration inhibitory factor (MIF) using PPD, SKSD, and candida as antigens. Antigen-induced mitogenic factor is produced by sensitized T cells but not by B cells and triggers both nonsensitized T and B cells to proliferate. In contrast, both sensitized T and B cell populations produce MIF. Inhibition of cell division by 5-bromo-2-deoxy-uridine and light decreases MIF production by T cells, but does not effect MIF production by B cells. These observations are consistent with the view that a significant number of MIF-producing T cells are proliferating, whereas B cells produce MIF in the absence of proliferation. Preliminary fractionation experiments by Sephadex G-100 chromatography show that T and B cell MIF are indistinguishable. Our data to date indicate that (*a*) both human T and B cells proliferate in response to mitogens; (*b*) T but not B cells proliferate in response to specific antigens; (*c*) B cells can be induced to proliferate by a mitogenic factor released by antigen-triggered T cells; and (*d*) both human T and B cells produce MIF in response to specific antigens. (Research supported by NIH Grants CA 05167, AI 12069, AI 10921, and AI 11729.)

184. Monospecific Human Antineutrophil and Anti-Eosinophil Sera. ADEL A. F. MAHMOUD,* ROBERT W. KELLERMEYER,* AND KENNETH S. WARREN, Cleveland, Ohio.

The essential role of neutrophil phagocytes in protection against infectious diseases was established before 1900. While eosinophils and basophils participate in immunopathologic reactions, their protective functions remain unknown. Furthermore, forms of leukemia are associated with large numbers of eosinophils and basophils. Availability of monospecific anti-granulocyte sera would establish separate immunologic identities for each cell type, provide a means of establishing their function, and offer a possible way of treating some immunologic and neoplastic diseases. Neutrophils obtained from a normal donor and eosinophils from patients with leukemia and Hodgkin's disease were sedimented in EDTA/Dextran and purified by centrifugation in suitably adjusted concentrations of Hypaque 50. Washed cells (10^7) in complete Freund's adjuvant were injected subcutaneously into rabbits thrice at weekly intervals. 1 wk later, rabbits were bled and serum separated, decomplemented, and stored at −20°C. Using test cells obtained from a variety of patients, agglutination and cytotoxicity titers were obtained on crude and absorbed sera. None of the antisera had antilymphocyte activity; low anti-erythrocyte and platelet activity were absorbed out. The antineutrophil (ANS) and anti-eosinophil (AES) sera had titers of 1/320 against only the specific cells. Absorption with 10^8 specific cells lowered titers of both antisera to 1/10, while the other cells had no effect. An attempt has been made to produce an antibasophil serum (ABS), as described above, using cells from a patient with leukemia of which 82% were basophilic. The serum had a low ANS titer (1/32) and no AES activity. It is now being tested for antibasophil activity using a basophil degranulation test. Availability of monospecific human ANS and AES has established the unique immunologic identity of neutrophils and eosinophils. These observations may soon be extended to basophils. (Research supported by NIH.)

185. Interrelationships Between Bile Acids (BA) and Fatty Acid (FA) Absorption Regulate CCK-PZ Release in Man. J. R. MALAGELADA,* V. L. W. GO,* E. P. DIMAGNO,* AND W. H. J. SUMMERSKILL,* Rochester, Minn. (introduced by E. E. Wollaeger**).

We have previously shown that in man fatty acids in the gut lumen induce release of CCK-PZ and thus stimulate pancreatic enzyme secretion. We have now investigated the influence of varying BA concentration, FA concentration, and FA chain length on pancreatic enzyme secretion, correlating the results with rates of FA absorption. Trypsin outputs were quantified in 30 healthy volunteers by our technique of perfusion at the second portion of the duodenum and aspirating 20 and 50 cm distally. Test solutions (containing polyethylene glycol) included: study 1, taurocholate (TC) 10 mM with FA 20 mM (either octanoic-C8-, lauric-C12- or oleic-C18-); study 2, TC 10 mM with C18 at either 10, 20, or 30 mM; study 3, C18 10 mM with TC at either 1, 10, or 20 mM. Results follow. In study 1, trypsin outputs correlated linearly with increasing FA chain length ($r = 0.975$). In study 2, trypsin outputs increased proportionally to C18 concentration ($P < 0.05$). In study 3, trypsin outputs decreased when TC concentration was increased from 1 mM to 10 mM ($P < 0.02$) then became elevated again when TC was augmented to 20 mM ($P < 0.01$). In contrast, absorption of C18 was highest with TC 10 mM and lower with TC 1 mM or 20 mM ($P < 0.02$). Thus, an inverse correlation ($r = 0.990$) was shown between C18 absorption and pancreatic enzyme secretion. Comparing the effects of all FA solutions, a significant correlation was found between the proportion of FA remaining unabsorbed at 20 cm ($r = 0.938$) or at 50 cm ($r = 0.964$) and pancreatic enzyme secretion. We conclude that in man absorption rates of perfused FA may determine the magnitude of pancreatic responses by modifying the surface area of the gut exposed to FA, thus regulating CCK-PZ release, and that there is an optimal concentration ratio between FA and BA for maximal long-chain FA absorption. (Supported by NIH Grant AM 6908.)

186. Depression of Delayed Hypersensitivity During Infectious Mononucleosis: Antigenic Competition? RICHARD J. MANGI,* JAMES C. NIEDERMAN,* JOSEPH E. KELLEHER,* JOHN M. DWYER,* ALFRED S. EVANS,* AND FRED S. KANTOR, New Haven, Conn.

Depression of delayed hypersensitivity (DH) in the course of viral infections may be prolonged and profound. Either the virus lyses a necessary cell type(s), or a viral-host interaction suppresses the response. Marked depression of DH during infectious mononucleosis (IM) was demonstrated

serially in 34 patients by the following parameters of immunological function: intradermal tests with *Candida albicans* (CA) and streptokinase-streptodornase antigens; T and B lymphocyte enumeration; lymphocyte stimulation to phytohemagglutinin and pokeweed mitogens, and to CA antigens; and lymphocyte response to pooled irradiated allogeneic lymphocytes. Skin tests were depressed during acute IM. Absolute number of B lymphocytes was elevated during the first week of symptoms and returned to normal in 3–4 wk. Absolute number of T lymphocytes increased early in illness to reach a peak in 1–2 wk and returned to normal in 4–5 wk. Therefore, the ratio of T/B lymphocytes was reversed during the first week and returned to normal in 3–4 wk. At no time was the absolute number of T or B cells diminished. Maximum depression of lymphocyte response in vitro occurred in 1–2 wk; while depression was noted in response to all stimulants, responses to CA and pokeweed mitogens were most diminished. Recovery of in vitro reactivity to CA required more than 4 wk in some cases. In selected cases lymphocyte stimulation by Epstein-Barr virus (EBV) was shown. Failure to demonstrate depression of absolute numbers of competent lymphocytes, and the observation that first B then T cells increase in number and spontaneous activity are consistent with the hypothesis that EBV produces infection, proliferation, and neoantigen formation in B cells which induce a T cell immune response rendering them incapable of reaction to other antigens. (Research supported by Grants NIH AI 11077 and NIH AI 08731.)

187. An Immunotherapy Trial in Acute Leukemia. DEAN L. MANN,* BRIGID LEVENTHAL,* AND ROGER HALTERMAN,* Bethesda, Md. (introduced by William D. Terry).

Prior studies demonstrated that crossreactive antigens are shared on acute lymphocytic leukemia (ALL) cells and on the tissue culture cell line RAJI. With this information, a combination immunochemotherapy study was undertaken using the RAJI cells as immunogens. 12 patients who had failed prior chemotherapy with combinations of a variety of drugs were randomized to chemotherapy or immunochemotherapy. Remission was induced with a combination of cytoxan, oncovin, cytosine arabinoside, and prednisone (COAP). 10–14 days after the last dose of drugs, one-half of the patients received three injections of 5×10^8 RAJI cells subcutaneously every other day. This combination of immunochemotherapy was repeated every 5 wk. Serums were obtained from these patients before and after chemotherapy or immunochemotherapy and tested for cytotoxic antibody to the RAJI cells, acute leukemia cells, and to normal peripheral blood lymphocytes. No antibody activity was detected in the serums from the chemotherapy group. Four of six patients receiving combination immunochemotherapy developed antibody to acute leukemia and RAJI cells but not to a panel of normal lymphocytes. During the course of immunochemotherapy antibody titers to leukemia cells rose and then fell to nondetectable levels 4–6 wk before relapse. Serums obtained from the four patients when their antibody titers were high were tested with their own cells in relapse and found to be positive. The results show that patients appropriately immunized can develop antibody to antigens on their own leukemia cells. The failure of these patients to continue to produce antibody with continued immunization is unexplained. Preliminary evidence suggests that antibody production fell because of elimination of antibody-producing cells by the cytotoxic drugs. This trial suggests new protocol models for combination immunochemotherapy.

188. Quantitative Continuous Automated Electrocardiographic and Hemodynamic Monitoring. JOHN A. MANTLE,* EUGENE M. STRAND,* JOHN B. BREINIG,* RICHARD O. RUSSELL, JR.,* AND CHARLES E. RACKLEY, Birmingham, Ala.

Quantitative continuous on-line 24 h electrocardiographic monitoring was evaluated in 15 patients with acute myocardial infarction, unstable angina pectoris, or recurrent ventricular tachycardia. A computerized system was used for the continuous on-line QRS recognition and R-R interval analysis from surface electrodes. Intracavitary electrodes mounted on the pulmonary artery thermal dilution catheter were used in three patients for bipolar atrial or ventricular spike detection and interval analysis. The results were displayed via a video terminal at the bedside and in 8-h summary graphs and tables of the minute statistics for heart rate, dysrhythmias, and signal noise. Serial on-line pulmonary artery pressures and thermal dilution cardiac outputs were monitored in five of these patients. The automated system of monitoring was more sensitive and objective than continuous visual monitoring by trained personnel. Dysrhythmias were often associated with daily activities; examples include sleeping, blood drawing, eating, excretory function, and family visitors. Hemodynamic disturbances were less sensitive to these activities, but were associated with sustained ventricular tachycardia and frequent premature ventricular contractions. Distinct cyclic patterns were observed in the R-R intervals of normal sinus rhythm and in the R-R coupling intervals of both premature ventricular contractions and ventricular tachycardia. Transient dysrhythmias were documented to precede both supraventricular and ventricular tachycardias. Automated continuous on-line electrocardiographic and hemodynamic monitoring provides quantitative data for the assessment and correlation of the electrophysiologic and hemodynamic disturbances in clinical heart disease. (Research supported in part by grants from NIH (MIRU, PPG) and NLM.)

189. Relative Contribution of Low and High Pressure Baroreceptors in Circulatory Adjustments to Venous Pooling in Man. ALLYN L. MARK,* DWAIN L. ECKBERG,* FRANCOIS M. ABBOUD, AND U. JAMES JOHANNSEN,* Iowa City, Iowa.

Lower body negative pressure pools blood in the lower extremities and produces vasoconstriction and tachycardia by activating reflexes originating in low pressure (cardiopulmonary) and high pressure (carotid sinus) baroreceptors. In eight normal subjects, we evaluated the relative contribution of low and high pressure baroreceptors to the reflex adjustments by comparing responses to (*a*) lower body negative pressure alone and (*b*) lower body negative pressure plus simultaneous neck suction to inhibit the contribution of carotid baroreceptors to the reflex vasoconstriction and tachycardia. Lower body negative pressure at 20 and 40 mm Hg increased forearm vascular resistance by 8 ± 2 (mean $\pm$ SE) and 12 ± 2 U, respectively, and increased heart rate. Simultaneous application of neck suction prevented reflex tachycardia during lower body negative pressure, but did not alter the forearm vasoconstriction; increases in forearm resistance during simultaneous neck suction and lower body negative pressure were 7 ± 2 and 12 ± 2 U at 20 and 40 mm Hg, respectively. Neck suction alone produced significant decreases in heart rate and arterial pressure, but did not significantly decrease forearm vascular resistance. The results indicate that low and high pressure baroreceptors may contribute selectively to autonomic adjustments during maneuvers such as lower body negative pressure:

high pressure baroreceptors trigger the reflex tachycardia and low pressure baroreceptors exert the predominant influence on forearm vascular tone.

190. Drug Sensitivity in Hepatic Porphyria: Relationship to Defect in Heme Synthesis. J. D. MAXWELL* AND U. A. MEYER,* San Francisco, Calif. (introduced by M. Sleisenger).

Intermittent acute porphyria (IAP) is an inborn error of porphyrin and heme synthesis characterized clinically by attacks of an acute neuropsychiatric syndrome. The syndrome is frequently precipitated by commonly used drugs and steroids, most of which are inducers of the hemoprotein cytochrome P450 (P450), the terminal oxidase in drug metabolism. The primary genetic defect in IAP (deficiency of uroporphyrinogen 1 synthetase activity) causes a partial block in heme synthesis which may result in secondary induction of δ-aminolevulinic acid synthetase (ALA-S) by derepression. As drug sensitivity in IAP may be related to the defect in heme synthesis, lead chloride (Pb) was used to produce partial blocks in heme synthesis in rats. Effects on ALA-S, P450, and drug metabolism (*p*-nitroanisole demethylation) were studied. Pb (10 mg/kg intravenously) alone produced significant changes (at 12 h) from controls in ALA-S +27% P450 −28%. Phenobarbital (Pheno) 100 mg/kg intraperitoneally alone: ALA-S +100%; P450 +30%. The rate of drug metabolism paralleled P450 concentration. Pheno in combination with Pb produced no increase in P450 (−16%) but resulted in a striking potentiation of ALA-S induction (+220%). The degree of potentiation varied with the dose of Pb (2.5–20 mg/kg) and was observed with doses of Pheno (10–25 mg/kg) or progesterone (100 mg/kg) which alone had no effect on ALA-S. As experimentally produced partial blocks in heme synthesis greatly increase the sensitivity of ALA-S to induction by drugs and steroids, these results suggest that the drug idiosyncrasy in IAP may be related to the underlying primary genetic defect in heme synthesis. Decreased drug inactivation as a consequence of impaired formation of P450 may contribute to this effect. (Research supported by Grants GM 16496 and GM 01791 from NIH.)

191. Terminal Deoxynucleotidyl Transferase (TT): a Thymus-Specific Enzyme in Acute Lymphoblastic Leukemia (ALL) Cells. RONALD P. MCCAFFREY,* ALLEN E. SILVERSTONE,* THOMAS A. HARRISON,* ROBERTSON PARKMAN,* AND DAVID BALTIMORE,* Cambridge and Boston, Mass. (introduced by David G. Nathan).

Terminal deoxynucleotidyl transferase (TT) is a unique DNA polymerase which has previously been found only in normal thymus. While studying polymerases of leukemic cells, we recently observed TT activity in cells from a child with acute lymphoblastic leukemia (ALL). TT was identified by dGMP polymerization onto oligo (dA_{14}) after phosphocellulose chromatography of cell homogenates. Studies on additional patients show TT activity in cells from six out of eight patients with ALL. Normal human thymus contains an identical enzyme in a subpopulation of lymphocytes which are recovered in the top layers of a 17–33% BSA gradient. In normal humans, as in other species, TT is restricted to the thymus. It is also absent in humans from cells of chronic lymphatic leukemia, lymphosarcoma, and acute myeloblastic leukemia. Cells cultured from infectious mononucleosis, lymphosarcoma, and Burkitt's lymphoma are also negative. In the AKR mouse a spontaneous lymphoblastic leukemia occurs with high frequency. This disease is known to originate in the thymus. AKR spleens infiltrated by leukemic cells contain TT, whereas in the nonleukemic state the enzyme is found only in thymus. These studies suggest a role for the human thymus in the development of many cases of ALL. TT would appear to be a potentially useful leukemic cell marker with diagnostic and/or prognostic significance. (Research supported by grants from ACS and NIH.)

192. A New Biologic Activity of Complement Associated with Its Intravascular Activation and Release of a Low Molecular Weight Factor. CHARLES MCCALL,* DAVID BROWN,* PETER LACHMANN,* AND LAWRENCE DECHATELET,* Winston-Salem, N. C., and London, England (introduced by Manson Meads).

Activation of the complement cascade in rabbits by intravenous administration of purified cobra venom factor (CVF), inulin suspension, or *Serratia marcescens* endotoxin results in a 95% reduction in circulating neutrophils (PMN's) within 15–120 s. Neutrophilia (200–600% increase) with appearance of immature cells follows within 3–4 h. Kinetic studies using $DF^{32}P$-labeled PMN's suggest sequestration rather than destruction. The infusion of 10 ml of fresh plasma into complement-depleted rabbits whose blood still contains circulating CVF reproduces the reaction. It can also be induced in C6-deficient rabbits but not in rabbits first depleted of C3 by intravenous injections of CVF, suggesting dependence on C3 and/or C5. Fresh rabbit plasma (10–20 ml) was incubated in vitro with CVF (1:10 v/v) or inulin (7 mg/ml) at 37°C for 10 min, and then filtered at 4°C through a UME 20,000 Diaflo membrane. Infusion of the low molecular weight filtrate reproduced the biologic activity. It is possible that release of this biologically active factor which follows intravascular complement activation is responsible for alterations in neutrophil kinetics seen in acute inflammatory states such as septicemia.

193. Globin Synthesis in Double Heterozygotes for α- and β-Hemoglobin Chain Abnormalities. PAUL R. MCCURDY,* ANITA S. SHERMAN,* H. KAMUZORA,* AND HERMANN LEHMANN,* Washington, D. C., and Cambridge, England (introduced by Dudley P. Jackson**).

To help understand protein synthesis from a single gene, we studied the incorporation of [^{14}C]leucine into the hemoglobin chains of five subjects doubly heterozygous for both an α- and a β-chain abnormality. All five had α^{G}Philadelphia (α 68 lys→Asp); three had β^{S}(all female) and two β^{C} trait (one female). The patients' hematocrit values were 38.9 ± 2.2 (mean ± SD; range 36.8–42.1) and the MCV's were 82.1 ± 4.1 (range 75.0–85.3). Hemoglobin protein synthesis was determined in reticulocytes concentrated from peripheral blood by high speed centrifugation. Globin prepared directly from whole RBC was chromatographed on CMC using a phosphate buffer system with 8 M urea (Clegg column). ^{14}C radioactivity was measured by liquid scintillation counting. Total α/total β radioactivity ratios were 0.51, 0.42, and 0.74 for S/G heterozygotes and 0.51 and 0.87 for C/G heterozygotes. Ratios of specific activities were similar. For the S/G subjects, the α^{A}/total β ^{14}C was 0.35, 0.29, and 0.52; for the C/G subjects it was 0.39 and 0.29. α^{G}/total β was less than 0.22 for all subjects. These double heterozygotes seem to have a deficit in α-chain synthesis of both normal and abnormal types. This might be the result of an associated α-thalassemia gene or because the α^{G}Philadelphia gene has an α-thalassemia-like effect on globin synthesis. (Research supported by a grant from NIH.)

194. Psoriasis and Occlusive Vascular Disease. CHARLES J. MCDONALD* AND PAUL CALABRESI, Providence, R.I.

Although the presence of microvascular abnormalities has been observed in the cutaneous blood vessels of normal and psoriatic skin in patients with psoriasis, to our knowledge, the association of psoriasis and large vessel disease has not been reported. The clinical records of 324 psoriatic and 325 nonpsoriatic patients admitted to the dermatology service of the Roger Williams General Hospital were examined to determine the occurrence of occlusive vascular disease (thrombophlebitis, myocardial infarction, pulmonary embolization, and cerebrovascular accident). Two approaches were pursued: (*a*) occurrence data from the psoriatic group were contrasted with that from the nonpsoriatic group; (*b*) data from the psoriatic population alone were analyzed to explore relationships between the patient's age, duration of psoriasis, extent and degree of disease, and the occurrence rate or likelihood of occurrence of occlusive vascular episodes. Detailed statistical analyses supported the following conclusions. (*a*) There is a threefold significantly greater occurrence rate of occlusive vascular episodes in the psoriatic patient. (*b*) The psoriatic patient appears to have a significantly higher predisposition to occlusive vascular disease. (*c*) Although age plays a significant role, duration of psoriatic disease does not have an effect on the patient's likelihood of experiencing an occlusive vascular disease. (*d*) Percent of body involvement by psoriasis appears to be directly related to increased occlusive vascular occurrence, particularly in the older age groups. (*e*) The treatment received by the psoriatic patient does not appear to bear any relationship to either the occurrence rate of, or predisposition to, occlusive vascular disease. (*f*) There appears to be no discernible difference between males and females with respect to disease occurrence. (Supported by NIH Grants GM16538 and CA 13943.)

195. Predicting Hormone Dependence in Human Breast Cancer. WILLIAM L. MCGUIRE, San Antonio, Tex.

We have previously reported that hormone-dependent breast cancer tissue in rats contains a cytoplasmic estrogen receptor protein (ER), while autonomous rat breast cancers commonly lack this receptor. Since only 20–30% of human breast cancers are hormone dependent, the presence of ER might identify these tumors for appropriate endocrine treatment. We have therefore initiated a prospective study to define the correlation of ER level in human breast tumors with objective response to endocrine therapy. The levels of ER in 182 primary and metastatic tumor specimens have been determined both by saturation analysis using Dextran-coated charcoal and by sucrose density gradient ultracentrifugation. 65% of the primary specimens and 50% of the metastatic specimens contained ER, ranging from 3 to 1100 fmoles/mg cytosol protein. These levels were generally higher in postmenopausal patients. An independent assessment of clinical response to endocrine additive or ablative therapy is available for 33 patients with metastatic disease. These judgments used the criteria of the Primary Cooperative Breast Group, without knowledge of the ER results. Of 16 patients with no detectable tumor ER, only one had an objective remission under endocrine treatment (6%), while 12 of 17 patients with measurable ER in either primary or metastatic sites experienced objective remissions (71%). Forthcoming clinical data will be needed to confirm these results and to compare the predictive value of ER levels for each separate type of endocrine treatment. These early results indicate that a reliable technique based on sound biochemical principles may soon be available for selecting breast cancer patients for endocrine therapy.

196. Quantitation of Platelet-Binding IgG (PBIgG) Produced In Vitro By Idiopathic Thrombocytopenic Purpura (ITP) Spleens. ROBERT MCMILLAN,* ROBERT LONGMIRE,* SAMUEL ARMSTRONG,* AND ROBERT YELENOSKY,* La Jolla, Calif. (introduced by William H. Crosby**).

Preliminary studies from our laboratory suggested that the spleen in idiopathic thrombocytopenic purpura (ITP) is an important site of antiplatelet antibody (APA) production; the present studies describe methods for measuring the magnitude of splenic APA production, as well as the quantity of APA attaching to target platelets. Splenic leukocytes from 19 control subjects and 20 ITP patients were cultured for 10 days, and the IgG synthesis rates were determined using the Fab-anti Fab assay technique. Total daily IgG production was calculated by multiplying the IgG synthesis rate per cell by the total number of splenic cells. Mean values (± SD) for daily IgG production by control and ITP spleens were 3.0 ± 1.5 and 23.2 ± 10.4 mg/day, respectively. Several cultures from each spleen were pooled and concentrated (30–60 μg IgG/ml), and aliquots were incubated with either homologous or autologous platelets. Platelet-associated IgG was significantly increased after incubation with each of the 19 ITP concentrates tested; incubations using 10 control concentrates were negative. Seven of the ITP concentrates were tested further. Platelets were incubated with graded quantities of ITP concentrate and the increase in platelet-associated IgG was plotted against the quantity of incubated IgG. This allowed calculation of the maximum amount of IgG which would bind to target platelets ($PBIgG_{max}$), as well as the percentage of the total synthesized IgG which was platelet-specific (% PBIgG). Values for $PBIgG_{max}$ averaged 1347 ng IgG/10^9 platelets or 5068 molecules per platelet. Mean % PBIgG was 2.2% (range 0.9–3.5), corresponding to a daily splenic PBIgG production averaging 627 μg IgG/day (range 114–2065). This amount of PBIgG would allow "maximal sensitization" of between 0.7 and 7.5 times the platelets normally produced in vivo per day. We conclude that (*a*) the spleen is an important site of antiplatelet antibody production in ITP, and in many cases the quantity produced explains in large part the clinical findings; and (*b*) the average $PBIgG_{max}$ of 5068 molecules per platelet is 10 times the calculated minimum concentration required for in vivo platelet destruction in other immune purpuras. (Research supported by NIH Grant AM 16125.)

197. Elevated Nonsuppressible Insulin-Like Activity, Soluble in Acid-Ethanol (NSILA-s), in Patients with Hypoglycemia and Extrapancreatic Tumors: Application of a New Radioreceptor Assay. KLARA MEGYESI,* C. RONALD KAHN,* JESSE ROTH, PHILLIP GORDEN, AND DAVID M. NEVILLE, JR,* Bethesda, Md.

Fasting hypoglycemia in patients with islet cell tumors is due to inappropriate insulin secretion, while the etiology of hypoglycemia in patients with nonislet cell tumors has remained obscure. Nonsuppressible insulin-like activity, soluble in acid-ethanol (NSILA-s), is a purified serum peptide that has insulin-like bioactivity in vivo and in vitro, but lacks insulin immunoreactivity. Using a new radioreceptor assay, we have detected elevated levels of NSILA-s in the plasma of five of seven patients with extrapancreatic tumors and hypoglycemia. Plasma was filtered on Sephadex G-50 in 1 M acetic acid. Each effluent fraction was neutralized and assayed for NSILA-s by competitive binding with [^{125}I]NSILA-s and purified rat liver plasma membranes. This assay is able to detect NSILA-s at 1 ng/ml (using an NSILA-s standard with 70 mU/mg of insulin-like activity provided by R. E. Humbel

and E. R. Froesch). In this assay, insulin is 1/300,000th as potent as NSILA-s and proinsulin is 1/1000th as potent. In all plasma, NSILA-s was detected as a discrete peak with an elution volume identical with that of purified NSILA-s. In five normal adults plasma NSILA-s was 900–2200 ng/ml. Elevated NSILA-s levels (2400–16,000 ng/ml) were detected in five of seven patients with hypoglycemia and nonislet cell tumors. Normal values were found in patients with similar tumors but without hypoglycemia and in patients with hypoglycemia due to insulinoma or exogenous insulin administration. Two acromegalics and two hypopituitary patients had normal values, while one hypopituitary patient had a value below the normal range. In view of the insulin-like properties of NSILA-s and its elevated levels (up to 8-fold) in patients with hypoglycemia and extrapancreatic tumors, we suggest a role for NSILA-s in the etiology of this form of hypoglycemia.

198. Growth Hormone Secretion: Evidence for an Essential Homeostatic Role in Women. THOMAS J. MERIMEE AND S. EDWIN FINEBERG,* Boston, Mass.

The subject of this investigation was the complex action of estrogen and growth hormone (HGH) upon insulin secretion. Circulating levels of HGH were increased in men to levels similar to those occurring normally in premenopausal women: first, by administering 1.5 mg of growth hormone nightly for 1 wk, and second, by treating with estrogen for an equal period of time. Insulin responses were studied after intravenous arginine, oral glucose, and a protein meal. The results follow. (*1*) Women and untreated men had similar increases of plasma insulin after each stimulus. (*2*) Despite obtaining comparable increases of HGH both by treatment with estrogen and by treatment with exogenous HGH, only the latter treatment increased insulin responses. (*a*) Mean peak insulin concentrations in plasma after arginine were 87 ± 12 μU/ml in women, 80 ± 16 μU/ml in untreated men, 82 ± 19 μU/ml after estrogen treatment, and 290 ± 65 μU/ml after treatment with growth hormone ($P < 0.01$). (*b*) After glucose the mean maximal insulin concentration in plasma was 75.4 ± 8.9 μU/ml in women, 68.5 ± 14 μU/ml in untreated men, 70 ± 8.6 μU/ml in men treated with estrogen, and 219 ± 71 μU/ml in men treated with growth hormone. A similar pattern was noted after protein meals. Since HGH increases insulin secretion, it can be viewed as a counterforce to estrogen, important in premenopausal women for maintenance of metabolic reactions dependent upon insulin. The results indicate a complex homeostatic mechanism involving estrogen, growth hormone, and insulin (Supported by NIH RR-533.)

199. In Vivo Survival of Cryopreserved Dog Granulocytes. DONALD S. MILLER,* Durham, N.C. (introduced by W. F. Rosse).

White blood cell (WBC) transfusion may be life-saving for infected granulocytopenic patients who are unresponsive to antibiotics. Cryopreservation of WBC concentrates has been attempted since sufficient numbers of granulocytes are infrequently available for transfusion. Isolated WBC have been frozen using glycerol, dimethyl sulfoxide, or dimethyl acetamide as preservatives. Cells were frozen at −3 to −5°C/min to −80°C and stored in the vapor phase of liquid nitrogen (LN_2). In vitro studies of phagocytic and bactericidal function of dog granulocytes preserved in LN_2 for 60–100 days revealed normal function. In vivo survival of cryopreserved autologous granulocytes was determined in splenectomized dogs. Circulating granulocytes were labeled in vivo by intravenous [^{3}H]DFP, harvested using an Aminco celltrifuge, and frozen. 10–12 U of WBC concentrate per dog were selectively removed during 7–10 days. At least 30 days after leukophoresis and storage in LN_2, rapidly thawed WBC concentrates were transfused into the cell donors. The number of granulocytes transfused was 10 times the circulating granulocyte pool (SA = 4.5 × 10^{-5} cpm/granulocyte). Granulocyte disappearance was random with a normal half-life of 6.8 h. The yield at 0 and 1 h posttransfusion was 14.5% and 12.4%, respectively. Transfusion caused an increase of total granulocytes at 0 and 1 h posttransfusion. Nearly 65% of transfused granulocytes were lost, presumably due to destruction in the lungs. Before such studies should be attempted in man, it is essential to determine the toxicity of preservative in animals. (Research supported by Leukemia Society of America and NCI CA 11265.)

200. Effect of Potassium Loading on Plasma Renin Activity. PAUL D. MILLER,* CHRISTINE WATERHOUSE,** ROGER OWENS,* AND EDWIN COHEN,* Rochester, N.Y., and Ann Arbor, Mich.

Hyperkalemia may suppress plasma renin activity (PRA) by either a direct effect of potassium or secondary to sodium retention mediated by a rise in aldosterone. Four patients with adrenal insufficiency were placed on a diet of 60 meq potassium and 100–150 meq of sodium while receiving a constant amount of cortisone acetate and fluorine. Upright noon PRA was determined for 2–3 days as controls after stable weights or sodium balance were obtained. The potassium intake was then increased to 200–240 meq/day for 2–3 days and 4–6 additional PRA determined at noon and 6–10 p.m. Potassium loading was accompanied by a naturesis. Hence, patients were either sodium replaced (four studies in three patients) or sodium depleted (two patients). Potassium loading without sodium replacement was associated with a decrease in weight (mean: −2.3 kg), negative sodium balance (mean: −181 meq), hyperkalemia (mean: 5.23 meq/liter), and positive potassium balance (mean: +114 meq). PRA rose 22.0 ng/ml per h (11 a.m. means: control vs. experiment), or 41.3 ng/ml per h (mean: last day control vs. last day experiment). Potassium loading with sodium replacement was associated with little change in weight (mean: −0.25 kg), sodium balance (mean: +23 meq), hyperkalemia (mean: 5.77 meq/liter), and positive potassium balance (mean: +119 meq). PRA (total mean: control vs. experiment) showed no significant changes: (+1.23 ng/ml per h; range +6.9 to −2.0). 11 a.m. values showed a significant rise in two studies: (mean: 42.5 ng/ml per h; range 31.0–54.0) and no significant change in the other two studies: (mean: +0.10 ng/ml per h; range +1.90 to −1.80). We conclude that hyperkalemia and/or a positive potassium balance does not suppress PRA in Addisonian man when sodium balance is maintained. Furthermore, potassium does not prevent a significant rise in PRA when sodium balance is negative. Potassium-induced suppression of PRA in previous human studies appears to be secondary to potassium-induced rise in aldosterone.

201. The Binding of Palmityl-CoA to Cytoplasmic Proteins: Binding to Z Protein in the Liver and Other Tissues of the Rat. SEYMOUR MISHKIN* AND ROLAND TURCOTTE,* Montreal, Canada (introduced by Jacques I. Kessler).

Z is a 12,000 mol wt cytoplasmic protein present in rat liver, myocardium, skeletal muscle, intestinal mucosa, adipose tissue, and kidney. Z protein binds bilirubin, various dyes, and long-chain fatty acids (FA) both in vitro and in vivo. We have observed that, as in the case of FA, [^{14}C]palmityl CoA (PA-CoA) added to liver cytosol (110,000 *g*), is reversibly bound almost entirely to Z protein. While 8% of a tracer dose of [^{14}C]palmitic acid (PA) is recovered on Z, 46%

of equimolar [^{14}C]PA-CoA is bound to Z protein. A single class of high affinity binding sites for PA-CoA with an apparent Kd of 2.4×10^{-7} moles (at 4°C) was identified by Sephadex chromatography. PA-CoA binding was significantly inhibited by the CoA derivatives of various long-chain FA's (C14:0, C16:0, C18:1, and C18:2) palmityl carnitine, and palmitic and oleic acids. No significant inhibition was detected with the CoA derivatives of short-chain FA's or with CoA alone. Bromopalmitin, a competitive inhibitor of FA metabolism markedly reduced the binding of FA-CoA to Z, while chlorphenoxyisobutyrate significantly enhanced the binding. PA-CoA binding to Z was reduced by fasting (24 h), while Z concentration was not detectably decreased. Z protein present in other tissues also showed a high affinity for PA-CoA. These results lend additional support to the hypothesis that Z protein is involved in the metabolism of long-chain FA's. (Supported by Canadian MRC Grant MA-4658 and Quebec MRC Grant 720054.)

202. The Binding of Calcium to Albumin in Normal Human Serum: a Unique Ion-Protein Interaction—The "Clam Effect." EDWARD W. MOORE, Richmond, Va.

Serum ionized calcium, [Ca^{++}], is normally maintained within narrow limits. Previous studies from this laboratory indicate that about 81% of protein-bound calcium [CaProt] is bound to albumin [CaAlb] and 19% to globulins in normal serum. Albumin is thus the major buffer against acute changes in [Ca^{++}]. The Ca^{++} electrode (Orion Research, Inc.) has allowed, for the first time, direct studies of Ca^{++}-protein interaction. We here report a previously unrecognized property of serum albumin and a new unique relationship for the binding of an ion to a macromolecule. A total of 77 studies were made by serial addition of $CaCl_2$ (n = 38) or serial dilution with NaCl (n = 26) of pooled samples of normal serum (n = 13) at constant temperature (25 ± 2°C), pH (7.34 ± 0.01, SE), and ionic strength (μ = 0.16 M). In each sample, [CaProt] and [Ca^{++}] were measured by techniques described previously (1970. *J. Clin. Invest.* **49**: 318–334). Total calcium ranged from 0.44 to 28.0 mM. The resulting Scatchard plot for $\bar{v}/[Ca^{++}]$ against $\bar{v}$ (where $\bar{v}$ = [CaAlb]/[Alb] molar ratio) was most remarkable: over the $\bar{v}$ range 1.0–9.4, the function was linear (r = 0.993) with K_a' = 69.4 ± 3.6 and an extrapolated maximum of 15–16 binding sites per molecule. Linearity indicates that the sites are identical and independent. Below $\bar{v}$ = 1, the ratio: $\bar{v}/[Ca^{++}]$ linearly decreased to zero, indicating that albumin has no affinity (K_a' = 0) unless a Ca^{++} is already on the molecule! This strongly suggests a conformational change ("jelly roll effect") for binding of the first Ca^{++}. Normal serum, at a mean $\bar{v}$ level of 1.10 ± 0.03 is thus at the pinnacle of a Scatchard plot and at a point where albumin is an ideal Ca^{++} buffer. We postulate that maximum protection against acute hypercalcemia is afforded by (n-1) available sites, while protection against hypocalcemia is afforded by a conformational change in which, upon dissociation of the last Ca^{++}, the albumin molecule "rolls up," with complete loss of available Ca^{++}-binding sites (the "clam effect"). We believe these findings are an important step towards understanding the relation of ion binding to specific conformational states, and are thus far unique for the interaction of a ligand with a macromolecule. (Supported by NIH grants.)

203. A Distinct Protein with Alkaline Phosphatase Activity in Serum of Patients with Acute Lymphatic Leukemia (ALL), Chronic Lymphatic Leukemia (CLL), and Infectious Mononucleosis (IM). EDGAR M. MORAN,* HAVA NEUMANN,* ROBERT M. RUSSELL,* AND IRWIN H. ROSENBERG, Chicago, Ill. (introduced by Harry A. Fozzard).

Serum alkaline phosphatase (APase) or normal mammalians catalyzes the hydrolysis of monoesters of orthophosphoric acid and of S-substituted monoesters of thiophosphoric acid with similar efficiency. APase derived from thymus of lymphatic leukemia mice does not catalyze the hydrolysis of the S-substituted monoesters of thiophosphoric acid. This distinct APase was arbitrarily named N-phosphatase (N-APase). The amount of N-APase can be expressed as percentage of the total serum APase activity. This study sought to determine whether N-APase appears also in human malignant and benign lymphoproliferative diseases and, if present, to observe its variations in relation to clinical evolution. N-APase was absent in normal human controls. 6 patients with ALL, 5 patients with CLL, and 22 patients with IM were studied. All patients had N-APase activity in the serum. N-APase in ALL ranged between 26 and 100%, in CLL between 35 and 39%, and in IM between 26 and 100% of the total APase activity. With achievement of remission in ALL, a decrease in N-APase activity was noted. Clinical deterioration in CLL was accompanied by a marked increase in N-APase activity. Clinical and serological improvement in IM was associated with disappearance of N-APase. The occurrence of N-APase in ALL and IM may be related to their presumed viral etiology, whereas in CLL it may be at least in part due to the neoplastic transformation of lymphocytes. The finding of N-APase may serve as a potential marker in lymphoproliferative disorders. (Research supported by grants from ACS IN-41 M, The Louis Block Fund—The University of Chicago, The Leukemia Research Foundation, and the Jules J. Reingold Fund.)

204. Relation Between Left Atrial Size and Development of Atrial Fibrillation in Patients with Mitral Valve Disease. JOEL MORGANROTH,* ALAN S. PEARLMAN,* WALTER L. HENRY,* AND STEPHEN E. EPSTEIN, Bethesda, Md.

Systemic embolization, a serious complication of mitral valve disease, usually occurs in patients in atrial fibrillation (AF) and particularly in those who recently have converted from normal sinus rhythm (NSR) to AF. It would therefore be important to identify patients in NSR who are a high risk of developing AF. In an attempt to define an index predictive of onset of AF, 92 patients with rheumatic mitral disease (53 with mitral stenosis, 21 with mitral regurgitation, and 20 with mixed disease) were evaluated by echocardiography (ECHO). 51 patients were in chronic AF. In all 51, left atrial (LA) size measured by ECHO was ≥40 mm (50 of the 51 ≥ 45 mm). This contrasts to a mean value of <30 mm found in normal adults. 43 patients were in NSR: 21 of 43 had LA size < 40 mm, and none had a history of paroxysmal AF; 22 patients had LA size ≥40 mm, and 9 (41%) had a history of paroxysmal AF. 2 of 9 patients (22%) with paroxysmal AF and 11 of 51 patients (22%) in chronic AF had a history of major systemic embolization. Cardiac catheterization in 54 patients demonstrated that LA pressure did not correlate with rhythm or LA size. No significant difference in LA size occurred in four patients studied immediately before and after cardioversion, suggesting that the LA size was a cause and not the result of AF. We conclude that LA size is an important factor in the development of AF. Moreover, LA size ≥40 mm by ECHO in patients with mitral disease and NSR appears to identify a group at high risk of developing AF, and possibly systemic embolization.

205. Response of Ischemic Myocardium to Changes in Contractility, Afterload, and Coronary Perfusion Pressure. HILTRUD MUELLER,* STEPHEN AYRES, ANNA RELIGA,* AND ROBERT EVANS,* Worcester, Mass.

Coronary sinus sampling and measurement of coronary blood flow (CBF) ([^{131}I]antipyrine) were performed in 50 patients

with acute myocardial infarction (AMI) not in shock and in 35 patients in shock (coronary shock, CS). Severity was estimated from metabolic (lactate, pyruvate, free fatty acids, glucose) and hemodynamic data. Oxygen extraction (Exo_2) was 67 ± 8% (mean ± SE) and 77 ± 7% and CBF was 88 ± 15 ml/100 g per min and 71 ± 9 in AMI and CS, respectively. Myocardial oxygen consumption (MVo_2) was correlated with arterial-coronary sinus oxygen difference ($r = 0.61$) and CBF ($r = 0.78$) in AMI but only with arterial-coronary sinus O_2 difference ($r = 0.91$) in CS. 15 patients with AMI and 25 with CS produced lactate; therapeutic interventions in this group were analyzed for efficacy. Raising mean aortic (coronary perfusion) pressure with *l*-norepinephrine (*l*-NE) increased CBF 27 ± 4 ml/100 g per min and reversed lactate production (LP) in five of eight patients with CS but increased MVo_2 and free fatty extraction while decreasing lactate extraction in AMI. Raising coronary perfusion pressure with intra-aortic counterpulsation (IACP) increased CBF 23 ± 3 ml/100 g per min in CS and reversed LP in all. External counterpulsation (ECP) in CS increased CBF 10 ± 1 ml/100 g per min but reversed LP in only two of ten patients. ECP in AMI increased CBF 21 ± 4 ml/100 g per min and reversed LP in all. Propranolol (PROP) (0.1 mg/kg intravenously) decreased CBF 14 ± 2 ml/100 g per min and reversed LP in all patients. Both nitroprusside (NITROP) and PROP reversed LP in experimental AMI: PROP in all dogs and NITROP in five of eight dogs who were lactate producers. We conclude the following. These data provide a metabolic basis for selection of interventions designed to salvage myocardium. PROP is indicated in patients exhibiting the hyperdynamic consequences of catecholamine stress, ECP, and/or NITROP in patients with left ventricular failure, and IACP or *l*-NE in patients with shock.

206. Griseofulvin, a Potent Inducer of Porphyric Hepatomas and Hyperhemopexinemia in Mice. In Vivo and In Vitro Study. URSULA MULLER-EBERHARD AND DEREK J. CRIPPS,* La Jolla, Calif., and Madison, Wis.

Hemopexin (Hx), a porphyrin-binding serum protein, is synthesized in the liver. In this study, variations in Hx on an erythrohepatic porphyria were determined with Swiss Webster mice on 1% griseofulvin in the feed. Hx concentrations, measured by radial immunodiffusion, were compared with protoporphyrin and coproporphyrin content of liver and erythrocytes. Liver weights rose remarkably with ratios of mean normal to porphyric hepars of 1:2.1, 1:2.8, and 1:3.8 after 10, 20, and 46 days, respectively. This weight gain was due to overproduction of porphyrins, bile duct proliferation, biliary obstruction, and an increase in number, size, mitosis, and binucleation of hepatocytes. Porphyrins appeared first in the hepar and reached 415 μg protoporphyrin and 6 μg coproporphyrin per g wet liver weight on the 46th day when erythrocyte content was also highest (41 μg/100 ml packed cells). Erythrocyte protoporphyrin was maximal at day 21 (1850 μg/100 ml packed cells). Increasing Hx levels correlated positively with the log of coproporphyrin ($r = 0.934$, $P < 0.05$) and protoporphyrin ($r = 0.969$, $P < 0.01$) in RBC and the log of coproporphyrin in the liver ($r = 0.999$, $P < 0.05$). Concentrations of albumin, the other serum porphyrin binder (1973. *J. Biol. Chem.* **248**: 3796), remained unchanged. Cells of porphyric liver explants resembled cytologically those of control mice but survived longer. Hepatocytes migrated into the medium on day 2 and divided after 10 days; and subcultures, made on day 21, survived for 2 mo. Microfluorospectrophotometry suggested protoporphyrin (peak at 637 nm) to be limited to the cytoplasma, most prominent in liver cells containing brown pigmented granules. The development of porphyric hepatomas and hyperhemopexinemia in mice warrants monitoring hemopexin levels in patients receiving griseofulvin therapeutically. (Research supported by NIH Grants HD-04445, HE-08660, AM-16737, and AMO-9995.)

207. A New Specific Stoichiometric Assay for 5-Fluorodeoxyuridylate (FdUMP): Use in Kinetic Studies of 5-FU Effect in Normal and Tumorous Tissue. CHARLES E. MYERS,* ROBERT C. YOUNG,* DAVID G. JOHNS, AND BRUCE A. CHABNER,* Bethesda, Md.

An understanding of the pharmacologic effects of 5-FU on normal and malignant tissue requires elucidation of the relationship between inhibition of DNA synthesis and the intracellular level of the active metabolite 5-fluorodeoxyuridylate (FdUMP). Kinetics of FdUMP generation can now be monitored by a new enzymatic assay based on the stoichiometric inhibition of *L. casei* thymidylate synthetase (TS) by FdUMP. This assay detects as little as 1×10^{-12} mol of FdUMP in tissue extracts. Other metabolites of FU, including FUMP and FUdR, do not interfere with the assay. Tissue dUMP pools can be measured by determining the total conversion of 5,10-methylenetetrahydrofolate to dihydrofolate in the TS reaction with tissue extract as the sole source of dUMP. These assays were used to follow serial changes in dUMP and FdUMP after 5-FU administration (50 or 100 mg/kg) to mice bearing P1534 ascites tumor. Intracellular FdUMP exhibited a two-phase disappearance with half-lives of 6 h and 9 days in the ascites tumor. dUMP levels rose steadily after 5-FU in both normal and tumor tissues. Recovery of DNA synthesis, as determined by [^{3}H]UdR incorporation into DNA, occurred when the dUMP: FdUMP ratio was 2–5 $\times 10^{+3}$ in both normal tissues (gastrointestinal mucosa, bone marrow) and tumor. This ratio is the same as that required to prevent inhibition of TS by FdUMP in vitro, and appears to be critical for recovery of DNA synthesis in vivo. These studies emphasize the importance of both dUMP and its competitive inhibitor, FdUMP, in determining the impact of 5-FU on mammalian cells and present sensitive methods for monitoring these nucleotides in tumor and normal tissues.

208. Preparative Chromatographic Technique for the Active Component of Transfer Factor. J. NEIDHART,* S. BALCERZAK,* AND A. LOBUGLIO,* Columbus, Ohio (introduced by J. V. Warren**).

Transfer factor (TF) is a low molecular weight dialyzable material which can transfer cellular immunity from an immune donor to a nonimmune recipient. Clinical trials have used a crude dialysate of leukocytes (TFD), and dosage has been expressed as the product of a certain volume or number of leukocytes. We have recently noted that the biologically active component of TFD adheres to Sephadex. This study describes a new method of TF preparation which utilizes direct chromatographic isolation of the active component and allows quantitation of material in terms of weight. Without prior dialysis, the leukocyte lysate is placed directly over a 100 × 5 cm G-25 Sephadex column and eluted with a volatile buffer (0.01 M NH_4HCO_3). The active component (TFC) elutes at 5/4 V_t and thus the lyophilized product is salt-free. TFC is relatively homogeneous in that it elutes as a single peak from DEAE and Bio-Gel P-10 and travels as a single band in 10% and 15% polyacrylamide-gel electrophoresis. It has a 280/260 ratio of 0.1–0.2 and contains ribose, phosphate, and amino acids. 250 μg of TFC prepared from donors immune to keyhole limpet hemocyanin, tuberculin, or coccidioidin successfully transferred delayed hypersensitivity to 10 of 10, 8 of 10, and 2 of 2 nonimmune recipients, respectively. The same dose of TFC from nonimmune donors did not transfer sensitivity. A single column run can accommodate cell volumes as large as $2–3 \times 10^{10}$ leukocytes with a yield of 5–6 mg of TFC. This simple, rapid method

of preparing large amounts of TFc allows a more quantitative and reproducible approach to clinical trials.

209. The Relation of Yeast Found in Sputum to Candida Pneumonia. HAROLD C. NEU AND MARGARITA SILVA-HUTNER,* New York.

The isolation of yeasts, particularly Candida species, has been the subject of increasing interest as more and more pulmonary infections in patients with immunological disorders are shown to be due to fungi. Sputum, bronchial, and tracheal specimens from hospitalized patients were examined over a 2 yr period. Clinical and epidemiological data were obtained on all patients with positive cultures. Of the sputum specimens 70% were positive for yeast. Tracheal samples were positive 39% but only 22% of bronchial washings. Only 24% of culture-positive specimens revealed either yeast or mycelial forms on direct initial examination. *C. albicans* accounted for 80% of isolates, *C. tropicalis* 6.3%, *C. krusei* 5.4%, *C. parapsilosis* 4.9%, and *T. glabrata* 2.7%. Mycelial forms were seen on direct examination in only 11% of specimens. Review of patient records and X rays showed that isolation of Candida even with mycelial forms did not correlate with pulmonary disease. Review of autopsy cases failed to demonstrate direct respiratory entry for Candida to produce the pulmonary pathology. A number of patients with generalized candidosis had lesions in kidneys, liver, and spleen, but not lungs. Most of the pulmonic disease due to Candida appears to be of hematogenous origin from intravenous or urinary sites. Sputum or tracheal isolation of Candida, even with serologic studies, does not differentiate patients colonized from those with yeast pneumonia.

210. Stimulation of Leukocyte-Derived Procoagulant Activity by Platelets and Platelet Membranes. JULIAN NIEMETZ* AND AARON J. MARCUS,** New York.

Thrombocytopenia frequently accompanies gram-negative sepsis, especially in the presence of disseminated intravascular coagulation (DIC), thereby suggesting increased platelet utilization. Since it is generally accepted that leukocytes can generate procoagulant (tissue factor) activity when incubated with endotoxin, the present studies were undertaken to determine whether platelets contribute to the procoagulant activity generated by leukocytes in the presence of endotoxin. Human or rabbit platelets and leukocytes were incubated with endotoxin, and the resultant procoagulant properties were evaluated in standard one- and two-stage assay systems. Platelets incubated with endotoxin alone had no clot-promoting properties, but a mixture of platelets, leukocytes, and endotoxin generated procoagulant activity which appeared rapidly and was fivefold greater than that produced by leukocytes alone with endotoxin. The enhancement produced by platelets was even more efficient if homogenates were used. Therefore we studied the effect of isolated human platelet membranes, granules, and the "soluble" fraction. The lipoproteins of the "soluble" platelet fractions were inactive, indicating that the stimulating activity was particulate in nature. Prior treatment of platelet membranes with phospholipase C or gangliosides or by extraction of lipid resulted in a loss of enhancing activity, whereas exposure to neuraminidase and trypsin had no effect. Incubation of human platelets with human leukocytes and endotoxin required the presence of serum (not necessarily containing clotting factors VII and X) for detection of the platelet stimulatory effect. These results suggest that platelets contribute a membrane lipoprotein to the procoagulant activity generated by leukocytes in the presence of endotoxin, and this phenomenon may aid in the understanding of some of the clinical and pathologic manifestations of gram-negative sepsis with DIC. (Supported by VA, NIH, and N.Y. Heart Association grants.)

211. Cell-Free Transcription of Human Globin Genes. ARTHUR W. NIENHUIS,* JUDITH A. KANTOR,* RAMON VELEZ,* ALAN STEGGLES,* GOLDER WILSON,* DANTE PICCIANO,* AND W. FRENCH ANDERSON, Bethesda, Md.

A mammalian cell-free transcriptional system was developed to study the expression of the globin genes. RNA was transcribed from human and rabbit erythroid chromatin by either mammalian (sheep liver) or *E. coli* DNA-dependent RNA polymerase. Globin messenger RNA (mRNA) sequences were detected in the in vitro transcribed product by hybridization with complementary DNA (cDNA) prepared with the enzyme, reverse transcriptase, using human or rabbit globin mRNA as template. Rabbit α- and β-cDNA's were prepared using isolated α-mRNA and β-mRNA. Results indicate that globin mRNA sequences were present in the RNA transcribed in vitro from erythroid but not from liver chromatin. Human erythroid chromatin from patients with sickle cell anemia or β-thalassemia directed the synthesis of globin mRNA sequences which hybridized efficiently with human globin cDNA ($Crot_{1/2} = 6 \times 10^1$ moles·s/liter) but less well with rabbit cDNA ($Crot_{1/2} = 1 \times 10^2$). RNA synthesized from rabbit erythroid chromatin could be annealed to both rabbit α- and β-cDNA, indicating that both globin genes were transcribed in this cell-free system. Although the polymerase from either sheep liver or *E. coli* was able to transcribe globin mRNA sequences from erythroid chromatin, the mammalian enzyme appeared to be more specific in that globin mRNA sequences represent a higher proportion (0.05% compared to 0.016%) of the RNA synthesized. Use of this cell-free system for gene transcription should facilitate the search for specific factors which regulate the expression of human genes and may aid in defining the defect in globin synthesis in thalassemia.

212. Chemotactic Activity in Cerebrospinal Fluid during Experimental Meningitis. CHARLES M. NOLAN,* ROBERT A. CLARK,* C. KENNETH MCALLISTER,* AND HARRY N. BEATY,* Seattle, Wash. (introduced by Robert G. Petersdorf**).

Chemotactic activity was measured in cerebrospinal fluid of rabbits with experimental pneumococcal meningitis. Meningitis was induced by intravenous injection of 10^6-10^7 *D. pneumoniae* after injection of 0.125% sterile mucin intracisternally. Meningeal infection, which progressed to spontaneous death within 3–4 days, was documented by clinical signs and positive CSF cultures at 24 h. Granulocytes from blood of normal rabbits were labeled in vitro with $Na_2Cr^{51}O_4$, and their migration in modified Boyden chambers incubated at 37°C for 3 h was quantitated by gamma counting techniques. Chemotactic activity was measured in pooled, sterile filtrates of CSF from groups of animals 24, 48, and 72 h after induction of meningitis. Simultaneous controls with normal CSF and proteinated Gey's buffer were routinely included. Normal CSF did not contain chemotactic activity greater than that of buffer solutions. Chemotactic activity in CSF from animals with meningitis increased exponentially with time ($r = 0.738$, $P < 0.01$) and at 72 h was 5-fold greater than that of controls ($P < 0.01$). Chemotactic activity was not decreased in CSF heated to 56°C for 30 min. Hemolytic complement was not detected in CSF of five animals 72 h after infection. Meningeal inflammation was quantitated by measurement of the area of granulocyte mass in serial microscopic sections of brain stained with hematoxylin and eosin and projected onto an XY plotter connected to a computer. Inflammation increased linearly to a peak at 72 h ($r = 0.835$, $P < 0.01$). Meningeal inflammation in experimental pneumococcal meningitis thus appears to parallel the development of CSF heat-stable chemotactic activity. (Research supported by grants from NIH.)

213. Hypothyroidism in Liver Patients Due to Failure of the Peripheral Conversion of Thyroxine (T_4) to Triiodothyronine (T_3). SETSUO NOMURA* AND CONSTANCE S. PITTMAN, Birmingham, Ala.

While the peripheral conversion of T_4 to T_3 was shown to be a major source of T_3 production, the role of liver to regulate this reaction is undefined. This study defines the role of liver by serial determinations of serum thyrotrophin (TSH), T_4, and T_3; by correlation of thyroid and liver functions; and by calculation of the conversion rate of T_4 to T_3. Our subjects included normal volunteers and patients hospitalized for liver cirrhosis with serum bilirubin 6 mg/100 ml or higher. Of the 37 patients studied, 9 showed reduced T_4, 4.3 ± 1.4 μg/100 ml (control 7.7 ± 1.2) and 22 showed reduced T_3, 45.5 ± 18.1 ng/100 ml (control 125.2 ± 23.4). TSH was elevated in 85.4% of the patients, 11–170 μU/ml (control 3.1 ± 0.7). These findings could not be explained by reduction of T_4-binding proteins, but were indicative of reduced effective concentrations of the thyroid hormones themselves. Serial sampling from patients with acute alcoholic hepatitis showed that TSH, T_4, and T_3 returned to near normal values when the liver function improved. The metabolic clearance rates of T_4 and T_3 and the percent of T_4 production metabolized to T_3 in the extrathyroidal pool were calculated by non-compartmental analysis after a simultaneous injection of [^{125}I]T_4 and [^{131}I]T_3. The conversion rates in the liver patients were reduced (7–20%) as compared to that in the normal controls (20–32%). Our results suggest that extrathyroidal deiodination of T_4 is the predominant source of T_3 and liver is a major site of T_4 conversion to T_3. Severe liver dysfunction may so impair this conversion as to render most of these patients hypothyroid. (Research supported by NIH Grant AM-15985.)

214. Chlorpropamide-Induced Inhibition of the Action of Parathyroid Hormone. PATRICIA NUMANN,* RICHARD COULSON,* AND ARNOLD MOSES, Syracuse, N.Y.

The effect of chlorpropamide on the action of PTH was investigated in parathyroidectomized rats because chlorpropamide augments the action of ADH, and because of similarities in the action of ADH and PTH. Chlorpropamide (20 mg/100 g) did not change serum calcium or phosphorus or urinary cAMP, but caused a slight phosphaturia. The subcutaneous injection of 40 U PTH increased serum calcium, urine phosphorus, and urine cAMP and decreased serum phosphorus. These PTH effects were less pronounced after chlorpropamide. Serum calcium was 8.0 ± 0.3 (SE) mg/100 ml 4 h after PTH, and 7.0 ± 0.2 after PTH and chlorpropamide. Serum phosphorus was 8.0 ± 0.3 mg/100 ml after PTH and 10.4 ± 0.5 after PTH and chlorpropamide. Chlorpropamide decreased cAMP excretion after PTH from 28.7 ± 2.9 to 15.7 ± 1.9 nmol/4 h. Chlorpropamide did not alter the phosphaturic effect of PTH. 2 min after intravenous injection of 40 U PTH into parathyroidectomized rats, the kidneys were removed and assayed for cAMP. Kidneys from untreated rats had 4.4 ± 0.6 nmol cAMP/g dry weight. This was unchanged by chlorpropamide injection, but increased after PTH to 21.7 ± 1.8 nmol/g dry weight. Chlorpropamide injection resulted in a lesser PTH-induced rise in renal cAMP, 13.7 ± 3.6 nmol/g dry weight. There were similar effects of 3.3 mM chlorpropamide on renal cAMP of kidneys from intact untreated rats which were perfused in vitro with 1 U PTH/ml. These data demonstrate that chlorpropamide inhibits PTH-induced increases in renal and urinary cAMP and the hypercalcemic and hypophosphatemic responses to PTH. The observations add to the reports of altered cAMP-mediated hormonal actions by chemotherapeutic agents. (Supported by VA Research Project 5855-01.)

215. Intestinal Fatty Acid Binding Protein (FABP): Studies of Physiological Function. ROBERT K. OCKNER* AND JOAN A. MANNING,* San Francisco, Calif. (introduced by R. J. Havel**).

FABP is a 12,000 mol wt protein in intestinal cytosol of rat and man, which noncovalently binds long-chain FA and in rats is immunochemically identical with FABP in liver, heart, and adipose tissue (other immunochemically distinct 12,000 mol wt FABP may exist). Intestinal FABP, which is highest in jejunum, increases during high fat intake, consistent with its postulated relationship to fat absorption. We have further studied its physiological role. By quantitative radial immunodiffusion, villus FABP concentration (996 ± 86 μg/g) exceeds that in crypts (356 ± 58 μg/g, $P < 0.005$), a distribution corresponding to lumenal fat absorption but opposite that of processes occurring mainly in crypts, including FA synthesis. Flavaspidic acid-N-methyl glucaminate (FLAV) is mutually competitive with oleic acid (OA) for in vitro binding to FABP. FLAV is absorbed but not activated or esterified by intestinal mucosa. In everted gut sacs, 1.8 mM FLAV (uptake: 247 ± 25 nmol/g per 4 min) did not significantly affect mucosal uptake of equimolar [^{14}C]OA from mixed micelles (control: 517 ± 25 nmol/g per 4 min; FLAV: 475 ± 16 nmol/g per min). In contrast, FLAV profoundly inhibited [^{14}C]OA incorporation into TG by gut sacs (control: 225 ± 13 nmol/g per 4 min; FLAV: 84.5 ± 7.2 nmol/g per 4 min; $P < 0.001$), resulting in a corresponding accumulation of mucosal unesterified [^{14}C]OA (control: 203 ± 20 nmol/g; FLAV: 318 ± 9 nmol/g; $P < 0.001$). Virtually identical effects were produced by 1.8 mM α-bromopalmitic acid. Inhibition by FLAV was partially reversed by increasing OA, and was not associated with significant change in conversion of [^{14}C]acetate to $^{14}CO_2$. In intestinal microsomes, 0.1 mM FLAV inhibited the monoglyceride acyltransferase-mediated incorporation of 0.04 mM and 0.08 mM palmityl CoA into glycerides by only 22% and 21%, respectively. Our studies suggest that FLAV, a competitive inhibitor of FA binding to FABP, inhibits intestinal incorporation of absorbed FA into TG at a step after mucosal uptake but preceding transacylation of monoglyceride in the endoplasmic reticulum. Although an effect of FLAV on microsomal FA activation is not excluded, these observations support the concept that FABP participates in the intracellular transport and metabolism of FA entering intestinal mucosa. (Research supported by grants from NIH.)

216. Ion Flux Across Canine Tracheal Epithelium. RICHARD OLVER,* BRIAN DAVIS,* MATTHEW MARIN,* AND JAY NADEL, San Francisco, Calif.

Changes in the net flow of water across the tracheobronchial epithelium are likely to modify the physical properties of its secretions. Since bulk water flow is coupled to net ion flux in many epithelia, the present study was performed as an initial step in characterizing water movement across the trachea. Potential difference (PD), short-circuit current (SCC), and the fluxes of Cl^- were measured across the posterior membranous portion of the canine tracheal epithelium. The epithelium was dissected free from muscle and interposed between two chambers containing Krebs-Henseleit solution maintained at 37°C and bubbled with 95% O_2-5% CO_2. After 3–10 h, PD and SCC reached stable maximum levels; PD = 23.9 ± 5.3 mV (SE, $n = 9$) with mucosa negative to serosa; SCC = 119 ± 16 μA/cm² (SE, $n = 9$). The addition of ouabain (10^{-4}M) to the serosal chamber ($n = 4$) or bubbling 100% N_2 ($n = 2$) abolished PD and SCC. Bidirectional fluxes of Cl^- were measured using $^{36}Cl^-$ under short-circuit conditions. In each of five studies there was a net flux of Cl^- from serosa to mucosa which accounted for 77.7 ± 6.8% (SE) of the SCC. This active transport of Cl^- may generate an

osmotic force which results in the movement of water into the tracheal lumen. (Supported by NIH Grant HL 06285.)

217. The Dynamics of Cystine Accumulation and Depletion in Cultured Skin Fibroblasts from Patients with Nephropathic Cystinosis. ROBERT OSHIMA,* JOHN C. CRAWHALL, POVEL N. PAUS,* AND JERRY A. SCHNEIDER,* La Jolla, Calif.

The rate of removal and reaccumulation of cystine in cystinotic fibroblasts was investigated. Maximal removal of cystine was achieved in 8–12 h by incubation of the cells in cystine-free medium or cystine-free medium containing dithiothreitol (DTT 1 mM). 60% of the cystine was removed by the former method and 90% by the latter if the DTT medium was replaced with freshly prepared reagent every 2 h for 6 h. Reaccumulation of cystine took place within 12 h, but this was prevented if the cells were maintained in cystine-free medium. Serum-free medium did not restrict cystine reaccumulation. Cystinotic fibroblasts which were not DTT treated were incubated in [^{35}S]cystine medium and showed rapid incorporation of isotope into glutathione and cystine, whereas normal cells showed incorporation only into glutathione. Isotope incorporation into the cystine pool of cystinotic cells which had been cystine depleted by DTT was reduced compared with untreated cells. This suggests that the percent of ^{35}S found as [^{35}S]cystine in such experiments reflects the total cystine pool of these cells and could result from a disulfide exchange reaction. *N*-ethyl-maleimide studies with cystinotic cells have shown that the intracellular glutathione is fully reduced and the cystine fully oxidized and hence they must exist in separate compartments in the cell. We have shown that the stored cystine can be quickly removed and reaccumulates at a comparable speed, indicating that the abnormal cystine storage is not due to a complete impermeability of a subcellular membrane. (Research supported by grants from the NIH, AHA, VA and NRCSH.)

218. Pyrimidine-Specific 5′-Nucleotidase in Human Erythrocytes: Diminished Activity in Lead (Pb) Overburden and Inhibition by Pb In Vitro. DONALD E. PAGLIA,* WILLIAM N. VALENTINE,** AND JAMES G. DAHLGREN,* Los Angeles, Calif.

This laboratory recently demonstrated a pyrimidine-specific 5′-nucleotidase in human erythrocytes (RBC) and its severe deficiency in an inherited hemolytic syndrome. The latter was associated with very prominent basophilic stippling and large accumulations of RBC pyrimidine nucleotides. The nucleotidase is inactive with purine nucleotides or β-glycerophosphate as substrates, is more active with UMP than CMP, has pH optimum of 7.5–8.0, and is inactivated by EDTA. The presence of basophilic stippling in both Pb poisoning and the hereditary deficiency prompted studies of Pb effects on the nucleotidase. In vitro, incubation of hemolysate with Pb for 10–20 min induced 90% inhibition of nucleotidase activity with 10^{-4} M Pb and 50% with 10^{-5} M. Incubation of *intact*, saline-washed RBC with glucose and 10^{-3} M Pb for 1 h, with subsequent Pb removal by washing, inhibited nucleotidase activity 50–90%. No reactivation occurred after prolonged dialysis (pH 8.0, saline-Tris buffer, $MgCl_2$ 0.01 M), a procedure not affecting nucleotidase activity in unexposed hemolysates. In seven subjects chronically exposed to industrial Pb (with variable general symptoms, minimal or no anemia, and lacking prominent basophilic stippling), recent blood Pb levels indicative of overburden ranged from 60 to 105 μg/100 ml, most of which is in RBC. Nucleotidase activity was reduced to 20–50% of normal, the lowest values occurring in subjects with highest blood Pb. Marked basophilic stippling, a highly variable phenomenon in Pb-induced anemia, may occur only on attaining inhibition of RBC pyrimidine-5′-nucleotidase activity to the severe degree present in the inherited deficiency. (Research supported by NIH Grant 12944.)

219. Liver Ultrastructure in Abetalipoproteinemia and Hypobetalipoproteinemia. JOHN C. PARTIN,* JACQUELINE S. PARTIN,* AND WILLIAM K. SCHUBERT,* Cincinnati, Ohio (introduced by Edward L. Pratt).

The liver is a main source of betalipoprotein, but virtually no published information is available concerning liver morphology and organelle pathology in abetalipoproteinemia (ABL), either from autopsied cases or from liver biopsy. We report liver morphology in three ABL and two hypobetalipoproteinemia (HBL) children. The five children derived from three families: ABL family I, ABL family II, and HBL family I, respectively. The three ABL children had grossly fatty intestinal epithelia; their liver biopsies were grossly fatty with large droplet cytoplasmic triglyceride and fatty lake formation. Hepatic Golgi apparatus was abnormal in all three, with virtual absence of *trans*-Golgi vesicles and *circum*-Golgi smooth endoplasmic reticulum. Very low density lipoprotein (VLDL) particles were absent. *Cis*-Golgi vesicle formation was apparently normal. The liver of the HBL sib derived from ABL family I was moderately fatty with excessive multivesicular body and lysosome formation adjacent to the Golgi apparatus; his intestinal epithelium was normal. The liver of the HBL infant from HBL family I was ultrastructurally normal with no fat storage, but her intestinal epithelium was grossly fatty and indistinguishable from that of "classical" ABL. These morphologic data show that (*a*) the hepatocyte ultrastructural defect in ABL is absent *trans*-Golgi vesicles and *circum*-Golgi smooth endoplasmic reticulum with absent VLDL formation; (*b*) there is a *hepatic* form of HBL with deficient VLDL formation and excessive multivesicular body formation but morphologically normal intestinal mucosa; and (*c*) there is an *intestinal* form of HBL with defective chylomicron formation but apparently normal hepatic triglyceride secretion. (Supported by NIH Grant RR-123.)

220. Time-Related Acceleration of Amino Acid Uptake in Human Leukemic Leukocytes; Inhibition by Amino Acids and Cortisol. WILLIAM A. PECK, PATRICK A. FRENGLEY,* AND MARSHALL A. LICHTMAN, Rochester, N.Y.

To study the regulation of amino acid transport in human leukemic leukocytes, we examined initial (10–20 min) rates of uptake (V_o) of [3-^{14}C]alpha-aminoisobutyric acid (AIB) periodically during 4 h of incubation in vitro. In amino acid-deficient medium (Hanks' salt solution plus 20% human serum), V_o increased progressively in lymphocytes from eight patients with chronic lymphocytic leukemia (CLL) (1 h = 3.1 ± 0.5 μmoles·kg cell water^{-1}·min^{-1}, 4 h = 5.6 ± 0.4) and in myeloblasts from five patients with acute myeloid leukemia (AML) (1 h = 6.8 ± 1.3, 4 h = 15.3 ± 3.8). Increases were due to enhancement of active (concentrative) AIB transport, were associated with increased capacity (increased V_{max}) and affinity (decreased apparent K_m) of the transport system, and did not reflect cell damage or changes in (*a*) intracellular free amino acid, ATP, or cyclic 3′,5′-AMP concentrations or (*b*) the composition of the medium. Exposure to high concentrations (4 mM) of AIB or alanine for 0.5–3.0 h reduced by 75% the time-related increases in V_o estimated after leukocytes were transferred to deficient medium. Simultaneous treatment with cortisol (1 μM) in vitro nullified increases in CLL but not in AML cells. Blockade of protein synthesis (10 μg/ml cycloheximide) was inhibitory in both cell types. 1

and 3 h V_0 were 60–70% lower in cells isolated from two patients (CLL) immediately after cortisol infusion (1 g, 4 h) than after control (NaCl) infusion. Time-related enhancement of active AIB transport appears to represent an adaptive response to environmental amino acid deprivation that requires continued protein synthesis and might influence leukemic leukocyte survival in vivo. Inhibition of adaptation could contribute to the therapeutic effects of glucocorticoids in CLL. (Supported by grants from NIH.)

221. The Influence of Food Deprivation on L-Dopa Metabolism. MARK A. PEPPERCORN,* BARRY R. GOLDIN,* AND PETER GOLDMAN,* Boston, Mass. (introduced by Irving H. Goldberg**).

After oral administration of L-dopa, m-hydroxyphenylacetic acid (m-HPAA) is found in the urine of conventional but not of germfree rats. The transformation of L-dopa to m-HPAA can proceed through either of two dehydroxylation reactions at the para position, that between dopamine and m-tyramine or that between 3,4-dihydroxyphenylacetic acid and m-HPAA. Although both reactions occur in conventional but not in germfree rats, only the former reaction is quantitatively sufficient to account for the overall transformation of L-dopa to m-HPAA. Attributing the conversion of dopamine to m-tyramine to the activity of the intestinal flora is consistent with alterations in L-dopa metabolism observed in patients fed antibiotics and with transformations of L-dopa by the intestinal flora cultivated in vitro. It has now been found that rats fed L-dopa (100 mg) excrete less m-HPAA in the urine after food deprivation for 4 days (0.4 mg) than when they are allowed to feed normally (1.8 mg) ($P < 0.01$). m-HPAA excretion is also significantly decreased in response to dopamine feeding when food is deprived for 1, 2, or 4 days. Food deprivation has no effect on the conversion of m-tyramine to m-HPAA. Thus it appears that food deprivation affects the conversion of dopamine to m-tyramine. During food deprivation the intestinal flora of the rat is altered. Since certain transformations of exogenous compounds attributable to the flora are not decreased during the period of food deprivation, it appears that selective changes in the flora occurring during food deprivation are responsible for the diminished conversion of dopamine to m-tyramine. (Supported by NIH Grants RR05479-10, CA10736-04, and CA15260-01.)

222. Pregnancy Serum Effects on Human Polymorphonuclear Leukocyte Functions. ROBERT H. PERSELLIN* AND JUNE K. RUSHING,* San Antonio, Tex. (introduced by Samuel J. Friedberg**).

The subsidence of rheumatoid arthritis in pregnancy has been related either to plasma cortisol or to a circulating lysosomal stabilizer. Since an experimental mode of inflammation—one mediated by lysosomes of the polymorphonuclear (PMN) leukocyte—can be suppressed by pregnancy serum, we have investigated the direct effects of pregnancy serum on PMN activity. Normal human peripheral blood PMN's in buffer containing serum either from females in the third trimester of pregnancy or from umbilical cords were studied for their ability to ingest both viable and heat-killed Staphylococci. Normal adult male and female serum pools were also studied. At ratios of from 2–5:1 organisms: PMN, pregnancy serum at various concentrations permitted efficient ingestion. At 15 min incubation with either pregnancy or normal female serum at 15% concentration, 65% of PMN's had ingested bacteria. Male and cord serum pools were not significantly different (62–72% phagocytosis). Matched individual pregnancy and cord sera equally affected phagocytosis, both inducing an 11- to 14-fold increase in uptake when compared to a control without serum. At 15 min, bacterial viability was 62.6% ± 5.1 SEM in pregnancy serum, 56.8 ± 6.3 in normal female, 56.3 ± 3.5 in cord, and 52.2 ± 6.0 in normal male serum. However, bacterial killing in pregnancy serum was significantly less efficient at 60 min incubation, 60.4% remaining compared with 39.2% in cord serum. PMN viability (dye exclusion or lactic dehydrogenase activity) was not affected. β-Glucuronidase released after phagocytosis in pregnancy serum was 7.7% of total and in cord serum was 7.8%; 11-hydroxycorticoids were 1.4×10^{-6} M in pregnancy serum and 2×10^{-7} in cord serum. Thus, PMN leukocytes show rapid and efficient interiorization of bacteria in all serum pools, but subsequent metabolic events are significantly altered in the presence of pregnancy serum.

223. Pumping Performance of the Hypertrophied Spontaneously Hypertensive Rat Left Ventricle. MARC A. PFEFFER,* JANICE M. PFEFFER,* AND EDWARD D. FROHLICH, Oklahoma City, Okla.

In systemic hypertension left ventricular mass progressively increases to meet its continuous demand to eject the stroke volume against an unrelenting increased afterload. However, the capability of this hypertrophying ventricle to perform its function is unresolved. The spontaneously hypertensive rat (SHR) provides a model for studying alterations of cardiac performance during hypertension-induced hypertrophy. External cardiac work was calculated from cardiac output (ascending aortic flow, electromagnetic) and arterial and venous pressures, in ether-anesthetized open-chest male normotensive Wistar (NWR), Wistar Kyoto (WKY), and SHR rats. Cardiac function curves were produced by rapid intravenous infusions (Tyrodes solution, 40 ml/min per kg) until output failed to increase further despite continued right atrial pressure elevations (usually 30 s). Peak stroke and minute work were also expressed per gram of ventricular tissue. Peak minute and stroke work of 10 wk old WKY and NWR were similar: 92 ± 8 and 87 ± 6, 0.252 ± 0.021, and 0.248 ± 0.014 g-m, respectively. However, age-matched SHR demonstrated greater ($P < 0.001$) minute and stroke work (160 ± 12 and 0.436 ± 0.035 g-m) than both normotensive controls. Despite the increased SHR ventricular weight, peak minute and stroke work per gram were also greater ($P < 0.01$). With growth (20 wk) the normotensives increased peak work; however, when expressed per gram ventricle work remained constant. In contrast, work per gram SHR ventricle declined during this time. Therefore, the early hypertrophying ventricle not only ejects its demanded volume, but its potential is greater. However, with continued demand this performance was not maintained and, unlike the normal, deteriorated.

224. The Relation between Enzymatic Maturation and Resistance to Hypoxia of Postnatal Rabbits. JAMES R. PHILLIPS,* JAMES THEODORE,* AND EUGENE D. ROBIN,** Stanford, Calif.

The remarkable ability of newborn rabbits to withstand anoxia (30 min) is lost rapidly reaching adult levels (1.5 min) by 18 days of postnatal life. This loss of resistance parallels neurological maturation and in other mammalian species also parallels increasing O_2 consumption of the brain. Little is known about the enzymatic dynamics responsible for these events. We have measured brain activity of pyruvate kinase (PK) and cytochrome oxidase (CO) (terminal enzymes in glycolysis and oxidative phosphorylation), as quantitative indices of glycolytic capacity and O_2 consumption capacity, respectively, in rabbit brain at approximately 3-day intervals from birth to 18 days. CO and PK activity increased from day 1 to 18 (CO day 1: [4.24 v ± 0.570 v]-day 18 [10.64 v

± 2.480 v]; PK day 1: [158.8 v ± 14.70]-day 18 [229.4 v ± 9.20]). The increases in CO and PK were not linear. CO increased most rapidly during initial 3 days of life, then a more gradual increase until day 13, and finally the slowest rate of increase. This pattern is the reciprocal of the three slopes of survival times in 100% N_2, suggesting that the resistance to hypoxia of postnatal rabbits is inversely related to the O_2 consumption of brain. PK activity (approximately 60% of adult levels at birth) is stable until day 9, then rapidly rises to day 14, followed by a terminal slow increase, suggesting that increasing glycolytic capacity may primarily provide support for oxidative phosphorylation. These data suggest that increased resistance to hypoxia in neonates is related to decreased energy requirements of the newborn brain and not to an increased capacity to generate ATP through anaerobic glycolysis. (Research supported by grant from NHLI.)

225. Effect of Antimetabolites on the In Vitro Synthesis of Fibrinogen by Embryonic Chicken Hepatocytes. JOHANNA PINDYCK,* M. W. ROOMI,* RICHARD D. LEVERE, AND MICHAEL W. MOSESSON, Brooklyn, N. Y.

A tissue culture system has been developed for investigation of the effects of hormones and other agents on fibrinogen production. Chicken embryo hepatocytes were established in primary culture after dissociation from 16-day-old embryo livers by a modification of the technique of Granick. After 24 h of incubation, the medium above the hepatocyte monolayer was removed. Fresh medium was added containing test agents in 1 μl propylene glycol/ml of medium, and the culture incubated for an additional 24 h. During this period control cells produced 1.2 ± 0.5 μg of thrombin-coagulable, immunoassayable fibrinogen/100 μg cell protein, which could be increased to 120–160% of control cultures by addition of certain natural and synthetic steroids. Cycloheximide or puromycin, at concentrations equal to or greater than 0.25 μg/ml or 2.0 μg/ml, respectively, abolished fibrinogen production. This indicated that the fibrinogen elaborated into the medium had been newly synthesized. Actinomycin D, at concentrations between 0.5 μg/ml and 2.0 μg/ml, caused progressive inhibition of fibrinogen production; at 4.0 μg/ml production was abolished completely. However, at an actinomycin D concentration of 0.25 μg/ml (sufficient to inhibit transcription of DNA in this system), the amount of fibrinogen secretion significantly increased (160% of control) during the first 12 h, followed by a marked decrease in production. This suggests that fibrinogen is synthesized from a relatively long-lived mRNA, and that as other proteins with shorter-lived mRNA's ceased to be formed, increased tRNA and amino acids became available to permit the increased fibrinogen synthesis. Alternatively, fibrinogen synthesis may be controlled by a relatively labile repressor protein with a short-lived mRNA. (Supported by NIH Grant HL-13767.)

226. Erythropoietic Protoporphyria and Pb Intoxication; the Molecular Basis for Difference in Cutaneous Photosensitivity. SERGIO PIOMELLI,* ANGELO LOMOLA,* MAUREEN POH-FITZPATRICK,* TETSUO YAMANE,* EDWINA CARLOS,* CAROL SEAMAN,* AND LEONARD HARBER,** New York.

The concentration of erythrocyte protoporphyrin in chronic lead intoxication (PI) may be greater than in erythropoietic protoporphyria (EPP), yet PI exhibits no cutaneous photosensitivity. This study investigated whether the clinical differences result from different states of protoporphyrin within the respective erythrocytes. The "in vivo" persistence of protoporphyrin was measured in erythrocytes separated according to age on arabino-galactan discontinuous gradients. In PI, erythrocyte protoporphyrin decreased exponentially at a slow rate ($t_{1/2}$ 120 days), with fluorescence detectable in all erythrocytes, independent of age; after therapy, protoporphyrin remained elevated only in the oldest erythrocytes. In EPP erythrocytes, protoporphyrin rapidly decreased with a slope parallel to the disappearance of reticulocytes; a small percentage of EPP erythrocytes was intensely fluorescent; their fluorescence disappeared in vivo at the same rate as protoporphyrin. All young EPP erythrocytes were fluorescent, suggesting a single population. Protoporphyrin was bound to Hgb in both diseases. However, the fluorescence emission of erythrocyte suspensions or Hgb solutions from EPP peaked at 624 nm, whereas those from PI peaked at 590 nm. Lauryl-dimethylamine-oxide shifted the peak of EPP Hgb solutions from 624 to 630 nm (free protoporphyrin) indicating detachment of protoporphyrin from Hgb; but PI Hgb solutions remained unchanged at 590 nm. These data indicate different binding of protoporphyrin to Hgb in each disease. Heme synthesis is defective in PI; therefore, the relative excess of protoporphyrin can bind firmly at available heme sites. Heme synthesis is normal in EPP; therefore the excess protoporphyrin binds loosely to the surface of Hgb; thus it may diffuse rapidly from erythrocytes into skin to induce photosensitivity. (Supported by NIH Grant 7-RO1-ES-01040-01 and New York City Health Research Council Contracts 1-383 and U-2282.)

227. Specificity of IgA Protease for Human Immunoglobulins of the IgA_1 Subclass. ANDREW G. PLAUT,* Boston, Mass. (introduced by Samuel Proger**).

IgA protease is a bacterial, neutral endopeptidase found in human saliva and feces into which it is elaborated under physiologic conditions by the resident microflora. In catalytic amounts the naturally occurring enzyme and the enzyme partially purified from culture filtrates of *Streptococcus sanguis*, a numerically important oral microorganism, are capable of cleaving the heavy chain of IgA myeloma proteins to yield intact Fabα and Fcα fragments. Under identical conditions the enzyme does not proteolyze numerous other peptide and protein substrates, including human IgG, IgM, and IgE paraproteins. To further study IgA protease specificity, six human myeloma proteins of the IgA_1 subclass and four of IgA_2 subclass were purified and incubated with *S. sanguis* enzyme at pH 8.1, 37°C for up to 18 h. All starting IgA substrates were in part polymeric. Digests were examined for proteolysis by immunoelectrophoresis using unabsorbed anti-IgA sera, 5% polyacrylamide disk-gel electrophoresis (PGE), and molecular seive chromatography on Sephadex G-200 in columns carefully calibrated with marker proteins of appropriate molecular weight. By these criteria, all six IgA_1 proteins had been cleaved to Fabα and Fcα fragments, but IgA_2 proteins showed no evidence of proteolysis by change in size or electrophoretic mobility and had lost no antigenic determinants when studied by gel diffusion analysis. The relative susceptibility of the two subclasses in secretory IgA has not been studied although immunoelectrophoresis shows that human colostral IgA is in part cleaved into Fabα and Fcα fragments by IgA protease. The differential susceptibility of myeloma IgA proteins reveals a potentially important biological difference between these two subclasses which, in the form of secretory IgA, are known to coexist in fluids containing active IgA protease. (Supported by NIH Grant AM16607).

228. Myocardial Electrolytes and Water in Thyroparathyroidectomized Rats. PHILIP I. POLIMENI,* Chicago, Ill. (introduced by E. Page).

Clinical and pathological data suggest that tissue fluid accumulates in the interstitium of the "myxedema heart."

Quantification of intracellular electrolyte and water contents requires accurate measurement of extracellular space (ECS). Alterations of interstitial volume could mask even marked changes in cellular electrolyte concentrations, or alternatively, concentrations could remain quite constant despite marked changes occurring in tissue and plasma. For this reason, in vivo ECS was measured in rat ventricular muscle by two independent methods: (*a*) equilibration with the extracellular tracer [^{35}S]sulfate after nephrectomy; and (*b*) morphometry (point-counting) of histological sections from tissues fixed by immersion into saline-glutaraldehyde isotonic with rat plasma and postfixed with OsO_4. The equivalency of the two methods was established by comparing measurements made on the same tissue sample under various conditions. 8 wk after thyroparathyroidectomy (TPTX) ventricular ECS measured by tracer distribution was 0.219 ± 0.004 compared to 0.177 ± 0.006 g extracellular H_2O/g ventricle in control tissue. This increase in ECS was confirmed morphometrically (0.209 ± 0.004 cm^3/cm^3). The ECS increment was due to expansion of interstitium, which increased relative to cellular volume; however, absolute volumes of both compartments decreased. Results are compatible with inhibition of cell growth rather than a cell-to-interstitium shift of water. Cell water content remained constant after TPTX. Tissue and/or plasma electrolyte (Na, K, Ca, Mg, Cl) concentrations were markedly altered by TPTX, but the tissue alterations were almost completely due to the expansion of ECS relative to the cellular compartment. Although nominal cellular concentrations of Na and K rose slightly, and that of Mg showed a small decline, cellular electrolyte concentrations were remarkably little changed. (Supported by grants from USPHS, MIRU, and Chicago Heart Association).

229. Abnormalities in Triiodothyronine Metabolism Induced by Starvation in Man. GARY PORTNAY,* JOHN O'BRIEN,* APOSTOLOS VAGENAKIS,* MERRITT RUDOLPH,* RONALD ARKY, SIDNEY INGBAR,** AND LEWIS BRAVERMAN, Boston, Mass., and San Francisco, Calif.

Studies were performed to ascertain whether the hypometabolism of caloric deprivation is associated with alterations in peripheral thyroid hormone economy. After a 3-wk control period, nine obese volunteers were fasted totally for a period of 4 wk. Peripheral thyroid hormone metabolism was studied during each period, with differentially labeled thyroxine (T_4) and triiodothyronine (T_3). Measurements of serum thyroid-stimulating hormone (TSH) and both total and free T_4 and T_3 concentrations were made throughout the study. Thyrotropin-releasing hormone (TRH) tests were performed before and during fasting in six patients. Metabolic clearance rates for T_4 and T_3, assessed by both single-compartmental and noncompartmental analysis, were unchanged by starvation. Since total serum T_4 concentration was also unaffected, T_4 production rate remained normal. Serum T_3 concentration, in contrast, decreased from 145 ± 7 ng/100 ml (mean ± SE) to 66 ± 9 during starvation. Hence, T_3 disposal rates decreased from 36.4 ± 4.5 to 11.2 ± 0.7 μg/day. During starvation, the free fraction of both T_4 and T_3 in serum increased. Free T_4 concentration increased slightly during starvation (1.09 ± 0.07 to 1.32 ± 0.13 ng/100 ml), but free T_3 concentration decreased by about 50%, from 227 ± 8 to 118 ± 15 pg/100 ml. Neither basal serum TSH nor the response to TRH was significantly altered by starvation. We conclude that prolonged starvation in man leads to decreased production of T_3 but leaves the secretion rate of T_4 unchanged. Hence, decreased production of T_3 must result from either selective inhibition of T_3 secretion or, more likely, decreased conversion of T_4 to T_3 peripherally. Lack of compensatory TSH hypersecretion despite decreased total and free T_3 concentration in serum suggests that T_3 is not the only active form of thyroid hormone.

230. Opsonin-Dependent Phagocytosis of *Mycoplasma pneumoniae*. DWIGHT A. POWELL* AND WALLACE A. CLYDE, JR., Chapel Hill, N.C.

Several species of mycoplasmas recently have been shown to resist phagocytosis by polymorphonuclear or mononuclear phagocytes of human or animal origin. The potential importance of this phenomenon in disease pathogenesis led to a study of the interaction between pulmonary macrophages and *Mycoplasma pneumoniae*, a common respiratory pathogen of man. Monolayers of normal guinea pig alveolar macrophages were overlaid with a suspension of *M. pneumoniae* radiolabeled with tritiated thymidine. After 18–24 h, filamentous, motile mycoplasmas were seen attached to the coverslip and to the macrophages. These infected phagocytes were rounded and demonstrated little phagocytic activity as viewed with time-lapse phase cinematography. Autoradiographs of infected monolayers illustrated a particularly heavy concentration of silver grains, representing mycoplasmas, directly adjacent to the macrophages in a cap-like fashion. Few cells contained appreciable intracellular grains. By electron microscopy, mycoplasmas were seen to attach, by their characteristic terminal organelles, directly to the macrophage membrane. Few intracellular organisms were seen. Addition of specific antimycoplasma serum to the infected monolayers lead to an immediate increase in phagocytic activity demonstrated cinematographically. Spreading pseudopodia engulfed large numbers of surrounding mycoplasmas. Within 40 min, macrophages in autoradiographs were vacuolated and spread, and contained large numbers of intracellular grains. Comparable cells visualized with the electron microscope contained vacuoles packed with organisms in various stages of degeneration. These studies illustrate a role for specific opsonins in countering an inherent antiphagocytic property of *M. pneumoniae*. Characterization of these opsonins and their role in host resistance are the subjects of continued investigation. (Research supported by Contract DADA-17-71-C-1095, U.S. Army Medical Research and Development Command.)

231. Forearm Tissue Metabolism in Postabsorptive and 60-h-Fasted Man: Studies with Glucagon. THOMAS POZEFSKY,* ROBERT G. TANCREDI,* RICHARD T. MOXLEY,* JOHN DUPRE,** AND JORDAN TOBIN,* Baltimore, Md., and Montreal, Canada.

Metabolic effects of glucagon on extrahepatic tissues in man have been postulated based generally on studies employing supraphysiologic glucagon concentrations. In this investigation glucagon was infused into the brachial artery to achieve a physiologic increment in hormone concentration within the forearm and effects on muscle and adipose tissue metabolism sought. Seven subjects were studied postabsorptively, and seven after a 60-h fast when the plasma insulin-glucagon ratio was low and increased forearm tissue glucagon sensitivity anticipated. In postabsorptive subjects a 2 h intraarterial glucagon infusion (400–600 pg/kg per min) elevated local concentrations by 1,156 pg/ml. Glucagon did not alter muscle balance of glucose or amino acids (acidics and neutrals) or adipose tissue glycerol balance. Fasting reduced basal insulin (13.0 to 7.5 μU/ml) and increased glucagon (116 to 135 pg/ml) significantly. Muscle output of eight amino acids increased significantly over postabsorptive values, averaging 87% for threonine, glycine, alanine, methionine, isoleucine, and tyrosine. α-Aminobutyrate release increased twelvefold. Serine, taken up postabsorptively, was released in large amounts. In these fasted subjects the 2-h glucagon infusion elevated glucagon locally by 748 pg/ml

but was without effect on muscle balance of glucose, lactate, amino acids, or acetoacetate. Glucose, lactate, free fatty acid, and glycerol balance across adipose tissue were also unchanged. Local and systemic insulin concentrations were unchanged during all glucagon infusions. We conclude that physiologic increments in glucagon are without effect on muscle and adipose tissue metabolism in postabsorptive or 60-h-fasted man. Short-term starvation is characterized by a dramatic *increase* in muscle amino acid release in contrast to the conservation of muscle nitrogen accompanying prolonged starvation. (Supported by NIH grants.)

232. Alterations in Aldosterone Metabolism Induced by Adrenocorticotropin (ACTH). J. HOWARD PRATT* AND JAMES C. MELBY, Boston, Mass.

In man, ACTH activates aldosterone biosynthesis and has been considered to produce a transitory acceleration of aldosterone secretion with prolonged administration. In this study, the effects of ACTH on aldosterone secretion, plasma aldosterone concentrations, and the aldosterone metabolic clearance rate were examined in healthy adult male subjects ingesting in excess of 200 meq of dietary sodium per day. Repository ACTH injected every 12 h for 6 days induced a fivefold increment in the aldosterone secretion rate and a fourfold increment in urinary tetrahydroaldosterone excretion by day 6, whereas plasma concentrations of aldosterone did not change significantly. Daily plasma aldosterone levels varied from 6 to 9 ng/dl before the subjects received ACTH and between 3 and 9 ng/dl during the course of ACTH treatment. Metabolic clearance rates of aldosterone determined before and on the third day of ACTH administration were found to increase substantially during treatment with ACTH (increased from 45 to 91%). Metabolic clearance rates of aldosterone determined in subjects receiving the same diet, but no ACTH, were unchanged, thereby demonstrating no effect of dietary salt intake alone on the metabolic clearance rate. It is apparent from these studies that increased mineralocorticoid activity observed after administration of ACTH is due almost exclusively to the increased production of non-aldosterone mineralocorticoids and the net biological effect of increasing aldosterone secretion can only be ascertained when metabolic disposition and plasma concentrations are carefully monitored. In addition, it is proposed that the augmented metabolic clearance rate for aldosterone after ACTH stimulation can to an extent regulate aldosterone activity and may be a mechanism for escape from aldosterone effect. (Supported by NIH.)

233. Mitogen-Induced Rearrangement of Lymphocyte Membrane Receptors. F. QUAGLIATA* AND A. TARANTA, New York.

Movement of surface Ig and antigen receptors toward one pole of the lymphocyte can be induced by specific antigen. Using receptors for C3 and sheep erythrocytes (E) as index molecules and rosettes formed with E coated with amboceptor and complement (EAC) or with E alone as indicators, we have noted a similar rearrangement to occur after incubation with various mitogenic preparations. Purified human lymphocytes cultured without mitogens formed EAC (B cell) rosettes and E (T cell) rosettes, indistinguishable from those obtained before culture, with the red cells evenly distributed around the lymphocyte. In contrast, lymphocytes incubated in the presence of purified, soluble phytohemagglutinin, culture filtrates of *Streptococcus pyogenes* (containing streptococcal mitogen), or *Staphylococcus aureus* (containing staphylococcal mitogen) formed rosettes with the red cells distributed eccentrically, covering the lymphocyte surface only partially, resulting in a "horse-shoe," "cap," or "extreme cap" appearance. EAC rosettes were fewer and smaller than E rosettes, before (as expected) and after culture with the mitogens. E rosettes, after culture with the mitogens, were enlarged more than could be accounted for by the cellular enlargement entailed by the lymphocyte-to-blast transformation. A direct relationship was noted between [^{3}H]thymidine incorporation and percentage of E, but not of EAC rosettes. It therefore appears that nonspecific mitogenic stimulation causes movement of receptors for C3 and E determinants which are apparently not functionally related to the mitogens. This phenomenon may be explained either by a rigid structural relationship between the receptors for the mitogens and those for C3 and E determinants, or by postulating that lymphocyte stimulation might cause rearrangement of a variety of membrane components including those devoid of receptors for the mitogens. (Supported by NIH Grants AM01431, AM05064, and HE1464, and LSA.)

234. Solubilization of Calcitonin-Responsive Renal Cortical Adenylate Cyclase (AC). SHERRY QUEENER,* JOHN FLEMING,* AND NORMAN BELL, Indianapolis, Ind.

Several laboratories have attempted to solubilize from a variety of tissues adenylate cyclase (AC) which retains hormone responsiveness. We have accomplished this with a partially purified membrane preparation from pork renal cortex. AC was determined by conversion of [α^{32}P]adenosine triphosphate ([α^{32}P]ATP) to cyclic [^{32}P]adenosine monophosphate ([^{32}P]AMP). Cyclic [^{3}H]AMP was used to estimate recovery. Creatine phosphate and creatine phosphokinase were added as an ATP generating system. Results are expressed as picomoles per milligram protein per 15 min (mean ± SE). Basal activity (77 ± 4) in the partially purified preparation was increased to 756 ± 25 ($P < 0.001$) by NaF (5 mM), 605 ± 10 ($P < 0.001$) by highly purified bovine PTH (1 μM), 190 ± 19 ($P < 0.001$) by glucagon (10 μM), and 94 ± 4 ($P < 0.05$) by porcine calcitonin (10 μM). After treatment with Lubrol WX (0.5%) and NaF (5 mM) and centrifugation at 37,000 *g* for 15 min, AC response to calcitonin increased from 197 ± 11 to 393 ± 31 ($P < 0.01$). Response to glucagon and PTH was lost. After centrifugation at 100,000 *g* for 2 h at 4°C, AC increased with calcitonin (10 μM) from 256 ± 11 to 655 ± 62 ($P < 0.001$) and to 961 ± 37 ($P < 0.001$) with NaF (5 mM). Gel filtration (Sephadex G-200) of the 37,000 *g* supernate was quantitative with dithiothreitol (1 mM) and NaF (5 mM). AC eluted immediately after the void volume as a single peak and was increased from 675 ± 23 to 1590 ± 16 ($P < 0.001$) by calcitonin (10 μM). These studies show that calcitonin-responsive AC can be solubilized from renal cortical membranes and provide a means for purification and further characterization of the enzyme, receptor, and calcitonin-receptor interaction.

235. Role of Heme Oxygenase in Intestinal Absorption of Hemoglobin Iron. STEVEN B. RAFFIN,* CHOONG H. WOO,* AND RUDI SCHMID,** San Francisco, Calif.

Hemoglobin and myoglobin are a major source of dietary iron in man. Heme, separated from these hemoproteins by intraluminal proteolysis, is absorbed intact by intestinal mucosal cells. Subsequently, intramucosal cleavage of absorbed heme (ferroprotoporphyrin) is accompanied by iron release. Although this "heme-splitting" reaction initially was ascribed to xanthine oxidase (XO), we investigated the possibility that it is catalyzed by microsomal heme oxygenase (MHO), an enzyme which converts heme to bilirubin, CO, and iron. Microsomes prepared from rat intestinal mucosa contain enzymatic activity similar to that of MHO in liver and spleen. Thus, the intestinal enzyme requires nicotinamide adenine dinucleotide phosphate,

reduced form (NADPH); is completely inhibited by 50% CO; liberates bilirubin IX-α, identified spectrophotometrically and chromatographically; and releases inorganic ^{55}Fe from [^{55}Fe]-heme. Intestinal MHO activity was highest in the duodenum (0.09 ± 0.01 nmol bilirubin/min per 10 mg protein) where hemoglobin-iron absorption is most active. In iron-deficient rats (serum iron < 40 μg/100 ml), duodenal MHO activity was increased (0.24 ± 0.02, $P < 0.001$). This enhancement of enzyme activity was paralleled by an increase in hemoglobin-iron absorption, from 2.5% in control rats to 5.8% of administered dose in iron-deficient animals ($P < 0.005$). After iron repletion, normalization of serum iron was accompanied by a decrease in duodenal MHO activity towards control values. In contrast to MHO, duodenal XO activity fell to 11% of control values in iron deficiency and increased towards base line upon iron repletion. Our findings suggest that intestinal MHO, rather than XO, is involved in the absorption of hemoglobin iron, that enzyme activity is increased adaptively in iron deficiency, and that the intestinal mucosa is a potential source of bilirubin formation. (Supported by NIH Grants AM-17365 and AM-11275.)

236. Binding of Gentamicin and Related Aminoglycosides to Human Serum. CARLOS H. RAMIREZ-RONDA,* RANDALL K. HOLMES,* AND JAY P. SANFORD, Dallas, Tex.

The binding of antibiotics to serum components may affect antimicrobial activity, pharmacokinetics, toxicity, and drug interactions. The binding of gentamicin (Gm) and related aminoglycoside antibiotics to human serum was measured by equilibrium dialysis. Assays for aminoglycosides were performed by an enzymatic method using gentamicin adenyltransferase and [8-^{14}C]adenosine 5'-triphosphate. In previous reports that 25–30% of Gm is bound to serum, the concentrations of Gm tested were ≥100 μg/ml (usual total serum levels in man are ≤10 μg/ml). In the present study serum binding was tested at therapeutic antibiotic concentrations. At total concentrations of Gm between 1.7 and 8.9 μg/ml (21 separate determinations), approximately 70% of gentamicin was bound. Tobramycin and the purified components gentamicin Cl, Cla, and C2 were each 70–80% bound at 10 μg/ml or less. With increasing concentrations of Gm (10 to 20 μg/ml) the percentage of Gm bound decreased progressively to less than 50%. Sodium cephalothin (Ce) at concentrations increasing from 25 to 300 μg/ml of free drug (usual free levels of Ce in man are approximately 2–140 μg/ml) caused a progressive decrease in gentamicin binding from 77 to 54%. Because more Gm is bound to serum at therapeutic doses than previously reported, the displacement of bound Gm (or related aminoglycosides) by drugs such as Ce might significantly increase the concentration of free Gm. Although Gm and Ce are often administered concurrently to provide a broad spectrum of antibacterial effectiveness, this combination of antibiotics might increase the potential risk of oto- or nephrotoxicity associated with Gm therapy. (Research supported by grants from NIH.)

237. Deficiency of Arginine Esterase in Cystic Fibrosis. G. J. S. RAO* AND HENRY L. NADLER, Chicago, Ill.

Cystic fibrosis (CF) is an autosomal recessive disorder in which the basic defect is unknown. We have previously reported (1972. *Science (Wash. D.C.)* **177**: 610) that plasma of patients with CF is deficient in arginine esterase activity and proposed that the deficiency might explain the presence of the anti-ciliary factors found in CF serum. We report here the resolution of arginine esterase activity in plasma by ion-exchange chromatography and polyacrylamide-gel electrofocusing and document a deficiency in several types of arginine esterase. Blood was collected in citrated plastic tubes from 13 CF patients, 12 age-matched controls, and 5 obligate heterozygotes. Arginine esterase in plasma from the samples was activated using chloroform-ellagic acid. In control plasma, arginine esterase activity, assayed by the hydrolysis of α-*N*-benzoyl-L-arginine ethyl ester, was resolved into major (60%) and minor (40%) components by separation on DEAE-Sephadex at pH 8.0. In CF plasma, the major fraction was reduced to 30% of control, while the minor component was unaffected. Electrofocusing on 5% polyacrylamide-gels at pH 5–8 (200 V, 12 h) and subsequent staining for activity (Fujimoto et al. 1972. *J. Biochem.* **71**: 751) revealed six activity bands in control plasma as contrasted by five bands in all CF samples. Eight CF samples had one activity band missing, two had another missing, and the remaining three had a third type missing. All missing bands were within a narrow pH range. No qualitative differences were observed between controls and heterozygotes. These data demonstrate the quantitative reduction in specific column eluates and the absence of a single type of arginine esterase in CF plasma. The absence of a different activity band in different patients is consistent with the concept of genetic heterogeneity of the disease. (Research supported by grants from NIH and NCFRF.)

238. Quadriceps Capillary Basement Membrane Width in Diabetic Children. PHILIP RASKIN,* JAMES F. MARKS,* HENRY BURNS,* AND MARVIN D. SIPERSTEIN,** Dallas, Texas.

The effect of childhood-onset diabetes mellitus on quadriceps capillary basement membrane (QCBM) width has been examined by the electron microscopic morphometric method previously developed in this laboratory. QCBM widths in 48 children with diabetes mellitus were compared to those in 31 non-diabetic controls of the same age. In addition 45 adults (ages 20–30 yr, 19 diabetics and 26 controls) were also studied. The overall prevalence (95% tolerance level) of QCBM hypertrophy in diabetic children from ages 4 to 20 yr was 40%. Before age 5 no QCBM thickening was demonstrated, but after age 6 there was a gradual increase in the prevalence of this lesion so that over the next decade between 27 and 53% of the diabetic children showed the abnormality. After age 20 over 90% of diabetics demonstrated QCBM hypertrophy. In confirmation of our previous findings, no effect of the duration of the diabetes on QCBM width was observed, but there was a striking relationship between age of diabetic child at biopsy and the thickness of his QCBM. The control children showed a small but significant effect of age on QCBM width up to the age of 20. Our finding that QCBM hypertrophy is present at the time of acute onset of juvenile diabetes mellitus in 30% of children coupled with the fact that this lesion is not affected by duration of hyperglycemia strongly supports our previous conclusions that diabetic microangiopathy is independent of the hyperglycemia of this disease. Barring the possibility that pancreatic microangiopathy precedes that in muscle, these data provide evidence against the suggestion that basement membrane hypertrophy represents the primary lesion of the diabetic syndrome. (Research supported by Grant AM 13866 from USPHS.)

239. Thrombopoietin Activity in Disorders of Platelet Production. ROBERT E. REED* AND JOHN A. PENNER,* Ann Arbor, Mich. (introduced by C. William Castor**).

A number of disease states are associated with changes in circulating platelet numbers, but the mechanisms involved are not well understood. A major problem has been the difficulty in developing a simple and reliable assay for thrombopoietin, the hormone controlling platelet production. Our initial

investigations indicated a surprisingly rapid effect of platelet-stimulating substances on the young megakaryocyte which permitted us to adopt a relatively simple rat assay for thrombopoietin, based on changes in [^{75}Se]selenomethionine incorporation into platelet protein. The assay was applied to patients with abnormalities in circulating platelet numbers. As expected, plasma obtained from humans or rats with normal platelet counts did not increase the rate of isotope incorporation. Stimulatory activity, however, was detected in plasma from rats made acutely thrombocytopenic by injections of anti-platelet antibodies; in plasma from patients with thrombocytopenia secondary to marrow aplasia, acute leukemia, and acute idiopathic thrombocytopenic purpura; and from patients with thrombocytosis associated with thrombocythemia or polycythemia vera or metastatic carcinoma (a single case). Activity was not found in plasma from patients with postsplenectomy thrombocytosis. These results suggest that the thrombocytosis of the postsplenectomy state is probably related to the elimination of splenic sequestration, and that the basic abnormality in thrombocythemia and polycythemia vera may be related to dysfunctions of the control mechanisms rather than the development of autonomous hematopoiesis, at least with respect to megakaryocytopoiesis. (Research supported by grants from VA and NIH [Grant 5M01 RR-42].)

240. High Incidence of Thyroid Carcinoma in Unselected Patients with a History of Irradiation to the Neck. SAMUEL REFETOFF, JOHN HARRISON,* BORISLAV KARANFILSKI,* EDWIN L. KAPLAN,* AND LESLIE J. DEGROOT,** Chicago, Ill.

93 consecutive patients with history of neck irradiation but no prior knowledge of thyroid gland abnormalities were examined. 42 were men (M) and 51 were women (F). Mean age was 29 ± 7 yr, and age at time of irradiation was 4.6 ± 5.1 (range 0.1–27) yr. Patients were irradiated for tonsils (39), adenoids (10), tonsils and adenoids (7), thymus (26), acne (7), and other conditions (4). Of 25 (27%) with palpable thyroid abnormalities (12 M, 13 F), surgery was recommended to 17. The remaining 8 patients who had either diffuse thyroid gland enlargement or firmness with positive thyroid antibodies were given hormone replacement. Of the 17 patients selected for surgery, 3 refused operation and 4 were operated upon elsewhere with pathological diagnosis unavailable in one. Only 10 of the 17 had abnormalities on thyroid scan. Of the 13 (7 M, 6 F) patients with known postoperative diagnoses, 6 (4 M, 2 F) had carcinoma, 6 had benign lesions, and 1 (F) had fetal adenoma. Extrathyroidal extension and/or lymph node metastases were found in 4 of 6. Average age at diagnosis, age of irradiation, and M/F ratio of the cancer group were not significantly different from the whole group examined. However, of the 7 out of 93 patients with irradiation for tonsils and adenoids, 3 (43%) had carcinoma and 1 had fetal adenoma. Though data on dosage level is incomplete, it appears that the incidence of carcinoma may be related to dose rather than age at the time of irradiation. The incidence of thyroid carcinoma in asymptomatic young patients with a history of irradiation to the neck is at least 6.5% and higher than previously suspected. (Supported by NIH Grants CA-14,599 and AM-15,070.)

241. The Effect of Digoxin on Sinus Node Automaticity and Sinoatrial Conduction (SAC) in Man. JAMES A. REIFFEL,* J. THOMAS BIGGER, JR., AND ELSA G. V. GIARDINA,* New York.

To determine digoxin's effect on sinus node automaticity and sinoatrial conduction (SAC), atrial and His bundle electrograms were recorded during atrial premature stimulation and rapid atrial pacing at varying rates (60–150/min) before and 30 min after 0.75 mg digoxin intravenously in each of 10 patients with sinus bradycardia. After digoxin, mean sinus rate decreased in 4/10, increased in 3/10 ($P < 0.01$ in each, range −41 to +89 ms), and was unchanged in 3/10; mean SAC ($\overline{SAC}$) prolonged in 3/10 and shortened in 5/10 ($P < 0.01$, range −22 to +37 ms), and none developed first degree sinoatrial block. Sinoatrial reentry was seen only before digoxin in 3/10 and only after digoxin in 1/10. Sinus recovery time (SRT) is the time from the last paced P wave to the next spontaneous P wave, providing it is of sinus origin. After digoxin, the maximal SRT (max-SRT), i.e., the longest SRT seen at any of the pacing rates, shortened in 7/10 (in all three where $\overline{SAC}$ increased), lengthened in 2/10 (both showed decreased $\overline{SAC}$), and changed >100 ms in 4/10, but did not necessarily follow in magnitude or direction any change in sinus rate. In 1/10 an ectopic beat terminated the maximal postpacing pause after digoxin. Max-SRT was unrelated to BP changes. The atrial pacing rate producing max-SRT varied among the patients; after digoxin, it increased in 3/10, decreased in 4/10, and was unchanged in 3/10. We conclude that electrophysiologic testing is useful in elucidating the complex and clinically unpredictable effects of digoxin in sinus bradycardia. (Supported by NIH Grants HL 12738, HL 05864, and HL 70204 and a NYHA Grant-in-Aid.)

242. Changes in Cyclic Nucleotide Metabolism in Aorta and Heart of Neurogenically Hypertensive Rats: a Possible Trigger Mechanism in Hypertension. DONALD J. REIS, NOBUTAKA DOBA,* AND M. SAMIR AMER,* Evansville, Ind., and New York.

We sought to determine if acute fulminating neurogenic hypertension produced in rats by bilateral electrolytic lesions of the nucleus tractus solitarii (NTS) in brainstem (1973. *Circ. Res.* 32: 584) would result in changes in cyclic nucleotide metabolism in aorta and heart. By 2 h after lesions, when hypertension is well developed, there was a reduction of cyclic AMP (cAMP) in aorta and heart and elevation of cyclic GMP (cGMP) in aorta. The decreased cAMP content results from increased degradation due to elevation in the activity of the high affinity (low K_m) form of phosphodiesterase. The elevation of cGMP results from enhanced synthesis due to activation of guanylcyclase. Adenyl cyclase in lesioned animals, although exhibiting normal basal levels and sensitivity to stimulation by sodium fluoride, is insensitive to stimulation by isoproterenol. NTS lesions placed after systemic administration of 6-hydroxydopamine and bilateral adrenelectomy fail to produce an elevation of blood pressure and changes in nucleotides. Ligation of the aorta elevating carotid blood pressure to levels comparable with NTS-hypertension resulted in increased rather than decreased levels of cAMP. Thus changes in cyclic nucleotide metabolism in aorta and heart of lesioned rats depend on catecholamine release and are not due to mechanical distortion secondary to the increased arterial pressure. The pattern of change in cyclic nucleotides correlates with the increase in smooth muscle contraction and peripheral resistance characteristic of NTS-hypertensive rats. The metabolic changes in blood vessels may be a link through which enhanced sympathetic tone leads from transient to relatively fixed states of elevated vascular resistance and arterial pressure. (Supported by NIH and NASA.)

243. Effects of Phorbol Myristate Acetate on Normal and Chronic Granulomatous Disease Polymorphonuclear Leukocytes. JOHN E. REPINE,* JAMES G. WHITE, C. CARLYLE CLAWSON,* AND BEULAH M. HOLMES,* Minneapolis, Minn.

The influence of phorbol myristate acetate (PMA) (the active principle of croton oil) and heat-killed bacteria (HKB) on the metabolism and ultrastructure of polymorphonuclear leukocytes (PMN) from 22 normals and 6 patients with chronic granulomatous disease (CGD) has been investigated. PMA at concentrations of 0.1, 1.0, and 10 $\mu g/4 \times 10^6$ PMN produced 2-, 10- and 15-fold increases in normal neutrophil oxygen consumption, and in amounts of 0.1 and 1 μg caused 4- and 40-fold rises in the rate of [1-^{14}C] glucose oxidation. Ratios of 1 HKB:1 PMN, 10 HKB:1 PMN, and 100 HKB:1 PMN accelerated oxygen uptake by normal PMN 2-, 5- and 12-fold and ratios of 1 HKB:1 PMN and 50 HKB:1 PMN caused 2- and 10-fold increases in the resting rate of [1-^{14}C] glucose oxidation. Both PMA and HKB caused normal PMN to reduce nitroblue tetrazolium dye (NBT) and to develop many vacuoles in their cytoplasm. Vacuoles in PMA-treated neutrophils were empty, while those in cells combined with HKB were filled with bacteria. Amounts of ascorbic acid, epinephrine, or phenazine methosulfate sufficient to increase oxygen consumption by normal PMN did not cause vacuoles. PMN from the six patients with CGD did not respond metabolically to stimulation by PMA or HKB. Both agents caused vacuoles to form in CGD neutrophils, but failed to stimulate oxygen consumption, [1-^{14}C] glucose oxidation, or the reduction of NBT. PMA-treated neutrophils from heterozygote parents of CGD patients developed increased rates of oxygen consumption approximately 1/3 those of normal PMN. The similarities in physical and metabolic responses of normal and CGD neutrophils to PMA and HKB indicate that PMA is a useful tool for studying basic mechanisms of phagocytosis. The chemical agent is as effective as particulates in detecting abnormal neutrophils from CGD patients and heterozygotes and may provide clarification of the underlying defect in this disease. (Supported by Grants HL-11800, AM-15010, AM-15317, H7-06314, CA-11996, and CA-08832 from USPHS, and by the American Heart Association and the Minnesota Medical Foundation.)

244. Hemoglobin Bushwick, Beta 74 (E18) Gly-Val: an Unstable Hemoglobin Found in Extremely Small Amounts. R. F. RIEDER, D. J. WOLF,* J. B. CLEGG,* AND S. L. LEE,* Brooklyn, N. Y., and Liverpool, England.

A 34-yr-old woman of Italian ancestry had mild chronic hemolysis punctuated by episodes of acute hemolytic jaundice occurring with infections and sulfonamide ingestion. Incubation of her blood with new methylene blue resulted in the formation of multiple intra-erythrocytic inclusions, whereas the heat denaturation test at 50°C provided no evidence of an unstable hemoglobin. Starch-gel electrophoresis at pH 8.6 showed a faint hemoglobin fraction migrating slightly anodic to hemoglobin A_2. Similar inclusion body production and electrophoretic patterns were demonstrated in five other members from three generations of the family. The abnormal fraction, constituting 0.7–1.2% of the total hemoglobin, was separated by DEAE-cellulose chromatography. Heme depletion of this component was suggested by an absorption spectrum with an A540:A280 ratio one-half that of hemoglobin A. Fingerprint patterns were normal, but amino acid analysis of peptide βTp IX revealed that glycine in position 74 was replaced by valine. Glycine β74 lies in the heme pocket in a tightly packed area near the EF corner. The bulkier valine side chain may move the E and F helices apart, weakening the binding of the heme group. After incubation of peripheral blood from the proposita with [^{3}H]leucine for 15 min, the total radioactivilty in hemoglobin Bushwick was one-third that in hemoglobin A. The specific activity of β-Bushwick was 18 times that of β^A, and the β-Bushwick:β^A synthesis ratio was 1:3. These results indicate that the minute amount of hemoglobin Bushwick found in peripheral blood is not primarily a result of defective synthesis but is the consequence of a markedly preferential destruction of the unstable hemoglobin.

245. The Alpha Adrenergic Receptor: Its Role in Abnormal Insulin Secretion in Diabetes Mellitus. R. P. ROBERTSON* AND D. PORTE, JR., Seattle, Wash.

Absence of acute insulin responses (AIR) to intravenous glucose in diabetic patients with fasting plasma glucose (FPG) levels >115 mg/100 ml has been reported. Closer examination of patients with FPG >170 now reveals a transient decrement in circulating insulin (IRI) 3–10 min after a glucose (20 g intravenously) pulse ($-34 \pm 14\%$ maximal decrement, $\bar{x} \pm$ SD, percent basal IRI, $n = 8, P < 0.001$). Since endogenous alpha adrenergic activity is a potent inhibitor of insulin secretion and thus a potential mechanism for this glucose-induced IRI decrement in diabetics, a glucose pulse (5 g) was given to normal subjects during alpha adrenergic stimulation with epinephrine infusion (6 μg/min intravenously) to determine whether the glucose-induced IRI decrement could be reproduced. Decrements occurred in 5/5 subjects ($-17 \pm 8\%$, $P < 0.02$), indicating that alpha adrenergic activity mediated the decrements and suggesting that endogenous alpha adrenergic activity may play a role in the abnormal AIR found in diabetic subjects with FPG >170. To test this hypothesis, a glucose pulse (20 g intravenously) was given to diabetic subjects before and during alpha adrenergic blockade (phentolamine, 0.5 mg/min intravenously). In all instances (6/6) an improved AIR resulted (before = $-34 \pm 16\%$, during = $156 \pm 26\%$, $P < 0.001$). To determine whether the alpha adrenergic stimulation after glucose pulsing could be due to endogenous catecholamine (CA) release, total plasma CA levels after a 20 g glucose pulse in six normal subjects were measured by a double-isotope derivative assay. All subjects had immediate increments in CA which were maximal within 3–5 min (470 ± 550,* pg/cm^3, $\bar{x} \pm$ SD, $P < 0.01$) and returned to basal levels by 10 min. It is concluded that alpha adrenergic receptors, perhaps through stimulation by endogenous catecholamine secretion, may counterregulate glucose-stimulated IRI responses in normal subjects and play a role in the abnormal AIR observed in some diabetic subjects. (Supported by NIH grant.)

246. Prostaglandin Concentrations in Synovial Fluids in Rheumatic Diseases. DWIGHT R. ROBINSON* AND LAWRENCE LEVINE,* Boston and Waltham, Mass. (introduced by Marian W. Ropes**).

Prostaglandins (PG) of the E and A series have been shown to have inflammatory properties experimentally, and nonsteroidal anti-inflammatory drugs are strong inhibitors of PG synthesis. We have investigated the role of PG's in human rheumatic diseases by determining PG concentrations in synovial fluids by radioimmunoassay. Levels of PGB, which arises from PGE and PGA, were measured in 83 patients. Untreated patients with inflammatory arthritis (rheumatoid, gout, pseudogout, and infectious) had elevated PGB levels in synovial fluids with a mean of 351 ± 105 pg/ml (SEM). Patients with noninflammatory disease, DJD, had a lower mean of 86 ± 7 pg/ml, ($P < 0.02$). Patients with inflammatory effusions treated with aspirin and/or indomethacin also had lower levels of PGB than the inflammatory group (88 pg/ml ± 9, $P < 0.02$). Analysis of 15 representative fluids for PGF_{2a} gave undetectable values (<1 ng/ml) in all cases. However, concentrations of the 15-keto metabolite of PGF_{2a} ranged from <0.2 to 34 ng/ml, but with no significant difference between inflammatory and noninflammatory groups, in contrast to PGB. Four patients with acute synovitis responding clinically to

indomethacin or aspirin had elevated PGB levels which fell dramatically after treatment parallel to clinical resolution of the synovitis. We conclude that elevated levels of PGB are associated with inflammatory arthritis and that prostaglandins of the E and A series contribute to the pathogenesis of inflammation in rheumatic diseases and may be a factor of major importance in acute synovitis, such as gout and pseudogout. The results also indicate that inhibition of PG biosynthesis by nonsteroidal anti-inflammatory agents may be a major mechanism of their effects on synovitis in man. (Supported by grants from NIH.)

247. An Improved Quantitative Assay for Epstein-Barr (EB) Virus-Induced Transformation of Lymphocytes. J. ROBINSON,* M. NEWMUIS,* L. HESTON,* AND G. MILLER,* New Haven, Conn. (introduced by D. M. Horstmann**).

Transformation by Epstein-Barr virus (EBV) of human lymphocytes into continuous lymphoblastoid cell lines is the basis for an infectivity assay for the virus. This assay lacks quantitative precision in its reliance on morphologic changes, and it does not measure the rate of transformation. An improved assay for active virus that also measures the rate of transformation is based on the relative rates of DNA synthesis in EBV-infected and uninfected human umbilical cord lymphocytes. EBV was prepared from a marmoset lymphoblastoid cell line which produces high titers of virus. The rates of DNA synthesis in periodic samplings of cell cultures were determined by measuring the incorporation of [^{3}H]thymidine into cellular material insoluble in trichloroacetic acid. In comparison to uninfected cells, EBV-infected cells showed a marked increase in DNA synthesis which preceded but correlated with morphologic transformation. Uninfected cells and cells treated with antibody-neutralized EBV failed to transform after showing a brief, lesser increase in DNA synthesis. A quantitative relationship between the amount of virus added and the rate of DNA synthesis was found; with undiluted virus, stimulation of DNA synthesis was detectable within 3 days. Decreasing the amount of active virus by serial dilution or by graded exposure to UV light was accompanied by a corresponding increase in the time required for stimulation to occur. This assay should facilitate more precise studies of the mechanisms by which EBV affects the growth properties of lymphoid cells. (Research supported by grants from ACS [VC107], DRG [1147], and NIH [CA12055, HD00177].)

248. Studies on the Mechanism of Lithium-Induced Impairment of Urinary Acidification. J. ROSCOE,* M. B. GOLDSTEIN,* M. L. HALPERIN, D. R. WILSON,* AND B. J. STINEBAUGH,* Toronto, Canada, and Panama, Canal Zone.

Previous studies have indicated that acute lithium administration impairs urinary acidification. This study was undertaken to define both the type of and the mechanism for the lithium-induced renal tubular acidosis (RTA). Rats were infused with $NaHCO_3$ at a rate of 5 μeq/min. The pH and P_{CO_2} were determined on arterial blood (B) and four anaerobically collected urine (U) samples before and after the rats received 4 meq/kg LiCl intraperitoneally. The mean control values for five rats were: urine pH 7.67 ± 0.07, urine P_{CO_2} 68 ± 3.4 mm Hg, (U-B) P_{CO_2} 24 ± 3.0 mm Hg, and plasma HCO_3 29.6 ± 1.4 meq/liter. This (U-B) P_{CO_2} gradient indicates distal nephron (dn) H^+ secretion. After lithium administration (30–90 min) the values in these five rats were: urine pH 7.51 ± 0.06, urine P_{CO_2} 39.6 ± 1.3 mm Hg, (U-B) P_{CO_2} 5 ± 1.9 mm Hg and plasma HCO_3 21.6 ± 1.2 meq/liter. This marked decrease in (U-B) P_{CO_2} indicates that there is a significant reduction in dn H^+ secretion induced by lithium. Therefore LiCl produces a distal RTA of the secretory rather than gradient-limited type since the alkaline urine minimized the possibility of a significant hydrogen ion gradient. In addition, the presence of proximal RTA was suggested by the reduced renal HCO_3 threshold (plasma HCO_3 21.2 meq/liter, urine pH 7.51). We conclude that LiCl induces a reduction in H^+ secretion in both the proximal and distal nephron, resulting in proximal and distal RTA.

249. Study of Human Hereditary Spherocytosis in a Heterologous Species. MICHAEL W. ROSEN,* OSWALDO CASTRO,* AND STUART C. FINCH,** New Haven, Conn.

The ^{51}Cr survival and liver-spleen uptake of erythrocytes from 12 patients with hereditary spherocytosis (HS) were studied after transfusion into rats pretreated with ethyl palmitate (EP) and a cobra venom factor (CVF). The CVF, a C3 inhibitor, prevented intravascular hemolysis of the human RBC's, whereas EP depressed the animals' RES and thus delayed phagocytosis of transfused RBC's. In this model, the survival of RBC's from eight splenectomized HS patients was consistently shorter ($t_{1/2}$: 18.4 ± 4.85 h, $P < 0.002$) than that of healthy controls (32.7 ± 8.31 h). In contrast, of five nonsplenectomized HS patients, only two had significantly shortened survival. In one HS subject, the postsplenectomy RBC survival was significantly less than that of the presplenectomy value. In animals sacrificed after transfusion with RBC's from splenectomized HS patients, the mean ratio of total spleen to liver (S:L) ^{51}Cr uptake was 0.31 (0.20–0.57). This ratio was significantly greater than the S:L ratio (mean 0.21, range 0.14–0.25) in rats transfused with control human RBC's. The S:L ratios in rats transfused with HS RBC's from nonsplenectomized patients was not different from the control value. The short ^{51}Cr survival and increased S:L uptake of RBC's from all splenectomized HS patients is consistent with the hypothesis that splenectomy permits survival of a greater proportion of spherocytes which are extremely susceptible to rapid removal in this animal model. The normal survival of RBC's from some of the nonsplenectomized HS subjects in the rat may be due to either a milder RBC defect in these patients and/or to removal of the most abnormal RBC's by the patients' spleens. (Research supported by grant from NIH.)

250. Assessment of Folate Malabsorption in Celiac Sprue Comparing Synthetic [^{3}H]Pteroylpolyglutamate ([^{3}H]PteGlu$_7$) and [^{3}H]Pteroylmonoglutamate ([^{3}H]PteGlu). I. H. ROSENBERG, H. A. GODWIN,* R. M. RUSSELL,* AND J. L. FRANKLIN,* Chicago, Ill., and Houston, Tex.

Folate deficiency is a common finding in patients with celiac sprue, and malabsorption of monoglutamyl folate has been documented by various techniques. Absorption of polyglutamyl folates, which require both digestion and transport, has received scant attention. Studies in sprue may help to determine whether deconjugation or transport are rate-determining for dietary folate absorption in man. We have employed synthetic [^{3}H]pteroylpolyglutamate ([^{3}H]PteGlu$_7$) and [^{3}H]pteroylmonoglutamate ([^{3}H]PteGlu) for comparative quantitative testing of polyglutamyl folate absorption in six patients with biopsy-proved celiac sprue. In untreated sprue (four patients), 24 h urinary excretion after 0.5 μmol oral test dose ranged from 5.3 to 31% for PteGlu$_7$ and from 18 to 33.8% for PteGlu. Normals absorbed 56.1 ± 11 and 70.8 ± 13, respectively. Both mono- and polyglutamyl folate absorption improved

proportionately after gluten withdrawal. Two patients with treated sprue in remission had low normal folate absorption with both test compounds. Jejunal biopsy conjugase was assayed in vitro using [^{3}H]PteGlu$_7$ as substrate with paper chromatographic and high voltage electrophoretic separation of substrate and products. Conjugase activity was only slightly depressed in celiac biopsies compared to normals. The sequence of formation of intermediate and final products during deconjugation of PteGlu$_7$ did not differ from that in normal biopsies. Severity of celiac sprue correlated best with monoglutamate malabsorption rather than with the discrepancy between monoglutamate and polyglutamate absorption or depression of biopsy conjugase. These data suggest that the defect in transport of folate across the diseased mucosa rather than the defect in mucosal deconjugation is rate limiting for folate absorption in celiac sprue. (Research supported by Grant 5-RO 1-AM 15351 from NIH.)

251. Hereditary Deficiency of Fifth Component of Complement (C5) in Man. STEPHEN I. ROSENFELD* AND JOHN P. LEDDY, Rochester, N.Y.

The first recognized human kindred with hereditary C5 deficiency (C5D) has been found through study of a young Black woman with lupus erythematosus (SLE), frequent bacterial infections, and absent serum hemolytic complement (C) activity. Further study revealed a constant, total lack of C5 by effective molecule titration. Other C components were normal during remission of SLE, although C4, C2, and C3 were decreased during relapses. A healthy half-sister displayed barely measurable hemolytic C5 (~1% of normal). Neither subject showed detectable C5 by immunodiffusion. Four additional family members displayed half-normal C5 by hemolytic and immunodiffusion assays; other family members had normal C5. Although family studies are still incomplete, autosomal recessive inheritance seems likely. Addition of purified human C5 to both C5D sera restored normal CH_{50} titers and titers of C3–C9 complex. Normal serum could be incubated with C5D serum and then diluted, without affecting titratable C5 (usual titers ~250,000 effective molecules/ml). However, when progressively higher concentrations (<1/10,000) of either C5D serum were mixed with purified C5, progressive reduction in the titer of the added C5 was noted. The site of action of this effect is under study. Incubation of aggregated IgG with C5D sera yielded neutrophil chemotactic activity which was clearly subnormal but not absent (studied with Dr. J. Baum); with endotoxin activation this function appeared less severely impaired. Bactericidal activity for *S. typhi 0 901* (with optimal antibody) was absent in the proband's serum and subnormal in the other C5D serum. The proband's serum did not lyse PNH red cells in acid hemolysis or "sugar water" tests. The ability of C5D serum to promote phagocytosis of baker's yeast by normal or proband's neutrophils appears normal. Because of this apparent conflict with data on patients with hereditary C5 dysfunction, exchanges of sera with interested workers are in progress. (Supported by grant from NIH.)

252. Repair of Normal Hematopoetic Tissue After Chemotherapeutic Suppression: Comparative Analysis of Biochemical and Cell Culture Techniques. STEPHEN ROSENOFF,* JOAN BULL,* AND ROBERT YOUNG,* Bethesda, Md. (introduced by Vincent T. DeVita, Jr.).

Cytotoxic effects produced by cancer chemotherapy in normal and malignant tissue is in part a function of their proliferative state. Several techniques have been utilized to assess the extent and duration of this injury. Among these are clinical techniques of serial white blood counts (WBC) and bone marrow (BM) cellularity; autoradiography of BM or tumor for labeling index (LI); the functional technique of granulocyte colony formation in vitro (CFU-C); and determination of DNA synthesis as reflected in [^{3}H]TdR incorporation into DNA ([^{3}H]TdR-DNA). Cyclophosphamide (CTX)-treated normal and L1210 tumor-bearing BDF^1 mice were employed to study the relative merits of these techniques for predicting optimal times for subsequent chemotherapy. After 200 mg/kg CTX intraperitoneally, nadir BM cellularity occurred at 48 h and nadir WBC at day 4. This time actually corresponded to a period of rapid recovery of the BM as reflected by [^{3}H]TdR, CFU-C, and LI studies; thus neither BM cellularity nor WBC were found preditive of the state of sensitivity of marrow to subsequent chemotherapy. Kinetic studies of LI, CFU-C, and [^{3}H]TdR-DNA after CTX proved to be more sensitive mirrors of the state of marrow and tumor injury. In the BM, nadir suppression occurred 1–6 h after therapy, returned to control by 48 h, and rose above control levels on days 3–5. However, recovery from initial suppression of DNA synthesis occurred between 12 and 24 h, whereas CFU-C did not reveal recovery until 24 h later. This lag in recovery of CFU-C obscured a therapeutically significant recovery event as demonstrated by divided dose toxicity studies ($P < 0.05$). Conventional methods for assessing proliferative state of normal BM and tumorous tissue did not prove as sensitive or simple as did the [^{3}H]TdR-DNA technique.

253. Abstract withdrawn.

254. Isolation of Testosterone-Estradiol-Binding Globulin from Human Plasma by Affinity Chromatography. WILLIAM ROSNER* AND REX N. SMITH,* New York (introduced by Nicholas P. Christy**).

Human plasma contains a protein, testosterone-estradiol-binding globulin (TeBG), which binds testosterone (T), dihydrotestosterone (DHT), and estradiol (E2) with high affinity. Earlier efforts to purify this protein in an active form have been unsuccessful owing to the lability of the binding activity. We have stabilized this activity by conducting the purification in Tris buffers, at low temperatures, and in the presence of Ca^{++}, glycerol, and excess DHT. Purification is achieved starting with Cohn fraction IV followed by ammonium sulfate precipitation, affinity chromatography, two gel filtrations, and two isoelectric focusing columns. The overall purification is 4,000-fold, and the recovery, based on what is obtained from the affinity column, is 35%. The affinity column we use is novel in that it consists of azodianiline coupled to Sepharose 4B with epicholhydrin. 5α-Androstane-3β,17β-diol, 3-hemisuccinate is then coupled to the free amino groups with a carbodiimide. There is a 300-fold purification of TeBG by affinity chromatography. The isolated protein has mol wt 95,000 (SDS electrophoresis) and an isoelectric point of 5.44. It is a glycoprotein which contains 32% carbohydrate by weight. The amino acid composition, not previously determined, has no special features. It contains both cysteine and cystine, consistent with the fact that binding activity is destroyed by both sulfhydryl reagents and reducing reagents, i.e. Cleland's reagent. In spite of the presence of disulfide bonds we are unable to demonstrate any subunit structure, so that we consider TeBG to be a single polypeptide chain. Using the isolated, purified TeBG, association constants were obtained for three steroids at 4°C and 37°C and are as follows: E2–6.0 × 10^8M^{-1} (4°C), 2.2 × 10^8M^{-1} (37°C); T-1.1 × 10^9M^{-1} (4°C), 3.5 × 10^8M^{-1} (37°C); DHT-2.45 × 10^9M^{-1} (4°C), 1.0 × 10^9M^{-1} (37°C). The availability of purified, active TeBG

will allow more precise studies of how the binding of appropriate steroids to this protein affects their activity. (Supported in part by USPHS Grants AM-11852, GRS-FR-0566, and T01-AM5531.)

255. Stereospecificity of Beta Cell Protection against Alloxan. ALDO A. ROSSINI,* MICHAEL A. ARCANGELI,* ARTHUR A. LIKE,* AND GEORGE F. CAHILL, JR.,** Boston, Mass.

Glucose stimulation of insulin release by the beta cell is mediated by a glucose receptor (Matchinsky) or by an intracellular metabolite (Ashcroft and Randle), or by a combination of the two. Inhibitors such as mannoheptulose (MH) or 2-deoxyglucose could act at either or both sites. Another approach to beta cell receptors takes advantage of the sensitivity of the beta cell to alloxan necrosis. Glucose (G) and its nonmetabolizable analogue, 3-O-methylglucose (3OMG), protect the beta cell against alloxan (40 mg/kg intravenously in 24-h fasted 200-g male rats) and are competitively displaced by MH. MH given to untreated rats (with endogenous steady-state G) does not increase beta cell sensitivity to alloxan. Protection was determined by blood glucose concentrations 24 h later and corroborated by beta cell morphology. G or 3OMG protection against alloxan is present at 6 s and persists for 2 but not 10 min. The α-glucose anomer is more active than the β- or mutarotated anomer, suggesting a highly specific receptor. No sugar other than G or 3OMG was effective at 6 s; therefore the protective effects of mannose, fructose, and others is probably via metabolic conversion to G and not by direct interaction with receptor. Thus ΔG or Δ3OMG initiate the formation of a highly stereospecific labile complex which protects the beta cells against alloxan toxicity. MH is capable of immediately altering the G or 3OMG receptor complex thus making the cell susceptible to alloxan.

256. Identification of the Delayed Reacting Cells in Cultures of Chronic Lymphocytic Leukemia (CLL) Lymphocytes. ARNOLD D. RUBIN, STEPHEN DAVIS,* AND EDWARD SCHULTZ,* Bethesda, Md., and New York.

We originally reported that phytohemagglutinin (PHA)-stimulated cultures of CLL cells initiate enlargement and division in 5–7 days rather than 2–3 days as seen in normal cultures. Subsequently reports from other laboratories raised the possibility that CLL is simply a B cell neoplasm and that the late reaction was caused by a slow growth of small numbers of normal T lymphocytes retarded by a large number of inactive B cells. We now have additional information which bears on this problem. (*a*) Many CLL patients developed an in vitro PHA response of normal magnitude despite a clear-cut delay in its initiation. (*b*) When small numbers of normal lymphocytes were mixed with large numbers of autologous cells rendered unreactive by treatment with Mitomycin C, the magnitude of the response was appropriately reduced but there was no delay. (*c*) The pokeweed mitogen response in normal and CLL cultures were identical, indicating that these cultures contained sufficient numbers of lymphocytes (both T and B) to mount a normal response to at least one mitogen. (*d*) When nonimmunoglobulin-bearing T cells were isolated from CLL patients by passing leukocyte suspensions through columns of particles coated with anti-immunoglobulin, the PHA response of these cells was still delayed. Therefore the delayed reaction to PHA appears to be an intrinsic property of circulating lymphocytes in CLL. (Research supported by grants from the NCI, AEC, and Leukemia Society of America.)

257. A Hemolytically Active Complex of Properdin Factors with Chemotactic and Deactivating Activity for Human Polymorphonuclear Leukocytes. SHAUN RUDDY, EDWARD J. GOETZL,* AND K. FRANK AUSTEN, Boston, Mass.

Interaction of C3b, the major cleavage fragment of the third complement component, with properdin factor B (B) and the activated form of properdin factor D ($\overline{D}$) generates a labile enzyme, C3bB($\overline{D}$), which cleaves C3 and, when bound to erythrocytes, leads to lysis by activating the terminal complement sequence, C3–C9. A functionally analogous more stable enzyme is made by substituting cobra venom factor (CoVF) for C3b. In a micropore filter assay with isolated human neutrophils, the maximum net chemotactic response to mixtures of highly purified CoVF, B, and $\overline{D}$ (CoVFB($\overline{D}$)) was 20–34 neutrophils per high power field. With a radiochemotactic assay (^{51}Cr-labeled human mixed leukocytes) CoVFB($\overline{D}$) attracted a maximum net of 4.2–7.8%. Control mixtures, containing CoVF, B, and $\overline{D}$ reacted in the absence of Mg^{++}, were hemolytically inactive and devoid of chemotactic activity. Over a range of doses, the chemotactic activity of CoVFB($\overline{D}$) correlated with its hemolytic activity. Since pretreatment of cells with chemotactic factors (kallikrein or C5a) renders them unresponsive to subsequent chemotactic stimuli, the deactivating capacity of CoVFB(D) was assessed. Leukocytes exposed to CoVFB($\overline{D}$) and washed were deactivated to kallikrein. Similar deactivation occurred after exposure to a mixture of C3b, B, and $\overline{D}$; with short incubation times and high cell concentrations this mixture also exhibited chemotactic activity. The C3-cleaving enzyme formed from properdin factors B and $\overline{D}$ is an active site chemotactic factor which can be bound to an immune complex, cell surface, or any of the substances, including endotoxin, which activate the classical complement or properdin sequences.

258. Release of Granulocyte Colony-Stimulating Factor from Thymus-Derived Lymphocytes. FRANCIS RUSCETTI* AND PAUL CHERVENICK, Pittsburgh, Pa.

Colony-stimulating factor (CSF) is essential for in vitro differentiation of bone marrow cells into colonies of granulocytes and mononuclear cells. Previous studies have indicated that blood monocytes and macrophages are a major source of CSF. Recent studies have reported that CSF may be produced by lymphocytes responding to immunologic stimulation. Lymphocytes, purified from spleens and thymuses of mice by glass wool columns, were incubated in CMRL-1066 medium with fetal calf serum at a concentration of 2.5×10^6 cells/cm^3 for 7 days in 5% CO_2. CSF activity in the conditioned medium (CM) was assayed by its ability to stimulate colony formation from murine marrow in the soft gel system. CM (0.1 ml) from thymic lymphocytes stimulated 22 ± 1.6 colonies/10^5 marrow cells, while CM from spleen lymphocytes stimulated 43 ± 2.0 colonies. In order to determine if B or T lymphocytes were responsible for CSF production, anti-mouse theta serum was prepared by injecting mouse thymocytes into rabbits. Anti-mouse B lymphocyte serum was prepared by absorbing rabbit anti-mouse lymphocyte serum with mouse thymocytes. Pure preparations of T and B lymphocytes were made by the differential killing of specific lymphocytes using these antisera. Suspensions of lymphocytes treated with anti-mouse theta serum stimulated 2 ± 0.3 colonies, while untreated cultures and cultures treated with anti-mouse B lymphocyte serum stimulated 48 ± 2.3 and 46 ± 2.6 colonies, respectively. Similar results were obtained with thymic lymphocytes. Addition of antisera to cultures stimulated with a known stimulus did not affect colony formation. These studies suggest

that thymocytes and thymus-derived lymphocytes can release CSF in vitro and may represent the increase in CSF observed in certain immunologic reactions. (Research was supported by ACS Grant ET10B.)

259. Intestinal Lipodystrophy in Normal Rats Induced by Prolonged Triglyceride (TG) Perfusion. SEYMOUR M. SABESIN,* SUSANNE BENNETT CLARK,* AI-LIEN WU,* AND PETER R. HOLT, Memphis, Tenn., and New York.

Prolonged (4-h) duodenal perfusion of fat ([1-^{14}C] triolein) in the unanesthesized rat induces triglyceride (TG) accumulation in distal (D) but not in proximal (P) intestine despite similar rates of uptake from the lumen. Such accumulation might result from a delay in intracellular fat transport or in release of mucosal prechylomicrons, or be associated with altered subcellular structures. Thick and ultrathin sections from multiple blocks of intestinal tissue were examined "blind" by light and electron microscopy after 0, 1, and 4 h TG perfusion and 3, 6, 9, 12, and 24 h postperfusion, and were compared with radiochemical analyses from the same animals. At 1 h the tissue TG concentrations and appearance of P and D intestine were similar. At 4 h D intestine contained fourfold more TG than P. In P intestine, fat droplets appeared rather uniform ranging in diameter from 80 to 600 nm, and 3 h after cessation of TG absorption P mucosal cells contained little fat. D intestine showed massive accumulation of fat droplets, ranging from 70 to 11,000 nm in diameter, distending the endoplasmic reticulum (ER) and grossly expanding the Golgi cisternae. Large droplets were seen for 9 h postperfusion with gradual reappearance of Golgi. After fat mobilization masses of hyperplastic ER were seen in D intestine. Despite massive D fat accumulation both P and D intercellular and lymphatic chylomicrons ranged between 60 and 750 nm. Preliminary studies of the composition of chylomicrons derived from regional perfusion of P or D intestine show significantly higher TG:phospholipid ratios in D chylomicrons. Other data from rats recovered from either P resection or reversal of P and D intestine indicate that the defect resulting in D fat accumulation is intrinsic. We suggest that the rat D intestine has a limited capacity for chylomicron formation. (Supported by NIH Grants AM 13436 and AM 17398, American Heart Association, and New York Heart Association.)

260. Release of Prostaglandins and Other Humoral Mediators during Hypoxic Breathing. SAMI I. SAID, TAKERO YOSHIDA,* SATOSHI KITAMURA,* AND CAROL VREIM,* Dallas, Tex.

The mechanisms by which hypoxia induces pulmonary vasoconstriction are incompletely understood. We searched for evidence that the hypoxic pressor response may be mediated by vasoactive substances released from the lung and the nature of such mediators. We induced alveolar hypoxia in 33 isolated cat lungs and 6 intact cats. The isolated lungs were ventilated with 2% O_2–5% CO_2 and perfused with Krebs-dextran at constant flow. Perfusion (PA) pressure was monitored continually and the perfusate was made to drip on a cascade of isolated smooth-muscle organs: trachea, ileum, and gallbladder of guinea pig; stomach and colon of rat; and chick rectum. The onset of hypoxic breathing was closely followed by contraction, most often of trachea, gallbladder, stomach, and colon. The contractions occurred with the rise in PA pressure and were largely unaffected by antagonists of acetylcholine, catecholamines, serotonin, and histamine. In the intact cats ventilated with 9% O_2, we measured PA, LA pressures, and cardiac output and calculated pulmonary vascular resistance. Hypoxic vasoconstriction was reduced by 58% ($P < 0.0025$) by the prior infusion of aspirin (>50 mg/kg), an inhibitor of PG synthesis. We conclude that (*a*) biologically active substances are released from the lung during hypoxic breathing; (*b*) the released substances include PG's or PG-like compounds; (*c*) inhibition of PG synthesis markedly attenuates the hypoxic pressor reaction; (*d*) other agents may be released; and (*e*) the failure in some instances to detect mediators in the perfusate may result from their inactivation before reaching the circulation. (Research supported by a Center Award, HL-14187, from NHLI.)

261. Immunological Reconstitution in Severe Combined Immunodeficiency Disease by Transplantation from a Noncompatible Donor. SYDNEY E. SALMON, OTTO SIEBER,* BRIAN DURIE,* AND VINCENT FULGINITI,* Tucson, Ariz.

We report the first successful immunological reconstitution of an infant with combined immunodeficiency disease (CID) after transplantation of cells from a donor incompatible by HL-A and mixed lymphocyte culture (MLC) typing. Previous long-term reconstitution in CID has been achieved by transplantation of bone marrow cells either genetically compatible by HL-A typing or, more recently, in MLC. A further step in reconstitution has been achieved in this infant with CID. No donor cells compatible by HL-A typing or MLC were available. However, paternal immunocytes reactive in MLC could be eliminated in vitro with a pulse of tritiated thymidine of high specific activity. The remaining immunocytes were MLC compatible with the infant's cells. Cells were prepared in vitro in this way on three occasions (twice from peripheral blood and once from bone marrow) and transplanted intraperitoneally over a 4-mo period, utilizing increasing numbers of donor cells each time. Now at 13 mo of age, the infant exhibits a moderate chronic graft versus host reaction. Immunoglobulins are normal. Cell-mediated responsiveness, measured in vitro by phytohemagglutinin, pokeweed, and MLC reactivity, is present. Skin tests have reverted to negative. B and T lymphocytes are present in normal amounts. This successful transplantation supports the potential use of cells from noncompatible donors for reconstitution of CID. (Research supported by Grant CA 14087 from the NIH.)

262. Thyroid Hormone Action: In Vitro Characterization of Solubilized Nuclear Receptors from Rat Liver and Cultured GH_1 Cells. HERBERT H. SAMUELS AND JIR S. TSAI, New York (introduced by Saul J. Farber**).

Triiodothyronine (T3) and thyroxine (T4) appear to regulate nuclear transcriptional events and induce a threefold increase in the rate of growth of cultured GH_1 cells. We previously reported that putative nuclear receptors for T3 and T4 can be demonstrated by incubation of hormone either with intact GH_1 cells or with isolated GH_1 cell nuclei and rat liver nuclei in vitro. We characterized further the kinetics of T3 and T4 binding and the biochemical properties of the nuclear receptor after extraction to a soluble form with 0.4 M KCl. In vitro binding of T3 and T4 with GH_1 cell and rat liver nuclear extract was examined at 0°C and 37°C. Equilibrium was attained within 5 min at 37°C and 2 h at 0°C. The binding activity from GH_1 cells was stable for at least 1 h at 37°C and 10 days at −20°C. Chromatography on a weak carboxylic acid column and inactivation by trypsin and pronase but not by DNase or RNase suggested that the putative receptor was a nonhistone protein. The estimated equilibrium dissociation constants (K_d) for hormone binding

to the solubilized nuclear binding activity was 1.77×10^{-10}M (T3) and 1.25×10^{-9}M (T4) for GH_1 cells and 1.57×10^{-10}M (T3) and 2.0×10^{-9}M (T4) for rat liver. These K_d values are virtually identical with those which we previously reported with isolated rat liver nuclei and GH_1 cell nuclei in vitro and similar to those determined with intact GH_1 cells. In contrast, the binding activity for T4 and T3 in GH_1 cell cytosol was markedly different from that observed with nuclear extract (K_d values were 2.87×10^{-10}M for T4 and 1.13×10^{-9}M for T3). Our results indicate that nuclear receptors for T3 and T4 can be isolated in a soluble and stable form with no apparent change in hormonal affinity. This should allow elucidation of the mechanisms of thyroid hormone action at the molecular level. (Research supported by grants from NIH and ACS and an RCDA from the USPHS.)

263. Abstract withdrawn.

264. Glucose Intolerance in α_1-Antitrypsin Deficiency. JULIO V. SANTIAGO,* THOMAS A. DEW,* MOREY HAYMOND,* JOSEPH R. WILLIAMSON,* CHARLES KILO,* DAVID M. KIPNIS,** AND JOHN A. PIERCE,** St. Louis, Mo.

α_1-antitrypsin deficiency (PiZZ) is associated with pulmonary and hepatic abnormalities, but disturbances of carbohydrate metabolism have not been reported. Seven patients (PiZZ) were investigated. They had a mean age of 43 yr, had no family histories of diabetes, were within 10% of ideal weight, and had normal liver function tests. Data were compared to normal adults. Five patients had abnormal oral glucose tolerance tests (OGTT) (Fajans and Conn criteria). Overnight fasting glucose was higher in deficient patients, whereas insulin and glucagon were similar to controls. During OGTT, insulin levels were elevated at 60, 90, 120, and 180 min, but not at 30 and 240 min. The mean disappearance rate (kg) after 25 g of intravenous glucose was 1.18 ± 0.07 vs. control 1.60 ± 0.09, $P < 0.01$. After arginine infusions (500 mg/kg over 40 min) insulin responses were normal but glucagon responses were elevated (9.77 ± 1.24 vs. 5.28 ± 1.23, respectively, ng/40 min, $P < 0.05$). While on a standard diet, five patients (four with abnormal OGTT) were monitored with hourly blood samples for 24 h. Mean glucose was elevated (100 ± 2 vs. 82 ± 1 mg/dl, $P < 0.01$), and mean glucagon was normal. These data clearly demonstrate carbohydrate intolerance and relative insulin deficiency during both physiological monitoring and glucose tolerance testing of antitrypsin-deficient patients. This is the first report of a pancreatic abnormality in these patients who lack the major serum proteinase inhibitor. Proteolysis of beta cell glucose receptors could have been responsible for the abnormal beta cell function. (Research support from NHLI N01-HL 12218 R, NIH RR 00036, and HL 13694.)

265. Control of Breathing during Methadone Addiction. TEODORO V. SANTIAGO,* ANTHONY C. PUGLIESE,* AND NORMAN H. EDELMAN,* New Brunswick and Metuchen, N.J. (introduced by Hadley L. Conn, Jr.**).

Although narcotic drugs are known to be potent acute ventilatory depressants, the effects of chronic narcotic dependence upon respiratory control are unclear. Therefore, we studied the prolonged and superimposed immediate effects of methadone upon former heroin addicts in a methadone maintenance program. We measured arterial blood gas tensions, ventilatory responses to CO_2 ($VRCO_2$) by a rebreathing method, and ventilatory responses to hypoxia (VRHypox) by an isocapnic steady-state method before and 75 min after intake of the daily dose (40–100 mg). Since the data indicated that duration of maintenance was the major determinant of responsiveness, two groups were identified. Long-term subjects ($n = 8$) had taken methadone for from 5 to 43 mo; short-term subjects ($n = 6$) for less than 3 mo. Before the daily dose in long-term subjects, alveolar ventilation ($PACO_2 = 39.7 \pm 0.8$ mmHg), $VRCO_2$ (2.55 ± 0.18 liters/min per mm Hg), and VRHypox ("A," the sensitivity parameter of the VE-PO_2 curve = 194 ± 50) were all within previously defined normal limits. In comparison, short-term subjects had elevated $PACO_2$ levels (44.7, $P < 0.01$) and reduced $VRCO_2$ (1.76, $P < 0.05$) and VRHypox (61, $P < 0.01$). After the daily dose, long-term subjects had unchanged $PACO_2$ (39.8) and $VRCO_2$ (2.60) values although VRHypox was lowered (84, $P < 0.01$). In contrast, short-term subjects exhibited increases in $PACO_2$ (49.1, $P < 0.01$) and decreases in both $VRCO_2$ (1.54, $P < 0.05$) and VRHypox (25, $P < 0.01$). We conclude that, for at least 3 mo, daily methadone intake causes both acute and persistent hypoventilation via suppression of central (CO_2) and peripheral (hypoxia) chemoreception. Beyond 5 mo, hypoventilation is abolished as the central receptors acquire full tolerance to the drug. However, the peripheral receptors remain sensitive to the acute effects of methadone.

266. Nitrogen-Sparing Induced by Keto-Analogues of Essential Amino Acids. D. G. SAPIR,* O. E. OWEN,* T. POZEFSKY,* AND M. WALSER,** Baltimore, Md.

Keto-analogues of essential amino acids are aminated in liver from glutamine and in muscle from alanine and other amino acids (1973. *J. Clin. Invest.* **52**: 2865). Hence they may spare nitrogen during fasting (*a*) by providing a nitrogen-free source of amino acids for protein synthesis and (*b*) by suppressing urea formation. Eleven obese subjects were fasted 3–5 wk; six were then given five ketoacids (analogues of valine, leucine, isoleucine, methionine, and phenylalanine, as sodium salts, 9 g) plus the remaining essential amino acids (2 g) as 3-h infusions. Plasma valine, leucine, isoleucine, alloisoleucine, methionine, and phenylalanine rose significantly 1 h postinfusion, while plasma glycine and blood glutamine fell ($P < 0.01$). During 1 wk of daily infusions, urinary urea fell progressively to a value 20% less than the uninfused controls ($P < 0.01$); during the ensuing 5 days it was reduced still further (38%; $P < 0.01$). Urinary ammonia, plasma FFA, glucose, ketone bodies, and insulin were unchanged. In a second group, branched-chain ketoacids alone (4.9 g) were given daily from the start of fasting in four subjects. Branched-chain amino acids in plasma rose sharply but most other amino acids fell. 8-day cumulative urinary urea nitrogen was 19 g lower ($P < 0.03$) during 1 wk of infusions than in uninfused control subjects. This is more than twice the nitrogen required to aminate the infused ketoacids. In the ensuing 14 days, one subject excreted 36% less urea than during a preceding fast without ketoacids. Thus these compounds are efficiently aminated in fasting man; more important, they reduce urea formation not only during administration, but also long after they are metabolized. Possible mechanisms include suppression of glucagon secretion.

267. Studies on the Porphyrin-Heme Biosynthetic Pathway in Cultured Human Amniotic Cells. SHIGERU SASSA,* RICHARD D. LEVERE, GEORGE SOLISH,* AND ATTALLAH KAPPAS,** New York.

The porphyrin-heme biosynthetic sequence has been well studied in hepatic and erythroid cells, but no work on this pathway has been reported in human amniotic cells. We report here studies relating to intermediates and enzymatic steps of porphyrin-heme synthesis in such cells. Cells obtained from 20–50 ml of human amniotic fluid between 13 and 22 wk of gestation were grown in tissue culture for 2–10 wk.

These cells generated porphyrins from δ-aminolevulinic acid (ALA) in dose-related fashion. The ALA concentration required to generate a half-maximum level of porphyrins was 0.3 mM, which is about 10 times greater than that required by embryonic avian liver cells. The potent inducer of ALA-synthetase in liver cells, allylisopropylacetamide, did not stimulate porphyrinogenesis in amniotic cells. The enzyme which catalyzes the condensation of the monopyrrole porphobilinogen to the tetrapyrrole, uroporphyrinogen (URO), was measurable, with levels of activity (mean 57 ± 26 pmol URO formed/mg protein per h, 60°C) being similar to those found in cultured skin fibroblasts from normal individuals. ALA-dehydratase activity could not be measured directly, but the formation of porphyrins from ALA was strongly inhibited by agents such as lead or levulinic acid, known to inhibit ALA-dehydratase activity. On the other hand, porphyrinogenesis was markedly enhanced by inhibitors of ferrochelatase activity, such as EDTA (Ca^{++}, Mg^{++}) or desferrioxamine. These studies demonstrate that enzymes which convert ALA to heme are operational in cultured human amniotic cells and that such cells may be usefully employed in studies of genetic and environmental factors known to result in derangements of heme synthesis in man. (Supported by NIH Grants ES-00621 and AM 09838.)

268. Liver Disease in Asymptomatic Hepatitis-B Antigen Carriers. ROBERT A. SCHAEFER,* NIALL D. C. FINLAYSON,* AND ALFRED M. PRINCE,* New York (introduced by Norman B. Javitt).

Liver biopsy was performed in 56 asymptomatic chronic carriers of hepatitis-B antigen (HB Ag) detected by routine screening of blood donors. Normal biopsies were found in 11 carriers; nonspecific reactive hepatitis (NSRH) in 18; fatty infiltration (FI) in 5; chronic persistent hepatitis (CPH) in 5; chronic aggressive hepatitis (CAH) in 15 (including 5 with suspected cirrhosis); and cirrhosis in 2. SGPT was elevated in 38%, compared to 23% of 319 unselected carriers. SGOT values (normal: <40) were elevated in all patients with CAH: mean values were 168 without cirrhosis, and 87 with definite or suspected cirrhosis (range 59–388). SGOT was elevated in 3/5 patients with CPH (mean: 89, range 31–186); 7/18 with NSRH (mean: 52, range 20–214); 3/5 with FI (mean 36: range 25–46); and 2/11 with normal biopsies (mean 28; range 21–42). Abnormalities in serum alkaline phosphatase and 5'-nucleotidase were infrequent. No patient had depressed serum albumin or elevated total serum globulin. Titers of HB Ag were measured by reverse passive hemagglutination, with mean values of 10, 800 with NSRH (8 patients); 21, 200 with normal biopsies (six patients); 21, 300 with CAH and suspected cirrhosis (three patients); and 69, 100 with CAH without cirrhosis (five patients). Subtyping of HB Ag was performed in 30 patients: 23 were ad, 1 was ay, and in 6 only the a specificity was detected. HB antibody titers, measured by hemagglutination, were <2 in all but one individual, where transient rise to 512 was associated with transient elevation of SGOT and transient disappearance of antigen. Symptomatic HB Ag carriers may have significant abnormalities on liver biopsy when SGOT is elevated, but severity of histologic change cannot be predicted from degree of SGOT elevation, and liver biopsy is warranted. Two patients with CAH had intermittently normal SGOT, indicating need for follow-up with sequential determinations. (Supported by NHLI Contract 72-2961 B and NIH CRC Grant RR-47.)

269. Successful Treatment of "Pancreatic Cholera" by Streptozotocin. PHILIP SCHEIN,* C. RONALD KAHN,* ARNOLD G. LEVY,* PHILLIP GORDEN, AND JERRY D. GARDNER,* Bethesda, Md.

"Pancreatic cholera" is a syndrome characterized by non-beta islet cell neoplasm, profuse watery diarrhea, severe hypokalemia, and absence of gastric acid hypersecretion. Patients with metastatic disease have proven refractory to conventional chemotherapeutic modalities and most have died within 1 yr of diagnosis with diarrhea-induced hypokalemia. Two patients with pancreatic cholera and histologically proven hepatic metastases were treated with streptozotocin. Before therapy the patients had stool volumes from 2 to 8 liters/day and required 200–800 meq/day of supplemental potassium. Both patients demonstrated a dramatic and prompt clinical response after three doses of streptozotocin (1.5 g/m² per wk) administered through a catheter in the hepatic or pancreaticoduodenal artery. Stool volumes decreased to less than 0.2 liters/day, and both patients had a return of serum potassium concentration to normal without need for supplementation. These clinical responses correlated with a major reduction in number and size of hepatic metastases as documented by liver scan or angiography. When arterial infusions were compared to peripheral venous infusions in these two patients, peripheral venous levels of streptozotocin were reduced by 50% and urinary excretion decreased by 33%. These pharmacologic studies indicate that the arterial route of administration increased tumor and/or hepatic extraction of streptozotocin and decreased renal exposure to this nephrotoxic drug. The findings in these patients indicate that streptozotocin is an effective drug in the management of malignant islet cell carcinoma with pancreatic cholera and that the intraarterial route of administration may increase therapeutic efficacy.

270. Production of Basement Membrane-Like Collagen and Smooth Muscle Actomyosin by Cultured Human Glomerular Cells (GLC). JON I. SCHEINMAN,* ALFRED J. FISH,* DAVID M. BROWN,* AND ALFRED F. MICHAEL, Minneapolis, Minn.

By immunochemical analysis cultured lines of GLC from isolated infant glomeruli have been reported from this laboratory to produce a material similar to human glomerular basement membrane (GBM) and distinct from that produced by skin fibroblasts. Other recent studies by us have investigated glomerular tissue antigens and show that actomyosin in the glomerular mesangium undergoes marked changes in quantity and distribution associated with glomerular disease. The present studies have investigated the biochemistry of the glomerular cell (GLC) collagen and immunofluorescent staining for antigenic smooth muscle actomyosin. Radiolabeled proline and lysine incorporation and metabolism were analyzed in cell layer and medium in whole and collagenase-digested fractions, by analyzer chromatography, and liquid scintillation. Significant [^{3}H]3-OH proline, high [^{3}H]4-OH proline, and [^{14}C]OH-lysine with [^{14}C]galactosylglucosyl hydroxylysine far in excess of [^{14}C]galactosylhydroxylysine were synthesized. Immunofluorescent staining of GLC and fibroblast lines using antisera against human uterine smooth muscle actomyosin, GBM, collagen, and fibroblast protein was performed. GBM, collagen, and fibroblast protein antigens were present in intracellular granules and the *extracellular* fibrillar matrix of fibroblasts and GLC. Actomyosin was present in long parallel *intracellular* fibers in GLC, far less in fibroblasts. Before fixation, treatment of GLC with low ionic strength (0.1) KCl, known to form a gel from soluble actomyosin in vitro, markedly increased the actomyosin fiber prominence. Absorption of the anti-actomyosin with purified actomyosin inhibited all fiber-staining reactions. These observations demonstrate for the first time synthesis of two proteins, basement membrane-like collagen and smooth muscle actomyosin, both known constituents of the glomerulus, by single glomerular cell lines. The specific relationship of GLC properties to known glomerular

cell types may further elucidate glomerular functions: epithelial cell podocyte motility, GBM filtration, endothelial cell slit pore permeability, and mesangial phagocytic potential. (Research supported by grants from Kidney Foundation of the Upper Midwest, USPHS, and AHA.)

271. Paradoxical Function of Excitation-Contraction Coupling Mechanisms in an Increased Inotropic State: Effects of Acute Uremia on the Rat Heart. JAMES SCHEUER, THASANA NIVATPUMIN,* SOMSONG PENPARGKUL,* ASHOK K. BHAN,* AND TADA YIPINTSOI,* Bronx, N.Y.

Left ventricular function of rats was studied in the open chest 24 h after bilateral nephrectomy. Severe uremia was present. During ejection (E), uremic hearts (U) had the same end-diastolic pressure (EDP) as sham (S) but left ventricular pressure (LVP) was 121 mm Hg in U and 93 in S. Max dP/dt was 5691 mm Hg/s in U and 4015 in S. During gradual aortic occlusion LVP and dP/dt were higher in U than S at each EDP. Isovolumic LVP was 237 in U and 213 in S, and Max dP/dt was 9627 in U and 7600 in S. After 1.5 mg propranolol intravenously the differences persisted. (All differences were $P < 0.05$.) Normal rats treated for 24 h with methoxamine Q6H SC had a blood pressure course similar to that found in U. Isovolumic LVP and dP/dt were the same in these hypertensive controls as in S. Thus hypertension could not account for the increased contractility in U. Membrane Na^+, K^+-ATPase activity was 16.4 μmol/mg protein per min in U and 22.0 in S hearts ($P < 0.005$). Ouabain 10^{-4} M lowered ATPase activity in S to the same level as found without ouabain in U. The Ca^{+2} binding and uptake (with oxalate) of sarcoplasmic reticulum (SR) and the V_{max} were depressed in preparations from U compared to S as was SR ATPase activity (all $P < 0.05$). An increased contractile state in acute uremia is implied by the isovolumic performance. A digitalis-like effect on Na^+, K^+-ATPase which presumably overrides the decreased function of the SR may be responsible. A circulating factor, but not a catecholamine, is implicated because hearts from acute uremic rats do not show increased performance when they are perfused in the absence of uremic blood. (Research supported by a grant from NIH.)

272. Role of Platelet Membrane Phospholipids in the Release of Reaction. P. K. SCHICK* AND B. P. YU,* Philadelphia, Pa. (introduced by Walter Rubin).

The structure and function of the platelet surface was probed by phospholipase C (*Cl. perfringens*) (PLC) which hydrolyzes membrane phospholipids (PL), particularly phosphatidylcholine. Platelet PL were susceptible to PLC and extent of hydrolysis was dependent on concentration of PLC and Ca^{++}. PLC (0.15 U/ml) with Ca^{++} (0.55 mM) hydrolyzed 15.6% PL during 5 min. PLC released platelet serotonin (5HT), ADP, and platelet factor 4 (PF4). Hydrolysis of 5% PL resulted in release of 70% 5HT. Platelet 5HT release was rapid, occurring within 2 min. PLC (0.2 U/ml) with Ca^{++} (0.55 mM) also released 10.35 nmol storage pool ADP per 10^9 platelets and 63% PF4 during 3 min. PLC did not cause leakage of cytoplasmic metabolic pool ADP, since only 6.6% [^{3}H]ADP was released. Ultrastructural analysis of PLC-modified platelets showed that platelets were intact. After 2% PL hydrolysis, centralization of granules and contraction of microtubules were evident. After 18% PL hydrolysis, there were morphological indications of degranulation. PLC-induced PL hydrolysis caused release of ADP and 5HT since (*a*) PLC was free of protease and neuraminidase activity; (*b*) heated PLC caused release but omission of Ca^{++} inhibited release, eliminating heat-labile and Ca^{++}-requiring enzymes as contaminants; (*c*) antitoxin (*Cl. perfringens*) neutralized PLC-induced 5HT release which rules out a contaminant; and (*d*) phosphorylcholine, the hydrolysis product, did not induce platelet 5HT release. This study demonstrates that minimal hydrolysis of platelet phospholipids triggers the release reaction. Our hypothesis is that phospholipids, presumably phosphatidylcholine, are situated at or near the active site or "receptor" on the platelet surface and function as the modulator for the release reaction. (Research was supported by grants from the American and Pennsylvania Heart Associations.)

273. Lithogenic Bile: a Consequence of Weight Reduction. PAUL H. SCHREIBMAN,* DEMETRIUS PERTSEMLIDIS,* GEORGE C. K. LIU,* AND E. H. AHRENS, JR.,** New York.

A close correlation exists between human adipocyte cholesterol (CH) and adipocyte triglyceride (TG). Weight reduction (WR) might therefore result in loss of both adipocyte TG and CH. If mobilized CH is not rapidly converted to bile acids, then bile might become supersaturated with CH. To test this hypothesis, six obese patients were fed control (C) eucaloric, CH-containing but squalene-free liquid formula diets for 6 wk; then they underwent WR for 6 wk, same diet at 20% of C levels. Body weight decreased by 13.7 kg (mean); plasma CH did not change significantly in any patient. Biliary lipid composition changed significantly during WR ($P < 0.01$) with the lithogenic index rising from 116% (C) to 158% of maximum CH solubility on the 35th day of WR. The biliary cholic: chenodeoxycholic ratio fell significantly from 1.8 (C) to 0.6 (WR). Decreased CH synthesis during WR was suggested by (*a*) fecal sterol balance of C = 1.34 and WR = 0.50 g/day, and (*b*) decrease in plasma squalene (an obligatory precursor of CH) from 32.9 to 24.1 μg/liter ($P < 0.005$). CH efflux is suggested by the following calculation: if adipose TG lost during WR is 34% of total weight lost and adipose CH constitutes 0.18% of adipose TG, then a 13.7 kg weight reduction could result in an 8.4 g CH efflux. The actual fecal sterol excretion during WR was 0.50 g/day × 42 days, or 21 g, suggesting that CH synthesis was not completely abolished. We conclude that during WR there is increased bile lithogenicity. This may be due to mobilization of adipose tissue CH through the biliary tract at a time of decreased bile acid (cholic) excretion. (Supported by NIH Grants HL-06222 and FR-00102.)

274. Enzymes of Human Erythrocyte Membranes. STANLEY L. SCHRIER, IRENE JUNGA,* MURIEL SEEGER,* CARL GRUMET,* AND ISAAC BEN-BASSAT,* Stanford, Calif.

Some enzymes of the human erythrocyte such as the ATPases are entirely membrane bound; some enzymes such as triosephosphate isomerase are wholly in the cytosol; however, several enzymes, including glyceraldehyde phosphate dehydrogenase (GAPD) and phosphoglyceric kinase (PGK) are found both in the cytosol and in association with the membrane. The coupled catalytic action of membrane-associated PGK and GAPD would generate membrane ATP which could be the substrate for membrane-bound ATPases. We tested the hypothesis that the membrane-associated GAPD and PGK were isozymes which differed so that membrane association was facilitated. Human erythrocytic PGK and GAPD were partially purified from cytosol and membrane sources and their kinetic properties, pH optima, and apparent molecular weights were compared. Cytosol enzymes were purified from chloroform-ethanol fractions, precipitated by ammonium sulfate, and subjected to agarose gel filtration chromatography. Shearing of white erythrocyte ghosts in the French pressure cell solubilized membrane PGK (but not GAPD) which was then chro-

matographed. Membrane GAPD was solubilized by addition of 0.1% sodium dodecyl sulfate, concentrated by velocity sedimentation on linear sucrose gradients and then chromatographed. Approximately 1% of PGK is membrane associated and the properties of the membrane and cytosol enzyme are not distinguishable, both having molecular weights of 33,000 daltons. In contrast, 50% of erythrocyte GAPD is firmly membrane associated. The kinetic properties and pH optimum of membrane and cytosol GAPD are the same, and both have a molecular weight of 143,000 daltons. Inhibitory antibodies produced against either cytosol or membrane GAPD inhibited GAPD prepared from the opposite source. GAPD and PGK obtained from membrane or cytosol fractions are not distinguishable. The reasons for their varying proportions of adherance to membranes and the varying firmness of that association have yet to be determined. (Research supported by grant 5R01 AM13682 from NIH.)

275. Vasoactive Intestinal Peptide (VIP) Stimulation of Adenylate Cyclase and Active Electrolyte Secretion in Intestinal Mucosa: Possible Role in the Pancreatic Cholera Syndrome. CHARLES J. SCHWARTZ,* DANIEL V. KIMBERG, HARLAND E. SHEERIN,* MICHAEL FIELD,* AND SAMI I. SAID, Boston, Mass., and Dallas, Tex.

Vasoactive intestinal peptide (VIP) has recently been identified in the plasma and tumors of patients with the "pancreatic cholera" syndrome. The role of VIP in the pathogenesis of the diarrhea in these patients was examined by determining the effects of this peptide on the cyclic AMP levels, adenylate cyclase activity, and ion transport of in vitro preparations of rabbit ileal mucosa. VIP (20 μg/ml) caused a prompt 5-fold increase in cyclic adenosine monophosphate (cAMP) level, whereas many of the other hormones which have been suggested as playing a role in this syndrome did not. An increase in mucosal cAMP level was observed at a VIP concentration of 0.1 μg/ml and appeared to be maximal at a concentration of 2.0 μg/ml. VIP stimulated adenylate cyclase activity in a membrane preparation from rabbit ileal mucosa. When added to the serosal side of isolated ileal mucosa clamped in an Ussing chamber, VIP (2 μg/ml) increased short-circuit current (SCC) and reversed the net Na flux from one of absorption to secretion. The difference between the SCC and the net Na flux indicates a marked enhancement of net anion secretion. These effects of VIP on mucosal cAMP metabolism and ion transport are similar to those observed with cholera enterotoxin and certain prostaglandins. VIP was also tested with normal human ileal mucosa. At a concentration of 2 μg/ml it caused a 5-fold increase in cAMP level and a maximal increase in SCC, similar to that seen with theophylline. The present study suggests that the diarrhea seen in patients with VIP-secreting tumors is due to stimulation by VIP of adenylate cyclase in the epithelial cells of the small intestine. (Research supported by grants from NIH.)

276. Cellular Localization of Hormone Action in the Toad Urinary Bladder. WALTER N. SCOTT* AND VICTOR S. SAPIRSTEIN,* New York (introduced by William A. Brodsky).

The toad urinary bladder responds to oxytocin (OT) and aldosterone (Aldo) with an increase in the rate of sodium transport. We have separated the mitochondria-rich (MR) and granular (GR) cells from the bladder by density gradient centrifugation (DGC). Because we had previously found that oxytocin causes an increase in the cyclic adenosine monophosphate (cAMP) content of isolated MR cells, but not GR cells, we examined the localization of Aldo in the two cell types. Intact hemibladders were incubated for 45 min in [^{3}H]Aldo (10^{-9} to 2×10^{-8} M), the cells were removed by incubation in EDTA Ringers, and the MR and GR cells were separated by DGC. The separated cells were sonicated and centrifuged, and the [^{3}H]Aldo was measured in each fraction. Aldo bound to ammonium sulfate precipitable protein was 5.4×10^{-14} M/mg protein in MR cells exposed to 2×10^{-8} M Aldo; binding was half saturated at approximately 5×10^{-9} M Aldo. Aldo binding in the GR cells was negligible. Bladders incubated in radioactively labeled nucleosides were treated for 1 h with 10^{-8} M Aldo and the RNA extracted with phenol. Aldo slightly increased the incorporation of label into total RNA. Messenger RNA contains a long (50–200 bases) stretch of poly(A), which binds poly(U). We polymerized poly(U) to glass fiber filters, filtered the RNA from the bladders, and measured the label bound by the poly(U) filters. Approximately 6 times as much RNA was retained from the Aldo-treated bladders as from the controls. These data indicate the MR cell is the major locus of action of Aldo and that Aldo induces the synthesis of mRNA. (Research supported by grants from NIAMD [AM 15205] and the American Heart Association.)

277. Prothrombin San Juan: a Complex New Dysprothrombinemia. SANDOR S. SHAPIRO, NORMAN I. MALDONADO,* JEAN FRADERA,* AND SUSANNAH MCCORD,* Philadelphia, Pa., and San Juan, Puerto Rico.

We have recently studied a family in which two siblings have prothrombin levels of 15–20% but essentially normal immunoreactive concentrations of this zymogen. Immunofixation electrophoresis demonstrated two prothrombin bands—a major band of rapid mobility and a minor band (±25% of the total) with approximately normal mobility. Each parent was found to be heterozygous for prothrombin deficiency. The mother showed two prothrombin bands of equal intensity, one normal and one of rapid mobility like that seen in the children. The father showed only one normal-mobility prothrombin band. Family studies identified other maternal relatives with half-normal levels of prothrombin activity and two electrophoretic prothrombin bands, and several paternal relatives with half-normal activity levels but only a single prothrombin band of normal mobility. Using Sephadex-DEAE gradient chromatography, we have separated the two prothrombins from propositus plasma and find them present in a ratio of approximately 3:1. Neither component behaves chromatographically like normal prothrombin. Each component was labeled with ^{125}I, mixed with normal [^{131}I]prothrombin and normal plasma, and rerun before and after recalcification. The major prothrombin component in propositus plasma does not activate, whereas the minor component, which has some biologic activity, undergoes some molecular changes. No interconversion of the two components was seen during clotting. It appears that these individuals are doubly heterozygous, having two distinct abnormal prothrombins. It is possible that this situation may not be uncommon in offspring of nonconsanguineous marriages. These findings have implications for the study of structure-function relationships in apparently homozygous individuals with genetically dysfunctional proteins. (Research supported by Grant HL 01963 from NIH.)

278. Evidence of a Direct Effect of 1,25-Dihydroxycholecalciferol (1,25-diOHD$_3$) to Promote Bone Mineralization in the Rat. F. H. SHEN,* D. J. BAYLINK, J. E. WERGEDAL,* D. J. SHERRARD,* AND A. W. NORMAN,* Seattle, Wash., and Riverside, Calif.

The inhibition of bone mineralization in vitamin D-deficient (D-) rats could be due to an absence of the effect of vitamin D to promote mineralization directly, or to the attending hypocalcemia, or to both. To evaluate this issue, bone mineral-

ization was studied in D- thyroparathyroidectomized (TPTX) rats in which serum Ca and Pi were not decreased. TPTX weanling Holtzman rats were fed diet containing 1.8% Ca, 0.65% P, and no vitamin D for 3 wk. Intact rats fed adequate vitamin D, Ca, and P had normal serum Ca and Pi and osteoid width in tibial diaphysis, 9.8 ± 0.3 mg/100 ml, 9.9 ± 0.7 mg/100 ml, and 8.4 ± 1.1 μm, respectively. In D-TPTX rats, serum Ca and Pi were not decreased, 10.7 ± 0.8 and 16.9 ± 1.3 mg/100 ml, respectively; but osteoid width was markedly increased, 16.3 ± 2.2 μm, $P < .001$. To determine if the mineralization defect was improved by vitamin D or 1,25-diOHD$_3$, two other groups of D- TPTX rats, after being fed the above diet for 3 wk, were either placed on a diet containing a normal amount of vitamin D or injected intraperitoneally with 4 U of 1,25-diOHD$_3$ and then were sacrificed 72 h later. Both 1,25-diOHD$_3$ and oral vitamin D caused a marked decrease ($P < 0.001$) in osteoid width to almost normal values, 8.5 ± 1.1 and 9.2 ± 2.3 μm, respectively. These results show that the promotion of bone mineralization by vitamin D is probably mediated by 1,25-diOHD$_3$. Additionally, since the mineralization defect in D- rats is independent of changes in serum Ca or Pi, it suggests that 1,25-diOHD$_3$ acts directly on bone cells to promote mineralization.

279. Conversion of Thyroxine to Triiodothyronine by Mouse Neuroblastoma Cells: Evidence for Possible Role of Tyrosine Hydroxylase. LOUIS SHENKMAN,* VALERIE PECK,* ALBERT HARARY,* COLETTE THAW,* HARRIET NADEL,* AND CHARLES S. HOLLANDER, New York.

In view of recent evidence by Dratman et al. (1973. *Endocrinology*. **92**: A-156), suggesting that thyroxine may serve as substrate for tyrosine hydroxylase in adrenergic tissue, we have studied the conversion of thyroxine to triiodothyronine by mouse neuroblastoma in tissue culture. The cell line C-1300, NIE 115 (kindly supplied by M. Nirenberg) was selected for study because of its known capacity to produce tyrosine hydroxylase. Cells were grown in Dulbecco's modification of Eagle's medium with 10% fetal calf serum and placed in serum-free medium for 48 h before study. To assess conversion, cells were incubated with [^{125}I]thyroxine for 2–24 h. Triiodothyronine and thyroxine in cells and medium were separated by Sephadex G-25 chromatography. Percent conversion of thyroxine to triiodothyronine (correcting for triiodothyronine contamination and spontaneous deiodination) was 3.7% at 2 h and 5.2% at 24 h. This was equivalent to the generation of 1.27×10^{-16} and 1.81×10^{-16} mol of triiodothyronine per μg cell protein at 2 and 24 h, respectively, at a T_4 concentration of 8.8×10^{-10} M. Addition of propylthiouracil (1 mg/100 ml) reduced conversion at 2 h to 2.2%, while 10^{-4} M α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase, reduced conversion to 0.49%. Preincubation for 2 days with hypothyroid serum resulted in 9.5% conversion at 2 h. Our studies suggest that mouse neuroblastoma cells convert thyroxine to triiodothyronine and that this conversion is enhanced by prior incubation in hypothyroid medium and inhibited by propylthiouracil and α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase.

280. Ketone-Induced Hypoalaninemia: Possible Mechanism of Decreased Gluconeogenesis and Protein Conservation in Starvation. ROBERT SHERWIN,* ROSA HENDLER,* AND PHILIP FELIG, New Haven, Conn.

In prolonged starvation conservation of body protein is dependent on decreased gluconeogenesis, mediated via a reduction in plasma levels and muscle outflow of alanine. This decrease in alanine cannot be explained on the basis of hormonal changes. The present study was undertaken to determine the role of ketones in the hypoalaninemia of starvation. 12 healthy volunteers (eight nonobese, four obese) were given a primed-continuous intravenous infusion of sodium DL-β-hydroxybutyrate (3 mg/kg per min) for 3 h. Blood ketones increased rapidly, reaching a plateau of 1.1 ± 0.1 mmol/liter in 30 min. There was a small (5–10 mg) decline in blood glucose, but no change in plasma insulin or glucagon. Plasma alanine (306 ± 17 μmol/liter preinfusion) fell within 1 h and reached levels 21 ± 2% below basal at 3 h ($P < 0.01$). In contrast, all other amino acids remained unchanged. Comparable responses were observed in nonobese and obese subjects. In four subjects the ketone infusion was continued for 6 h resulting in a further decline in plasma alanine to 212 ± 20 μmol/liter, levels 37 ± 3% below basal and comparable to those observed after 3–5 wk of starvation (195 ± 11). When ketones were infused after 3–5 wk of starvation, blood ketones rose from 5.7 ± 0.6 mmol/liter (preinfusion) to 10.4 ± 0.9 and plasma alanine fell an additional 30% to 134 ± 11 μmol/liter in response to the infusion ($P < 0.025$). Equimolar control infusions of saline and/or bicarbonate failed to alter plasma alanine. We conclude that acute increases in blood ketones result in a decline in circulating alanine comparable to that observed in prolonged fasting. The hypoalaninemic effect occurs in nonobese and obese subjects and persists in prolonged starvation. The data suggest that in starvation, ketones not only replace glucose as major energy substrates, but also contribute to protein conservation by limiting amino acid availability for gluconeogenesis. (Supported by NIH grants.)

281. Calorimetric Studies of Glycolysis in Human Erythrocytes. S. SHOHET, D. EATOUGH,* L. HANSON,* S. REHFELD,* AND W. MENTZER,* Provo, Utah, and San Francisco, Calif.

Glycolysis in human erythrocytes was examined using high sensitivity isothermal calorimetry. Fresh erythrocytes were depleted of glucose by exhaustive washing with Krebs-phosphate buffer and resuspended in buffer with albumin and antibiotics at pH 7.4 in the calorimeter. Glucose (5 mM) was added to rekindle glycolysis; heat production, glucose consumption, adenosine triphosphate (ATP) production, and pH were monitored for 24 h. After a brief lag period, four distinct early peaks in the heat production were observed. When corrected for pH change effects, the heats associated with these peaks were 94, 171, 140, and 146 m-cal/cm^3 erythrocytes, respectively. The evolution of these peaks occurred over a 5 h period and was correlated with a rise in cell ATP from 0.85 μM/cm^3 cells to 1.35 μM/cm^3 cells and with a glucose consumption of 22 μM/cm^3 cells. The observed total heat production of 550 m-cal agrees well with the amount theoretically expected to be evolved from the glycolysis of 22 μM of glucose (506 m-cal). After approximately 6 h a long-term stable region of heat production was reached which, without further glucose, gradually decayed to no heat production. Addition of 10 mM sodium fluoride altered the early heat production pattern with elimination of the distinct peaks, loss of cell ATP, and 70% reduction of total heat. Addition of 0.3 mM adenosine to glucose-depleted cells transiently duplicated the heat effects of glucose re-feeding with a shorter lag period and with resynthesis of 0.35 μM ATP/cm^3 cells. The data suggest that we are observing specific exothermic reactions of glycolysis and that calorimetry is a valuable tool for the study of intermediary metabolism in red cells. (Supported by NIH Grants AM16095, AM37237, and GM18816C.)

282. Hyperresponsiveness to the Phosphaturic Effect of Parathyroid Hormone in X-Linked Hypophosphatemic Vitamin D-Resistant Rickets (FHR). ELIZABETH SHORT,* ANTHONY SEBASTIAN,* MARTIN SPENCER,* AND R. CURTIS MORRIS, JR., San Francisco, Calif.

In FHR, it has been inferred that the parathyroid hormone-responsive component of renal phosphate transport is defective because in hemizygotes the magnitude of reduction in renal phosphate reabsorption remained unchanged over a tenfold change in plasma concentration of endogenous parathyroid hormone (PTH) and despite experimental administration of PTH. But if the renal tubule in FHR were hyperresponsive to the phosphaturic effect of normal (or subnormal) concentrations of circulating PTH, a further response to even supernormal concentrations of circulating PTH might be precluded by an already maximal response. We investigated this possibility in two adult hemizygotes with FHR who had not received vitamin D or phosphate therapy and in whom the concentration of circulating PTH was normal (radioimmunoassay). Hypercalcemia (13–14 mg/100 ml) was induced by intravenously administered calcium gluconate (1800–2400 h). At 0800, when normocalcemia had recurred but plasma PTH concentration and urinary excretion of cyclic adenosine monophosphate (cAMP) were significantly reduced, parathyroid extract (PTE) was administered intravenously at successively increasing rates of 0.1, 0.4, and 0.8 units/kg per h, each rate lasting 90 min. Minutes after initiating PTE, fractional phosphate excretion increased from a negligible value to values significantly greater than those of similarly studied control subjects. This phenomenon persisted throughout PTE administration and could not be attributed to volume expansion, decreases in serum calcium concentration during the study, differences in percent of administered calcium retained, or hemodynamic changes. At any cumulative dose of PTE, urinary excretion of cAMP in hemizygotes was indistinguishable from that of controls. These findings indicate that in FHR the renal tubule is hyperresponsive to the phosphaturic effect of PTH. (Supported by NIH 16764-01.)

283. Platelet Production in W/Wv Mice. DAVID P. SHREINER,* Pittsburgh, Pa. (introduced by Dane R. Boggs).

W/Wv mice are genetically anemic but have normal numbers of circulating granulocytes and platelets. An intrinsic stem cell defect has been suggested by numerous experiments, and a reduced number of megakaryocytes is found in the bone marrow. Platelet production in W/Wv mice and +/+ littermate controls was studied by measurement of the incorporation of [^{75}Se]methionine (SeM) into platelets after intravenous injection of 2 μCi of SeM. Mean incorporation of SeM into platelets of W/Wv mice was 13.6% greater than that of +/+ controls at 18 h and 17.2% greater at 66 h ($P < 0.05$). The mean platelet count of the W/Wv mice was 1,297,000 ± 140,000/mm^3 (±SD), which was not significantly different from that of controls (1,338,000 ± 154,000). The mean hematocrits of the W/Wv mice and controls were 37.5 ± 2.6% and 45.5 ± 1.9%, respectively. W/Wv mice and controls were made thrombocytopenic (Tpenic) with antiplatelet sera in order to study platelet production during a thrombopoietic stimulus. 16 h after SeM administration and 40 h after induction of Tpenia, mean SeM incorporation into platelets was 70.4% greater in the W/Wv mice than in controls ($P < 0.0025$). However, the mean platelet count of the control group was 52.9% greater than that of the W/Wv mice ($P < 0.025$). 90 h after induction of Tpenia, no significant differences were found in SeM incorporation or in platelet counts between W/Wv mice and controls. These results suggest that W/Wv mice have an increased rate of production of platelets from a reduced pool of megakaryocytes in order to maintain a normal platelet count. A Tpenic stimulus leads to a greater labeling of platelets but a slight delay in recovery of the platelet count until 90 h after the thrombopoietic stimulus. (Research supported by VA.)

284. Functional and Hemodynamic Adaptation to Progressive Renal Ablation. NORMAN J. SIEGEL,* JOEL KAUFMAN,* AND JOHN P. HAYSLETT,* New Haven, Conn. (introduced by V. T. Andriole).

Although compensatory changes after uninephrectomy are well known, the functional and hemodynamic adaptations to progressive loss of renal mass are less well studied. In the present investigation, changes in renal function and blood flow were examined 4 wk after surgery in three groups of adult rats (200–350 g): sham-operated controls (Grp A), 50% ablation (Grp B), and 75% ablation (Grp C). Inulin clearance and extraction were used to determine whole kidney glomerular filtration rate (GFR) and blood flow (TRBF), respectively; mean nephron GFR (MNGFR) and blood flow (MNRBF) were estimated from the GFR and TRBF divided by the total glomerular count. Despite the marked reduction in renal mass, GFR was 68.4% of control values (1.14 ± 0.4 ml/min per 100 g body weight; mean ± SEM) in Grp B (0.78 ± 0.04, $P < 0.01$) and 49.1% in Grp C (0.56 ± 0.04, $P < 0.01$), while TRBF was maintained at 81.3% of control values (5.29 ± 0.19 ml/min per 100 g body weight) in Grp B (4.31 ± 0.29, $P < 0.02$) and 67.7% in Grp C (3.58 ± 0.41, $P < 0.02$). These adaptive changes resulted in a marked increase in MNGFR, a disproportionate rise in MNRBF, and a fall in filtration fraction (FF) as shown (* indicates $P < 0.05$ compared to Grp A):

	MNGFR	MNRBF	FF
	nl/min	*nl/min*	
Grp A	47.5 ± 2.4	215.2 ± 10.1	38.9 ± 2.0
Grp B	73.2 ± 4.9*	404.2 ± 25.7*	35.3 ± 4.0
Grp C	113.8 ± 9.2*	724.3 ± 53.4*	28.7 ± 2.8*

Using the radioactive microsphere technique, proportional renal blood flow to outer and inner cortical zones was determined. While there was a marked increase in absolute blood flow to all zones, there was a relative redistribution of flow toward inner cortical areas in both groups of experimental animals. These data demonstrate that, in response to progressive loss of renal mass, the compensatory changes in renal hemodynamics exceed the adaptation in filtration rate; a marked increase occurs in both MNGFR and MNRBF; and there is a relative redistribution of TRBF toward inner cortical areas.

285. Enzymatic Collagen Cross-Linking Requires Collagen Fibrils: Implications for Inherited Human Connective Tissue Diseases. ROBERT C. SIEGEL,* San Francisco, Calif. (introduced by Lloyd H. Smith, Jr.**).

Collagen solubility is increased in several inherited human connective tissue diseases and animal models, presumably because collagen cross-linking is deficient. The mechanism for such a deficiency is unknown in patients with hydroxylysine-deficient collagen and the Marfan syndrome. In experimental osteolathyrism the cross-linking enzyme, lysyl oxidase, is irreversibly inhibited. In penicillamine-animal disease and human homocystinuria the cross-linking aldehyde intermediates are trapped into a thiazolidine ring. The present study was designed

to investigate additional possible biological mechanisms for deficient cross-linking. Chick embryo lysyl oxidase was purified 2000-fold by collagen affinity and DEAE chromatography. A tritium release collagen assay with 5 times the sensitivity of the conventional elastin assay was used. The following results were obtained: native collagen fibrils, verified by electron microscopy, 2,000 ± 50 cpm ^3H released in 3 h per 200,000 cpm substrate; K_m 2 × 10^{-9} M. There was 3 times the affinity for hydroxylysyl as lysyl residues. Denatured collagen (131 cpm), isolated α1 (68 cpm) and α2 chains (64), pepsin-treated collagen (6.3 cpm), and isolated cross-linking peptide α1-CB1 (5.0 cpm) were much poorer substrates. 0.05 M arginine inhibition of fibril formation resulted in 98% inhibition of lysyl oxidase activity with collagen as substrate but in negligible inhibition with elastin. These in vitro results provide evidence that collagen cross-linking requires fibril formation and lysyl hydroxylation before the enzymatic reaction proceeds. Hydroxylysine-deficient collagen probably does not cross-link normally because of decreased affinity of lysyl oxidases for nonhydroxylated lysyl residues. Abnormal fibril formation due to the observed increase in proteoglycans may be the cause of the cross-linking abnormality in the Marfan syndrome. (Research supported by NIH Grants GM 19527, AM 16424, and AM 09406-10; a grant from the Orthopaedic Research and Education Foundation; and the Helen Hay Whitney Foundation.)

286. Sucrase Precursor in Human Jejunal Crypts. EDWARD R. SILVERBLATT,* KENNETH CONKLIN,* AND GARY M. GRAY, Stanford, Calif.

Enzymatically active sucrase (S_a) is absent in intestinal crypts but becomes detectable as cells migrate onto the lower villus, suggesting that sucrase is synthesized as cells mature. When pure human sucrase was injected into rabbits, a precipitating antibody, monospecific for sucrase, was produced. Indirect immunofluorescence of human jejunum with this antiserum revealed fluorescence not only of sucrase-containing brush borders of villus cells but also, surprisingly, of crypt cell surfaces. This suggested that crypts contain a protein having structural similarity to S_a but lacking enzymatic activity. Experiments to determine whether an inactive form of sucrase (S_i) exists in intestinal crypts were undertaken. Quantitative immunoprecipitation with the rabbit antiserum demonstrated that 0.01 ml of antiserum precipitated 40 mU of S_a from either crude or pure enzyme preparations, forming the basis for an immunoassay of sucrase protein (S_a + S_i). Crypts free of villus cells and sucrase activity, obtained from frozen longitudinal sections of human jejunum, were homogenized and separated into soluble and papain-solubilized, crypt protein fractions. Analysis of papain-solubilized (37°C; 60 min) crypt protein revealed that 5.6 μg displaced 20 mU (1.0 μg) of pure sucrase in the precipitin immunoassay. In contrast, neither cytoplasmic protein nor short-term solubilized papain (37°C; 15 min) protein from crypts possessed the cross-reacting material. Sucrase protein appears to be synthesized in crypts and is firmly bound to cell surfaces as an inactive precursor (S_i) of the villus enzyme (S_a). Hence maintenance of villus sucrase activity probably depends upon two factors: (*a*) synthesis of primordial sucrase protein (S_i) and (*b*) conversion of S_i to active enzyme (S_a). Sucrase deficiency in primary (genetic) or secondary (intestinal) disease must be reconsidered in light of these findings. (Supported by NIH Grants AM 11270, K4-AM 47443, and T01 AM 05418.)

287. Augmentation of Human LDA Activity by Pretreatment of Attacking Lymphocytes with Mitogens. JANET SMITH,* BARRY S. HANDWERGER,* RONALD H. SCHWARTZ,* AND JOHN R. WUNDERLICH,* Bethesda, Md. (introduced by William E. Paul).

In antibody-dependent cell-mediated cytotoxicity, antibody-treated target cells are destroyed by nonsensitized lymphoid cells. To investigate whether activated lymphocytes destroy antibody-treated target cells better than lymphocytes which have not been activated, we pretreated attacking cells from normal nonsensitized donors in vitro with a variety of mitogens. Peripheral blood lymphocytes from normal human donors were prepared by Ficoll-Hypaque gradient centrifugation. Cells incubated in Eagle's minimum essential medium (MEM) with 20% AB+ human serum were treated for 48 h with phytohemagglutinin (PHA), pokeweed mitogen (PWM), or concanavalin A (Con A). After thorough washing, untreated and mitogen-treated cells were tested for cytotoxic activity against freshly prepared target cells which were autologous to the attacking cells. Half of the target cells were pretreated with polyspecific allogeneic antiserum from a multiply transfused patient; the other half were left untreated. During 4 h ^{51}Cr release cytotoxicity tests, attacking cells destroyed antibody-treated but not untreated target cells. Attacking cells pretreated with PHA, PWM, or Con A were 30–530% more active than untreated cells in destroying antibody-coated target cells. Cells treated with mitogen also incorporated more [^3H]thymidine than did untreated cells. Augmentation of cytotoxicity appeared not to result from the presence of mitogen during the cytotoxicity test or from conditioning of the medium during the 48 h that cells were treated with mitogens. These results argue that mitogen treatment of lymphocytes from human peripheral blood increases their capacity to directly damage antibody-coated target cells or to recruit cells to carry out this function.

288. Hyperosmolal Mannitol and Myocardial Anoxia. CHARLES SMITHEN,* JAMES CHRISTODOULOU,* THOMAS KILLIP,** AND NORMAN BRACHFELD,* New York.

Recent reports have suggested that elevated extracellular osmolality has a beneficial action on myocardium deprived of oxygen. The mechanism of action of hyperosmolal mannitol was evaluated by hemodynamic, metabolic, optical and electron microscopic, and latex vascular injection studies in the isovolumic nonrecirculating paced perfused rat heart. 72 studies were performed during 15-min periods of aerobic, anoxic, and reoxygenated perfusion. Mannitol significantly aided recovery of hemodynamic function during reoxygenation. Thus with isosmolal perfusion left ventricular systolic peak pressure (LVSP) decreased 32% from 127 ± 5 to 86 ± 6 mm Hg; maximum dP/dt fell 50% from 3513 ± 328 to 1758 ± 172 mm Hg/s. With hyperosmolal mannitol added to perfusate LVSP decreased only 23% from 132 ± 5 to 102 ± 7 mm Hg and dP/dt fell only 21% from 3817 ± 215 to 2998 ± 234 mm Hg/s ($P < 0.01$). No effect of mannitol on energy metabolism could be demonstrated. Thus, postanoxic total coronary flow, lactate and glucose metabolism, tissue glycogen, creatine phosphate, or adenine nucleotide concentrations did not significantly differ with or without mannitol in perfusate before or after anoxia. Injection studies failed to reveal significant areas of capillary obstruction induced by anoxia. No physical effect of mannitol on arteriolar or capillary filling was observed. Ultrastructural analysis showed that mannitol significantly reversed the postanoxic mitochondrial and myofibrillar edema consistently observed during reoxygenation in the absence of increased osmolality. These changes were strikingly reduced by mannitol perfusion even in samples taken immediately after anoxia. A positive correlation between reduction in total myocardial water content (wet:dry wt ratio) and enhanced mechanical perfusion during reoxygenation was observed in the mannitol perfusion experiments. Thus, coronary perfusion with hyperosmolal mannitol enhanced postanoxic myocardial performance, preserved ultrastructural integrity, and minimized tissue swelling. Since neither energy production nor coronary flow were

improved, the most tenable explanation for the mechanism of action of mannitol during myocardial anoxia is its direct effect on interstitial water content. (Research supported by Grant 1-HL-71439 from NIH.)

289. Cytoplasmic Polymerases: a Newly Discovered System for Genetic Control. ANTERO G. SO,* KATHLEEN M. DOWNEY,* AND JOHN J. BYRNES,* Miami, Fla. (introduced by William J. Harrington**).

It is generally held that the synthesis of DNA and RNA is restricted to the nucleus and that only translational control mechanisms are operative in the cytoplasm of eukaryotes. We have isolated both DNA polymerase and RNA-dependent RNA polymerase from the cytoplasm of erythroid cells and propose that these cytoplasmic polymerases serve a pivotal role in regulation of gene expression and amplification. The RNA-dependent RNA polymerase utilizes hemoglobin mRNA as template. The DNA polymerase can be regulated by ATP and citrate, ATP acting as an allosteric activator. Both cytoplasmic polymerases are extremely sensitive to heme; complete inhibition occurs at 20 μM (the same concentration optimal for globin synthesis). Heme blocks nucleic acid synthesis instantaneously by dissociating the polymerases from nucleic acid templates. Heme promotes globin synthesis by (*a*) preventing association and promoting dissociation of RNA-dependent RNA polymerase from hemoglobin mRNA template, making mRNA available for translation; and (*b*) by inhibiting synthesis of double-stranded RNA intermediates which inhibit initiation of globin synthesis. Heme thereby regulates hemoglobin synthesis by regulating synthesis of cytoplasmic DNA and RNA. The demonstration of regulation of hemoglobin synthesis by cytoplasmic DNA and RNA polymerases affords a new approach to studies of disordered hemoglobin formation (e.g., thalassemias). Analogous controls in immunoglobulin and other protein syntheses are likely, providing fruitful areas for research in immune deficiency states and cancer. (Research supported by grants from NIH and AHA.)

290. Transitory Appearance of a New Bence Jones-Related Protein Associated with Corticosteroid Therapy. ALAN SOLOMON AND CARLA L. MCLAUGHLIN,* Knoxville, Tenn.

Urine specimens from patients with multiple myeloma and Bence Jones proteinuria frequently contain low molecular weight proteins which correspond to either the amino-terminal half (variant, VL) or the carboxyl-terminal half (constant, CL). The VL and CL of a Bence Jones protein (BJP) can be produced in vitro by specific proteolytic cleavage. Recently, we have observed that the urine specimens from such patients who have received high doses of corticosteroids as part of their treatment regimen contain a low molecular weight protein related to the BJP but not identical with the VL or CL. Analyses of daily urine specimens obtained over an extensive time period have revealed that a reproducible chain of events occurs during the treatment regimen which includes oral administration of 75 mg of prednisone daily for 7 consecutive days. Namely, there is a progressive decrease in the amount of BJP excreted, and by the sixth day the amount of BJP is usually <10% of the pretreatment value. The urine specimen obtained on the seventh day of treatment is characterized by a virtual absence of BJP and the striking appearance of a new protein whose electrophoretic mobility is distinct from that of the BJP or its VL or CL. With cessation of treatment there is a prompt disappearance of the new protein and a progressive increase in amount of BJP to the pretreatment level within 7 days. 24-h urine specimens have been obtained daily for 10 mo from one patient, and analyses of specimens obtained during 10 treatment regimens have revealed that the striking decrease-increase of BJP and the appearance of the new protein are associated with prednisone therapy and not with chemotherapy. The new protein was isolated and purified for comparative studies with the BJP and with the VL and CL prepared from the BJP. Physicochemical and immunochemical studies have shown that the new protein is antigenically related to but is smaller than the monomer BJP, and that it is more closely related to the CL than to the VL. (Supported by NIH Grant CA-10056.)

291. Beta-Adrenergic Receptor: Direct Interaction of Guanyl Nucleotide with Regulatory Site of Adenylate Cyclase. ALLEN M. SPIEGEL* AND G. D. AURBACH,** Bethesda, Md.

Purine nucleotides influence hormone-adenylate cyclase interactions in several tissues in vitro. An earlier study showed that GTP augments catecholamine-responsive adenylate cyclase (6 × basal vs. 4 × basal without GTP) activity in turkey erythrocyte membranes. The nature of the guanyl nucleotide interaction was investigated further using 5'-guanylyl imidodiphosphate (GMPPNP), an analog of GTP with a terminal phosphate resistant to enzymatic cleavage. GMPPNP augments isoproterenol-stimulated adenylate cyclase causing greater activation (over 50 × basal) than with sodium fluoride. The K_m for the effect of GMPPNP is 10^{-7} M. A similar K_m was observed for the slight stimulation (threefold) of the enzyme by the nucleotide in the absence of added catecholamine. The greatly enhanced stimulation of adenylate cyclase activity by isoproterenol in the presence of GMPPNP is blocked completely by propranolol ($K_i = 5 \times 10^{-6}$M). Propranolol, however, does not abolish the stimulation of basal activity produced by the nucleotide. GMPPNP did not affect binding of [^{3}H]isoproterenol to a catechol-specific site on the membranes. Radioactive GMPPNP bound to the same cell membrane preparation with a K_m equivalent to that found for effects on adenylate cyclase activity. Propranolol does not inhibit binding of the nucleotide, but unlabeled GMPPNP and other purine nucleotides competitively inhibit binding of GMPPNP with apparent affinities comparable to the order of potencies for augmenting catecholamine-stimulated adenylate cyclase (GTP > GDP > ITP > ATP). Thus, a purine nucleotide site with apparent affinity for GTP much higher than for ATP may be involved in regulating the catalytic function of the adenylate cyclase complex. This finding suggests that, although ATP is clearly the substrate for the enzyme, GTP and not ATP interacts at the regulatory site (identified as a GMPPNP binding site) in vivo.

292. The Catabolism of a Human IgG Half-Molecule. HANS L. SPIEGELBERG,* La Jolla, Calif. (introduced by Frank J. Dixon**).

The elimination from the circulation and excretion into the urine of a ^{131}I-labeled myeloma IgG half-molecule was studied in man and rhesus monkeys and compared to the catabolism of 3 γ heavy-chain disease (HCD) proteins. The half-molecule had a molecular weight of 75,000 daltons and consisted of one covalently linked heavy (γ) and one light (κ) chain. In contrast to the HCD proteins, the half-molecule was very rapidly catabolized and, in part, excreted into the urine. The average plasma half-lives were 4.5 days for the half-molecule and 26 days for the HCD proteins. The fractional turnover rates (amount catabolized per day as percent of intravascular pool) were 160% for the half-molecule and 7–11% for the HCD proteins. On the average, 7% of the half-molecule and 26% of the HCD proteins remained in the intravascular compartment. 7% of the intravascular pool of the half-molecule was excreted into the urine, but only 0.4–1.5% of the HCD proteins was excreted. The patient who formed

the half-molecule also formed four chain 7S IgG myeloma protein characterized by a spontaneous dissociation into half-molecules after mild reduction and alkylation. Although the 7S myeloma protein could not be isolated free of normal IgG, the turnover rate of the mixture indicated that the 7S myeloma protein was, like the half-molecule, very rapidly catabolized. These data indicate that the rare form of human IgG myeloma protein that produces half-molecules lacks the structures responsible for the slow rate of catabolism characteristic of the four known subclasses of IgG and of γ HCD proteins. (Supported by NIH and AHA.)

293. Hypocalciuric Action of 1,25-Dihydroxycholecalciferol in the Phosphate-Depleted Rat. THOMAS H. STEELE,* JOHN E. ENGLE,* ROMAN S. LORENC,* YOKO TANAKA,* KATHRYN L. DUDGEON,* AND HECTOR F. DELUCA,* Madison, Wis. (introduced by Edwin C. Albright**).

The effects of 1,25-dihydroxycholecalciferol (1,25 $(OH)_2D_3$) and inorganic phosphate (Pi) on renal calcium handling during Pi depletion were compared in rats maintained on a high calcium (1.2%), low phosphorus (0.1%) rachitogenic diet for at least 10 days and thyroparathyroidectomized 2 days before use. Clearance experiments were performed 14 h after single intraperitoneal injections of either propylene glycol (PG) or 1,25 $(OH)_2D_3$ (1300 pmol) in PG. In rats receiving only PG, calcium excretion initially averaged 198 ± 32 neq/min per 100 g body weight (mean ± SEM) and decreased during Pi infusion (0.7 mg/kg per min) to 85 ± 18 ($P < 0.001$). Fractional calcium excretion (FE_{Ca}) declined from 9.2 ± 1.4% to 4.9 ± 1.1% ($P < 0.001$) when plasma Pi was raised from 3.9 ± 0.4 mg/100 ml initially to 11.3 ± 1.1 mg/100 ml by Pi infusion. After 1,25$(OH)_2D_3$, calcium excretion averaged only 76 ± 28 and FE_{Ca} 4.1 ± 1.6%, values significantly less than those in the PG rats ($P < 0.02$ and <0.05). Absolute and fractional calcium excretions did not decrease further in 1,25$(OH)_2D_3$ rats when plasma Pi was raised from 5.8 ± 0.9 mg/100 ml to 12.8 ± 1.5 mg/100 ml. Both groups avidly reabsorbed Pi and had similar glomerular filtration, sodium excretion, and filtered calcium values. Within the 1,25$(OH)_2D_3$ group, absolute and fractional calcium excretions varied inversely with plasma Pi. In those 1,25$(OH)_2D_3$ rats with plasma Pi less than 6 mg/100 ml, FE_{Ca} ranged from 4.0 to 7.9%; in the remainder (plasma Pi greater than 7 mg/100 ml, FE_{Ca} ranged from 0.5 to 1.5%. These results indicate that 1,25$(OH)_2D_3$ increases renal calcium reabsorption in the phosphate-depleted rat, possibly in part through elevation of plasma Pi and consequent repletion of Pi in previously depleted kidney tissue. (Supported by NIH grants.)

294. Immuno-Epidemiologic Significance of the Mumps Virus Skin Test. JOSEPH W. ST. GEME, JR., TERRY YAMAUCHI,* JON M. AASE,* AND GEORGE R. NOREN,* Torrance, Calif.; Anchorage, Alaska, and Minneapolis, Minn.

The biologic validity of cell-mediated immunity (CMI) to mumps virus (MV) was evaluated in 395 children, adolescents, and adults. The study protocol included the determination of cutaneous delayed hypersensitivity to viral and avian control antigens and a very essential double bleeding before and after MV skin test for assay of neutralizing antibody. 7% of subjects expressed sufficient CMI to the control antigen to erase an apparently positive MV skin test. Anamnestic conversions from seronegativity to seropositivity, elicited by MV skin test, increased from 4% in children to 25% in adults, which suggests waning B cell recognition of prior MV infection in adults. Although pregnancy diminished the difference ($P < 0.001$), adults showed greater CMI than children ($P < 0.001$), suggesting that MV reinfection or persistence induces the escalation of more sensitive T cell recognition with increasing age. The larger CMI of Minnesota than California or Alaska school children ($P < 0.01$) may reflect variable potency of viral antigen or differences in skin pigmentation. Humoral immunity (HI) rose from 16% (1–4 yr), 45% (5–9 yr), and 80% (10–14 yr) to 94% in adolescents and adults. Ordinarily 90% in other age groups, the decline of immunologic correlation between MV CMI and HI to 70% in school children may result from prior parainfluenza virus infection, concurrent immunosuppressive infection, disease, or treatment, in addition to the other factors cited above.

295. The Opsonically Active Fragment of C3. THOMAS P. STOSSEL,* RONALD J. FIELD,* CHESTER A. ALPER, AND FRED S. ROSEN, Boston, Mass.

C3 is the major heat-labile opsonin of serum. *E. coli* lipopolysaccharide-coated paraffin oil particles (LPSP) containing oil red O were opsonized in fresh human serum by reactions involving the properdin system, and during opsonization in the presence of [^{125}I] C3, radioactivity was fixed to the LPSP. The initial rate of ingestion of the opsonized particles, assayed spectrophotometrically, was proportional to the amount of radioactivity fixed. Radioactivity and ingestibility of opsonized LPSP were not diminished by treatment with high salt concentrations, boiling, 8 M urea, 6 M guanidine, extremes of pH, 1% Triton-X-100, or 2% deoxycholate. Trypsin or purified C3 inactivator but not phospholipases C, A, and E removed radioactivity from and impaired ingestibility of opsonized LPSP. Opsonized LPSP treated with 10^{-4} M iodoacetate or *N*-ethyl maleimide and then washed to remove the free reagents had decreased ingestibility and unaltered radioactivity. Ingestibility of LPSP was destroyed and radioactivity was eluted by 1% dodecyl sulfate (SDS). The eluates contained one radioactive peptide with a molecular weight of about 70,000. Tryptic digests of these eluates revealed at least two radioactive peptides which corresponded to two of the more than 10 tryptic peptides of C3 or of C3b. C3 denatured in SDS, urea, mercaptoethanol, and propionic acid was resolved into subunits of approximately 110,000 and 70,000 mol wt by gel filtration. The lighter subunit comigrated on SDS-acrylamide gels with the radioactive peptide eluted from opsonized LPSP with SDS. The opsonically active fragment of C3 is extremely stable and bound to LPSP by a firm hydrophobic bond. It requires intact SH groups for opsonic expression. It has a peptide structure that suggests it is a 70,000 mol wt fragment or subunit of C3.

296. Uptake and Excretion of Tritiated Thymidine in Man. MARC J. STRAUS,* FRANCISCO TEJADA,* SUSAN KREZOSKI,* AND LEWIS BATTIST,* Washington, D. C. (introduced by C. Gordon Zubrod**).

Doses of tritiated thymidine (^{3}HTdR), >10 mCi intravenously, are needed for optimal autoradiographic cell kinetic studies in human solid tumors. Rubini (1960. *J. Clin. Invest.*) reported that >½ the injected dose of ^{3}HTdR is incorporated into DNA. We injected 11 patients with advanced cancer with 0.2 mCi/kg ^{3}HTdR intravenous push.* Radioactivity was determined by liquid scintillation. 33% of the injected dose was excreted in urine in 24 h and 55% total in 10 days. The plasma activity decreased to 20% of initial levels in 10 min. Plasma activity decay was then constant. Urine activity peaked in 6 h, at 10–20 times peak plasma activity. Urine levels equaled plasma's at 1–2 days. Activity of air vapor and sputum increased to plasma levels in 1 h. The regression of plasma, air, and sputum activity after 1 h and urine after 1–2 days measured over 2 months was linear; $t_{½}$ = 10–12 days. This $t_{½}$ is the biological half-life of tritiated

H_2O (^3H_2O) distributed in the total body water. Further studies indicate that the high urine values are due to excretion of ^{3}HTdR and metabolites. Approximately 10% of activity is unaccounted for when urine and insensible H_2O losses are measured. The amount of ^3H_2O inspired by investigators working close to these patients up to 6 h/day resulted in no measurable activity in plasma or urine. Activity in other patients sharing the room was unmeasurable with adequate ventilation. There was no short-term radiotoxicity in our patients. Use of intravenous ^{3}HTdR potentially yields valid and more complete kinetic data than in vitro studies and appears justifiable in selected patients.

* With permission of Radioisotope and Human Rights Committees (NIH:Wash., VA Hospital).

297. Effect of Spironolactone on the Biosynthesis of Testosterone. BITTEN STRIPP,* RAYMOND MENARD,* LYNN LORIAUX,* ADDISON TAYLOR,* JAMES GILLETTE,* AND FREDERIC BARTTER,** Bethesda, Md.

Prominent side effects limiting the usefulness of spironolactone are gynecomastia and loss of libido. Studies with closely related compounds suggest that the effect may be attributable to decreases in plasma testosterone. Spironolactone was given to human volunteers and to dogs, rats, mice, rabbits, and guinea pigs; testosterone biosynthesis was estimated from effects on plasma testosterone and in some studies on testicular 17-α-hydroxylase and its obligatory electron acceptor, cytochrome P450. Infusion of spironolactone (100 mg/kg) intravenously to dogs over 1 h lowered spermatic vein testosterone by 57–85% in 4–5 h; the treatment lowered testicular 17-α-hydroxylase (progesterone→17α-OH-progesterone and testosterone) activity by 60%, compared to no decrease in vehicle-treated dogs. Cytochrome P450 in testis decreased to the same extent, whereas other microsomal enzymes (NADPH cytochrome c reductase, 17-β-dehydrogenase, , cytochrome b_5) were unaffected. The effects of spironolactone on cytochrome P450 and 17-α-hydroxylase were fully reversible. The same effects were seen in mice, rabbits, guinea pigs, and rats. In rats, testicular microsomal cytochrome P450 had decreased by 16 h after only 10 mg/kg (intraperitoneally), and by 0.5 h after 100 mg/kg. No effect on spermatogenesis and no cell necrosis was seen in 30 days on a daily dose of 100 mg/kg. In vitro studies with testicular microsomes suggest that cytochrome P450 converts spironolactone to a metabolite, which in turn destroys cytochrome P450. Thus, the cytochrome P450 is not broken down if its action on spironolactone is prevented by withholding NADPH or by addition of competing quantities of progesterone. In providing a mechanism for inhibition of testosterone synthesis by spironolactone, these studies help to explain its depression of libido and perhaps its production of gynecomastia.

298. Insulin-Induced Augmentation of Lymphocyte-Mediated Cytotoxicity. T. B. STROM,* R. BEAR,* C. B. CARPENTER, AND J. P. MERRILL,** Boston, Mass.

After sensitization with an allograft, the recipient develops thymus-derived lymphocytes that are selectively cytotoxic for cells bearing donor alloantigens (lymphocyte-mediated cytotoxicity, or LMC). The effect of insulin upon the cytotoxic action of allosensitized splenocytes harvested 7 days after skin grafting upon donor thymocytes during a 4 h in vitro incubation was studied quantitatively using a ^{51}Cr release technique. Attacking and target cells were interacted in RPMI media supported by 10% heat-inactivated serum harvested from insulin-deficient rats rendered diabetic by streptozotoxin. Addition of crystalline bovine insulin in the physiologic dose range of 10^{-8} M to 10^{-12} M resulted in markedly augmented LMC: 10^{-8}M, 10^{-9}M, 10^{-10}M, 10^{-11}M, and 10^{-12}M produced 35 ± 5%, 75 ± 4%, 64 ± 5%, 52 ± 3%, and 26 ± 6% enhancement, respectively, of LMC in triplicate samples. Insulin-induced augmentation of LMC is completely dependent upon a period of preincubation of the sensitized attacking cells with this agent. Augmentation was not observed if the attacking cells were preincubated for more than 5 min with insulin before introduction of the target cells, nor if the agent was added after both attacking and target cells were mixed. These data indicate that insulin augments LMC via an effect upon the attacking cells alone, since the addition of insulin after mixture of attacking and target cells fails to enhance LMC. The time-dependent enhancement of LMC is similar to previous studies indicating that LMC may be augmented by elevations of intracellular cyclic guanosine 3',5'-monophosphate (cGMP) or depletion of cyclic adenosine 3',5'-monophosphate (cAMP) within the attacking lymphocytes at the time of initial attacking-target cell interaction. Furthermore, these studies are consistent with other observations, indicating that the action of insulin is mediated by increased levels of cGMP or depletion of cAMP. (Supported by NIH grants.)

299. Big and Little Prolactin in Human Plasma and Pituitary Glands. HAN K. SUH* AND ANDREW G. FRANTZ, New York.

In order to determine whether size heterogeneity exists for human prolactin, as has been demonstrated for several other polypeptide hormones, plasma samples and pituitary extracts and culture media were fractionated on 92 × 1.7 cm columns of Sephadex G-100, and the fractions measured by homologous radioimmunoassay. The major peak in all specimens, termed "little" prolactin, appeared in the same effluent fractions as purified [^{131}I]prolactin. A less retarded peak, termed "big" prolactin, emerging between human albumin and [^{131}I]prolactin, was also seen in all specimens. Big prolactin accounted for 6–20% of the total immunoreactivity in plasma samples from normal subjects, patients with chromophobe adenomas and idiopathic galactorrhea, and in pituitary culture media. Neither TRH stimulation nor L-dopa suppression produced major changes in the plasma ratios of big to little prolactin, which were similar in normals and tumor patients. Significantly higher amounts of big prolactin (range 26–31%) were seen in plasmas from pregnant subjects. Big and little prolactin fractions were indistinguishable by radioimmunoassay when tested over a wide range of concentrations. A third component of larger molecular size, eluting close to the void volume and also possessing immunoreactivity, was seen in small amounts in some specimens. Repeated freezing and thawing induced considerable conversion (25–50%) of big to little prolactin, as did treatment with 8 M urea; some conversion of big prolactin to the high molecular size (void volume) form was also seen under these conditions. Conversion of little to big prolactin was not observed under any circumstances, including incubation of little prolactin fractions with hypopituitary plasma. We conclude that human prolactin, like other polypeptide hormones, exists in at least two separate forms, and that big prolactin is probably secreted by the pituitary gland.

300. Characteristics of L-Triiodothyronine (T_3) Binding by Isolated Rat Hepatic Nuclei In Vitro. MARTIN I. SURKS, DIONA KOERNER,* AND JACK H. OPPENHEIMER,** Bronx, N.Y.

We have described high-affinity, limited-capacity T_3 binding by nuclei from rat tissues in vivo and recently demonstrated

limited-capacity T_3 binding by isolated hepatic nuclei in vitro. To determine whether nuclear sites observed in vitro were identical with those studied in vivo, hepatic nuclei isolated from euthyroid rats were incubated in isotonic medium at pH 7.0 with tracer [^{125}I]T_3 and increasing doses of nonradioactive T_3. Scatchard plots showed a single class of binding sites: mean apparent association constant (K_a) = 6.1×10^8 M^{-1}; mean binding capacity (M) = 1.7 pmol/g liver nuclei. Measurements of M in vitro were virtually identical with in vivo determinations. The avidity of the nuclear sites for five iodothyronine analogues determined in vitro was the same as described in vivo. Nuclear T_3 binding in vitro was exchangeable. Moreover, the release rate of T_3 bound in vivo or in vitro was indistinguishable. Similar to in vivo studies, Triton X-100 or 0.15 M NaCl treatment failed to extract in vitro-bound T_3, whereas 0.4 M KCl readily extracted the T_3-nucleoprotein complex. Finally, G-100 chromatography of 0.4 M KCl extracts from nuclei with receptor-T_3 complex labeled with [^{131}I]T_3 in vivo and [^{125}I]T_3 in vitro showed parallel elution peaks of the chromatin nonhistone protein-T_3 complex. Thus, similar stereochemical specificity, binding kinetics, extraction properties, and Sephadex elution profiles establish the identity of the in vitro nuclear sites to those described in vivo. The requirement for specific cytosol receptor was then investigated. K_a for T_3 was unchanged by extensive washing of nuclei. Addition of cytosol to nuclei or preincubation at 37°C did not alter the amount of T_3 bound. Moreover, K_a and M of nuclei from athyreotic rats was similar to euthyroid animals. Thus, in contrast to steroid hormones, prior formation of a T_3-cytosol protein complex does not appear necessary for the binding of T_3 by the nuclear chromatin receptor. (Supported by NIH and DOD.)

301. Effect of Antihistamine on Induced Hypoxic Pulmonary Hypertension in Man. ARMANDO SUSMANO,* Chicago, Ill. (introduced by John S. Graettinger**).

Pulmonary hypertension (PH) can be induced acutely in man or animals by hypoxia. Previous work conducted in our laboratory lent strong support to the hypothesis that histamine (H) may be the mediator of hypoxic pulmonary hypertension (HPH), by demonstrating that antihistamines (AH) prevented and abolished this effect in dogs. Our study tests whether a similar effect can be obtained in man. Eight patients breathed room air (RA) and then 12% oxygen for 10 min. Arterial blood gases, mean pulmonary artery (PA), mean aortic (AO) pressure, and heart rate (HR) were measured at RA and during hypoxia, before and again after 0.2 to 0.3 mg/kg of chlorpheniramine (CT) was given into the PA. One additional patient, a child, with adenoidal cor pulmonale and severe PH, studied at RA, dropped mean PA pressure by 10 mm Hg 15 min after and by 19 mm Hg 30 min after CT. During hypoxia all eight patients increased PA pressure by an average of 8 mm Hg ($P = 0.0002$) before CT. During RA breathing, CT did not affect blood gases, HR, PA, or AO pressure. During a second hypoxic exposure and after CT, prevention of HPH was seen in three patients and significant attenuation in three, and no effect was observed in two. The mean increase in PA pressure during hypoxia was significantly less after CT than before ($P = 0.008$). This study demonstrated that prevention or attenuation of HPH by a potent AH is consistent with the hypothesis that H mediates or plays an important role in HPH.

302. Control of Cardiac Sarcolemmal Adenylate Cyclase by Calcium Ions. MICHIHIKO TADA,* MADELEINE A. KIRCHBERGER,* JO-ANNA M. IORIO,* AND ARNOLD M. KATZ, New York.

A morphologically recognizable plasma membrane preparation, obtained after osmotic shock and KCl extraction of guinea pig ventricular cell segments, was used to study the Ca^{2+}-control of sarcolemmal adenylate cyclase activity (ACase). Basal ACase measured at 37°C and pH 7.5 in 0.5 mM ethylene glycol bis (β-aminoethylether)-*N*, *N'*-tetraacetic acid (EGTA) was 200–250 pmol mg^{-1} min^{-1}. ACase activity at 25°C and pH 6.8, conditions that facilitate use of Ca-EGTA buffers, was 30 pmol mg^{-1} min^{-1} in 0.5 mM EGTA ("zero" Ca^{2+}). This basal activity was markedly inhibited by increasing Ca^{2+} concentration. ACase at 10^{-7}, 10^{-5}, and 10^{-3} M Ca^{2+} was 15, 8, and 5 pmol mg^{-1} min^{-1}, respectively. Epinephrine-stimulated ACase under these conditions was similarly inhibited by increasing Ca^{2+}. ACase at "zero," 10^{-7}, 10^{-5}, and 10^{-3} M Ca^{2+} was 45, 40, 28, and 15 pmol mg^{-1} min^{-1}, respectively, in the presence of saturating concentrations (10^{-4} M) of *l*-epinephrine. Ca^{2+}-inhibition of epinephrine-stimulated ACase was less marked than that of basal ACase, so that net stimulation by 10^{-4} M *l*-epinephrine increased from ~40% in "zero" Ca^{2+} to ~400% in 10^{-5} M Ca^{2+}. Increasing Ca^{2+} concentration caused very little shift in apparent K_m for epinephrine stimulation of ACase, from 2 μM in 10^{-7} M Ca^{2+} to 5 μM in 10^{-5} M Ca^{2+} (both of which are similar to the value of 5 μM measured in 0.5 mM EGTA at pH 7.5 and 37°C), suggesting that Ca^{2+} does not alter the affinity of epinephrine for the β-receptor. In view of electrophysiological evidence that β-adrenergic stimulation increases a slow inward Ca^{2+} current across the sarcolemma, inhibition of ACase by Ca^{2+} may reflect the existence in the intact heart of a negative feedback mechanism by which excessive Ca^{2+} influx into the myocardium would depress subsequent Ca^{2+} entry. Such a mechanism could, for example, protect the ischemic myocardium from the detrimental effects of the high rate of energy utilization that results when large amounts of Ca^{2+} become available for binding to the contractile proteins. (Supported by New York Heart Association, NIH Grants HL-13191 and HL-15764 and Contract NIH-NHLI-72-2973-M, and the Jack Martin Fund.)

303. Influence of Exogenous Fat on the Metabolism of Low Density Lipoprotein (LDL) in Man: Possible Mechanism for Rise in LDL Level After Intravenous Fat. G. R. THOMPSON,* R. SEGURA,* H. HOFF,* AND A. M. GOTTO, Houston, Tex.

The amount and type of fat ingested in the diet are known to exert major influences on the level of low density lipoprotein (LDL) circulating in plasma. To investigate the mechanisms whereby fat intake exerts these effects, a fat emulsion (unsaturated triglyceride/saturated phospholipid) was administered to four healthy subjects on a low fat diet, both by the intravenous and intragastric routes. There was an insignificant fall in LDL-cholesterol and LDL-protein (apoLDL), as determined by radioimmunoassay, after IG fat but a 20% rise in both after intravenous administration. By contrast the level of very low density lipoprotein fell after intravenous fat. After intravenous fat there was persistence of exogenous phosphatidylcholine (PC) in plasma, demonstrable by electron microscopy and by changes in the fatty acid composition of circulating PC. In vitro and in vivo studies showed exchange between exogenous PC and LDL-PC resulting in the latter becoming more saturated. This did not occur after intragastric fat, due to intestinal hydrolysis of ingested PC. To ascertain the mechanism of the rise in LDL after intravenous fat, the turnover of [^{125}I]LDL protein was determined in six other subjects on a low fat diet. Intragastric fat had no effect on plasma decay or fractional catabolic rate (FCR) of ^{125}I, but intravenous fat increased ($t_{\frac{1}{2}}$) from 4.7 to 5.4 days and decreased FCR by 18%. These findings suggest that the catabolism of LDL-protein (apoLDL) is

inversely related to the degree of saturation of the fatty acids in LDL-PC. (Research supported by grants and a contract from NIH.)

304. Binding Characteristics of Cell Membrane Receptors and Antibodies for Radioligand Assays. JAN I. THORELL,* STEVEN M. LARSON,* PEDRO CUATRECASAS, AND HENRY N. WAGNER, JR.,** Baltimore, Md.

The kinetic characteristics of two different types of insulin binding receptors, cell membrane receptors and antibodies, were compared. Both types of receptors were in an insoluble form so that an identical assay technique could be used. The cell membrane receptors were purified from human placentas and the antibodies, produced in rabbits, were coupled with Sephadex. The binding reagents were incubated with [^{125}I]-insulin and with varying amounts of unlabeled insulin. After incubation, the receptors or the antibodies were isolated by centrifugation with a separating oil phase, and the bound radioactivity was measured. In the receptor assay, equilibrium was reached rapidly: 50% binding was achieved in less than 1 min. In the antibody assay, 50% binding required 18 min. The dissociation rates were measured after addition of excess amounts of insulin. Both systems showed a rapid and a slow component. The dissociation constants for the membrane receptors were 1.4 min^{-1} and 2×10^{-2} min^{-1} and for the antibodies they were 6×10^{-2} min^{-1} and 5×10^{-3} min^{-1}. The average association constants (K_m) as calculated from displacement curves were approximately 10^8 L/M for the receptors and 10^9 L/M for the antibodies. The cell membrane receptors showed binding capacities for insulin of the same order as that of the antibodies, binding up to 60% of the [^{125}I]insulin. This is considerably higher than has been previously reported for cell membrane receptors. The high association rate of the membrane receptors provides the basis for the development of more rapid radioligand assays than are possible with conventional radioimmunoassay techniques.

305. Hepatitis B Antigen (HB Ag) and/or Antibody (HB Ab) in Fulminant Hepatitis. Pathogenic and Prognostic Significance. CHRISTIAN G. TREPO, JEAN MOTIN, DOMINIQUE ROBERT, AND ALFRED M. PRINCE, Lyon, France, and New York (introduced by Stephen D. Litwin**).

Sera were obtained from 43 consecutive fulminant hepatitis cases in adults observed in Lyons. HB Ag was detectable by agar gel diffusion in 8/43 cases (18.6%); counterelectrophoresis in 12/43 (28%); and solid-phase radioimmunoassay in 24/43 (55.8%). In 14 patients the antigen subtype could be determined by passive hemagglutination inhibition. 11 were Y and 3 were D. HB Ab was found in 2/43 cases by agar gel diffusion but in 21/43 (48.8%) by passive hemagglutination (titer $\geq$ 1:4). Coexisting HB Ag and HB Ab was found in 14/43 (32.5%), whereas HB Ag and/or HB Ab were present in 31/43 (72%) of the cases. 5 of 12 patients without HB Ag or HB Ab (41%) survived whereas only 5/31 of those with HB Ag and/or HB Ab did so (16%) ($P > 0.05$). All 14 patients with both HB Ag and HB Ab died, compared with 4/7 of those with HB Ab and 8/10 of those with HB Ag. Serial specimens were available in seven cases of this series. Two patients repeatedly positive for HB Ag and one for HB Ab survived on serial testing, whereas in four other patients decrease in HB Ag titer with increasing HB Ab titers was followed by death, in spite of total clearance of HB Ag and presence of high titer of HB Ab alone in two cases. Similarly termination of chronic HB antigenemia in one hemodialyzed and one mentally retarded patient was associated with fatal fulminant hepatitis and appearance of HB Ab in both cases. These data emphasize the high frequency and severity of fulminant hepatitis associated with HB Ag and underline the role of the immune response in its pathogenesis. They suggest that development of HB Ab in fulminant hepatitis is not associated with improved prognosis. (Supported by NIH Grant HL 09011.)

306. Phytohemagglutinin (PHA) Stimulation of Axillary Lymph Node Cells from Patients with Operable Breast Carcinoma. VASILIS TSAKRAKLIDES,* EVANGELIA TSAKRAKLIDES,* AND ROBERT A. GOOD, New York.

Morphology of the axillary lymph nodes in patients undergoing radical mastectomy for breast carcinoma was classified in four patterns designated lymphocyte predominance (LP), germinal center predominance (GCP), lymphocyte depletion (LD), and unstimulated (U). Correlation of the lymph node histologic pattern with the survival data showed that LP was common in cases with high survival, LD was common in cases with low survival, and GCP and U patterns were associated with intermediate prognosis. In vitro analysis showed that the proportion of T lymphocytes was higher in lymph nodes with LP pattern than in those with GCP pattern. We now report the results of the in vitro stimulation of the axillary lymph node cells with PHA in 19 patients. Lymphocytes from 34 lymph nodes were taken by mincing half of the node; the second half was processed for routine histology. The tests were performed with PHA P (Difco) in three dilutions in microtiter plates. The cultures were labeled with [^{14}C]thymidine and were terminated at 84 h. Results were expressed as stimulation index (SI: stimulated response/unstimulated response). We found that SI varied from 4 to 255. Lymph nodes with LP showed a high SI (106 ± 14), and cases with GCP showed a low SI (28 ± 2.5). The SI of lymph nodes with U pattern was intermediate (68 ± 17.5). Lymph node with LD was examined and showed a very low SI (4). The morphology of regional nodes in patients with cancer correlates well with the functional status. (Research supported by grants from ACS and NCI [CA-08748-08S1].)

307. Adenosine Deaminase Deficiency in Severe Combined Immunodeficiency: Evidence for a Posttranslational Defect. MARTIN B. VAN DER WEYDEN* AND WILLIAM N. KELLEY, Durham, N. C.

A deficiency of adenosine deaminase (ADA) activity has been reported in a group of patients with severe combined immunodeficiency. Since this represents the first association of a specific enzyme defect with an inherited disorder of immune function, the nature of this association is of special interest. We have demonstrated the following. (*a*) Crude extracts of human spleen, thymus, and leukocytes, based on sucrose density ultracentrifugation and gel filtration, have four molecular species of ADA with approximate molecular weights of 30,000, 110,000, 280,000, and $>1.5 \times 10^6$ daltons. The relative proportions of these four forms in splenic extracts, as determined by sucrose ultracentrifugation, are 86:2:10:4, respectively. (*b*) The larger molecular weight species ($>1.5 \times 10^6$, 280,000, and 110,000) isolated from splenic tissue by gel filtration are convertible to the small molecular weight form (30,000), and the small form is convertible to the form with mol wt 280,000. (*c*) The small molecular form of the enzyme is located in the cytosol, whereas the larger species are associated with mitochondrial and microsomal fractions. (*d*) The specific activity of ADA in splenic tissue obtained from a patient with severe combined immunodeficiency was 0.5% of normal. In contrast to the finding in control extracts, the relative proportions of the molecular forms of ADA in splenic tissue from this patient, as observed by sucrose ultracentrifugation,

are 0:75:0:25. We conclude from these studies that: (*a*) the deficiency of ADA in severe combined immunodeficiency is not due to a large genetic deletion involving the structural gene for ADA; (*b*) molecular heterogeneity of ADA results from posttranslational events; and (*c*) the deficiency of ADA activity in severe combined immunodeficiency may be related to an alteration in these posttranslational events.

308. Role of Arterial Baroreceptors in the Cardiovascular Response to Exercise. STEPHEN F. VATNER,* ROBERT J. MCRITCHIE,* THOMAS A. PATRICK,* GUY HEYNDRICKX,* AND EUGENE BRAUNWALD, Boston, Mass.

Arterial baroreceptors are thought to be essential in mediating the circulatory response to exercise. It has been generally considered that during exercise, after arterial baroreceptor denervation (*a*) arterial pressure would rise markedly and (*b*) the normal rise in heart rate and fall in total peripheral resistance would not occur, and as a consequence of the inordinate rise in afterload cardiac output would not rise normally. In order to evaluate these hypotheses, six dogs were instrumented with aortic electromagnetic flow probes for cardiac output, miniature arterial pressure gauges, and iliac flow probes. Measurements were radio telemetered from the untethered dogs during spontaneous exercise (running 5–10 mph in the field) before and after arterial baroreceptor denervation, accomplished at a second operation by bilateral cervical section of the carotid sinus and aortic nerves. Arterial baroreceptor denervation was confirmed by altering arterial pressure equivalent amounts before and after arterial baroreceptor denervation with nitroglycerin and methoxamine, drugs which normally elicited striking reflex heart rate changes but failed to change heart rate after arterial baroreceptor denervation. Before arterial baroreceptor denervation, exercise increased mean arterial pressure from 98 to 123 mm Hg, heart rate from 100 to 262 beats/min, cardiac output (+245%), and iliac flow (+490%) and decreased total peripheral resistance (−64%). After arterial baroreceptor denervation exercise increased mean arterial pressure from 95 to 136 mm Hg, heart rate from 123 to 248 beats/min, cardiac output (+236%), and iliac flow (+440%) and decreased total peripheral resistance (−55%). Thus, arterial baroreceptors, while essential for regulating abrupt alterations in arterial pressure, play little role in mediating the cardiovascular response to exercise.

309. Derangement of Vitamin B_6 Metabolism with Ethanol: a Direct Demonstration in Isolated Perfused Livers. ROBERT L. VEITCH,* LAWRENCE LUMENG,* AND TING-KAI LI,* Indianapolis, Ind. (introduced by David R. Challoner).

Chronic alcohol abuse is associated with lowered serum levels of pyridoxal phosphate (PLP), the coenzyme form of vitamin B_6. Since the liver is responsible for serum PLP synthesis, we have examined the effect of ethanol on PLP metabolism in isolated perfused livers from rats both sufficient and deficient in endogenous B_6 stores. The liver PLP content of B_6-sufficient animals, 55 ± 7 ng/mg protein (mean ± 1 SD), did not change during a 4 h perfusion and PLP was released into the perfusate at 500 ± 130 ng/g liver per h. Addition of 18 mM (82 mg/100 ml) ethanol to the perfusate did not alter PLP release but a decrease in tissue PLP of 19 ± 10 ng PLP/mg protein was observed ($P < 0.005$). When B_6-deficient livers containing 13 ± 3 ng PLP/mg protein were perfused with 1.2 mg/100 ml pyridoxine, tissue PLP increased by 23 ± 5 ng PLP/mg protein and PLP was released into the perfusate at 1100 ± 150 ng/g liver per h. Addition of 18 mM ethanol decreased both the rate of PLP release and the rise of tissue PLP to 660 ± 150 ng/g liver per h ($P < 0.02$) and 12 ± 5 ng PLP/mg protein ($P < 0.02$), respectively. These data demonstrate directly that ethanol acutely deranges hepatic PLP metabolism. Our previous studies suggest that this effect of ethanol is mediated by acetaldehyde. Since PLP is essential in numerous metabolic processes, including protein synthesis, this biochemical aberration may be significant with regard to cell damage and delayed hepatic regeneration in alcoholic liver disease. (Supported by USAMRDC 17-72-C-2132 and the VA.)

310. Group A Streptococcal M-Protein Vaccine: Protection After Immunization Via the Respiratory Tract. R. H. WALDMAN,* E. N. FOX,* A. DORFMAN,* M. K. WITTNER,* AND S. M. POLLY,* Gainesville, Fla. (introduced by L. E. Cluff **).

Previous studies have shown the efficacy of parenteral immunization of volunteers with purified type 1 M-protein against challenge with homologous streptococci (1973. *J. Clin. Invest.* **52**: 1885). A double-blind study was conducted on 21 adults immunized by aerosol spray into the nasopharynx and 23 controls who received saline placebo. Two booster doses were given at monthly intervals and approximately 30 days later vaccinees and controls were challenged with homologous streptococci (10^6/ml) by swabbing the pharyngeal-tonsillar areas. Throat cultures, leukocyte counts, temperatures, and physical signs and symptoms were followed to assess infection. Illness was defined as a positive throat culture, oral temperature of ≥38°, a WBC count of twice base line or greater than 10,000/mm³, exudative pharyngitis, and adenopathy. Of the 43 subjects, 13 were ill by all criteria (10 controls, 3 vaccinees [$P < 0.02$]); 21 were well by all criteria (6 controls and 15 vaccinees); and 10 exhibited some but not all positive criteria (7 controls, 3 vaccinees). Positive throat cultures after challenge were obtained in 19 controls and 5 vaccinees ($P < 0.001$). There was no correlation between the prechallenge serum antibody titer and the development of subsequent illness. It is concluded that local topical immunization with a M-protein vaccine offers significant type specific protection against challenge with streptococci. (Supported by USPHS Contract AI-32519 and NIH-RR-82.)

311. Effects of a High Insulin:Glucagon Ratio (I:G) on Ammonia Levels in Cirrhotics and on DNA Synthesis by Fetal Hepatocytes. C. WALKER,* W. PETERSON,* H. LEFFERT,* AND R. UNGER,** Dallas, Tex., and La Jolla, Calif.

Glucagon increases hepatic ureagenesis, while insulin inhibits it and decreases amino acid release and deamination. A high level of insulin relative to glucagon might, therefore, reduce ammonia generation from urea and from amino acids, sparing them for protein synthesis and, ultimately, cell growth. Because such actions would be desirable in liver disease, studies were designed to determine if a high I:G (*a*) reduces hyperammonemia in advanced cirrhosis, and (*b*) increases DNA synthesis by hepatocytes. In five hyperammonemic cirrhotics blood NH_3 and I:G were measured during 9 h on 2 separate days while on their usual 40–60 g protein diet. NH_3 rose 21.7 μg/100 ml (±8.3) and I:G rose 4.3 (±1.8). When the same diet was supplemented with 20 g of glucose hourly, in two separate 9 h studies in the five patients insulin was invariably higher ($P < 0.05$) and glucagon usually lower, the I:G rising 22.8 (±5.1) ($P < 0.001$) than in the unsupplemented control studies. NH_3 not only didn't rise—it declined 8.1 μg/100 ml ±3.6 ($P < 0.001$). Thus, in every one of 10 profiles with glucose I:G was higher and NH_3 lower than in the controls, suggesting a possible causal relationship. To determine if I:G influences hepatocyte DNA synthesis, [³H]thymidine

incorporation by cultured quiescent fetal rat hepatocytes was measured at I:G's ranging from 0.5 to 580. At I:G's below 5 no increase in [^{3}H]thymidine incorporation occurred; at I:G's of 50 or above incorporation increased threefold. It is concluded that a high I:G reduces NH_3 levels in advanced cirrhosis and increases hepatocyte replication in vitro. Chronic elevation of the I:G might, therefore, have therapeutic value in certain forms of liver disease. (Research supported by VA and Grant AMO-2700-15 from NIH.)

312. The Role of Local Antibodies on the Intestinal Uptake of Antigens. W. ALLAN WALKER,** KURT J. ISSELBACHER,* AND KURT J. BLOCH, Boston, Mass.

Patients lacking secretory IgA frequently have elevated levels of serum antibodies to ingested antigens, suggesting that the intact secretory immune system may have a role in limiting the absorption of antigens. We have previously reported that protein antigens (horseradish peroxidase [HRP] and bovine serum albumin [BSA]) are absorbed from the small intestine of the rat by a pinocytotic mechanism and that after either oral or parenteral immunization, there is specific inhibition of antigen uptake. In order to investigate the mechanism whereby immunization interferes with absorption, everted jejunal and ileal gut sacs from rats previously injected intraperitoneally with BSA or HRP were incubated with the corresponding radiolabeled antigen for intervals up to 3 h. In comparison with controls, gut sacs from immunized rats showed: (*a*) rapid binding of labeled antigen to antibody which is associated with the surface of the intestinal cell; (*b*) enhanced (10-fold) breakdown of the specific protein antigen; (*c*) no increase in breakdown of unrelated antigens; and (*d*) decreased binding of antigen to the surface membrane of the intestinal epithelial cells. From these and other data, the alternative mechanism for antibody control of antigen uptake can be proposed. Initial exposure of gut sacs from immunized rats to soluble antigen appears to lead to the rapid association of antigen with antibodies on the mucosal surface which in turn results in a decrease in the pinocytosis of antigens by intestinal epithelial cells. Antigen-antibody complexes immobilized on the surface of the gut are then subject to degradation by local proteases. While the site of antigen-antibody binding and degradation remains to be determined, it would appear to be either at the level of the glycocalyx or the cell surface membrane. Thus, just as immunologic mechanisms may decrease bacterial proliferation in the gut, local antibodies may also appear to be important in controlling and influencing uptake of antigens by the intestine.

313. Evidence for Catalytic Regulation of Iron Absorption by Intestinal Mucosa. MARILYN S. WELLS,* KAI-FU CHOW,* AND WILLIAM M. AWAD, JR.,* Miami, Fla. (introduced by David S. Howell**).

The regulation of intestinal iron absorption is of great clinical and theoretical importance, but remains incompletely defined. The possibility has been examined that the mobilization of iron from its various liganded forms may be catalytically mediated for its eventual binding to transferrin, the macromolecule with an extraordinarily high affinity for ferric ions. To this end we have utilized as an in vitro model, the exchange of ^{59}Fe into chicken conalbumin (egg white transferrin), previously saturated with unlabeled iron. This protein was used because of its ease of purification and lack of genetic heterogeneity. Exchange was measured in the absence and presence of mucosal homogenates from rats in different ferremic states. The reaction included (ATP) and ascorbate and was carried out in Ringer's lactate (pH 7.4). The excellent chromatographic resolution of protein components required for our analyses after reaction was achieved by including glycerol (20% vol/vol) in the incubation. Negligible exchange of iron was noted in the absence of mucosa. Substantial exchange was noted in the presence of mucosal homogenate. Enhanced exchange was demonstrated with mucosa from bled rats as compared to that seen with mucosa from normal rats. Moderately suppressed iron exchange was noted with mucosa from animals after parenteral iron loading. The finding of persistance of iron exchange in iron-loaded animals is in accordance with the hypothesis that mucosal apoferritin regulates excess iron absorption; this protein is extruded with associated iron into the intestinal lumen. No conclusions can be made regarding iron exchange as to whether it is mediated by reduction or chelation of bound iron. Inappropriate excess iron absorption may be due to either derepressed catalytic mobilization of iron or suppressed apoferritin synthesis.

314. Regional Pulmonary Distribution and Clearance of Particles in Hypogammaglobulinemia. PETER WERNER,* PETER S. LEE,* FRANK J. HASS,* AND RUY V. LOURENÇO, Chicago, Ill.

Lung infections are frequent in patients with hypogammaglobulinemia. Because mucociliary clearance of inhaled matter is an important defense mechanism of the lung, we investigated deposition and clearance of particles in five asymptomatic patients (three nonsmokers, two former smokers), ages 18–53. Cellular immunity as determined by skin testing and in vitro lymphocytic stimulation was present in all. To assess regional deposition and clearance, a monodisperse aerosol of labeled (^{198}Au) iron oxide particles (mass median diameter = 2.5 μm) was inhaled; external imaging and counting with an Anger camera were followed for 5 h with a final measurement at 24 h. Based on chest radiographs, bronchograms, and an outline of the lung by ^{133}Xe inhalation, the right lung was divided into a central, an intermediate, and an outer zone. The ^{133}Xe ventilation studies also permitted comparison of regional ventilation and deposition of particles. Data were stored on tape for computer analysis. In addition, we obtained in all subjects pulmonary function tests, including specific airway conductance, dead space, alveolar-arterial oxygen gradients, closing volume (CV), and static (C_{Lstat}) and dynamic (C_{Ldyn}) compliances of the lung. In two nonsmokers only C_{Ldyn} and CV were abnormal, suggesting peripheral airway disease; the other three subjects showed central airway obstruction. Abnormal deposition of particles correlated with the degree of airway obstruction: three patients showed abnormal deposition in central airways. In the dependent zones of the lung, areas with decreased deposition of particles were associated with regional hypoventilation. Abnormal clearance was observed mainly in the larger airways; in two patients the initial movement of particles in these airways was towards the periphery. Four patients failed to clear particles for as long as 1 h as a result of clearance arrest in the central zone; cough appeared to be an important mechanism of clearance from this zone. Clearance from the outer zone was intact. These results indicate major abnormalities in particle deposition and clearance in large airways of patients with hypogammaglobulinemia even when only small airway obstruction was detected. (Research supported by Grant HL 13824 from NHLI.)

315. Alterations of Myosin in Ventricular Hypertrophy: Greater Quantitative Proportion of Heavy Chains Relative to a Light-Chain Inhibitor Resulting in Elevation of Myosin ATPase Activity. JOAN WIKMAN-COFFELT,* MATTHEW LOTYSH,* ROBERT ZELIS,* AND DEAN T. MASON, Davis, Calif.

Considerable attention has been focused on possible dysfunctions of contractile proteins in ventricular hypertrophy and heart failure. To evaluate enzymatic integrity and subunit composition of myocardial myosin in the pressure-overloaded ventricle, pulmonary artery banding was carried out in 12 dogs to elevate right ventricular systolic pressure 30% for 3 wk. Cardiac myosin ATPase activity (μmol Pi/mg per min) was elevated 29%: K+ activated system 0.57 in normal right ventricle (NRV) and 0.72 in hypertrophied right ventricle (HRV) ($P < 0.01$); Ca++ activated system 0.32 in NRV and 0.42 in HRV ($P < 0.01$). Augmented enzymatic activity in hypertrophy was not due to elevation of myosin concentration since myosin ATPase activity with NH_4+ as the activator cation was the same in NRV and HRV. Concomitant with rises in K^+- and Ca^{++}-activated ATPase reactions were changes in proportions and types of myosin subunits. On one-dimensional disc gels, NRV contained twice the number of moles of light chains ($C_1 + C_2$) per mole of heavy chains than in HRV ($P < 0.01$); these differences in chain proportions were reflected in sedimentation velocities. When labeled light chains were added to myosin, they were incorporated into myosin; with excess added light chains, myosin ATPase activity in HRV was lowered 60% compared to NRV in which the same amount of added protein was albumin. Dissociated myosin light chains removed immunologically indicated ATPase activity was mainly in the heavy chains. When the light chains were separated on two-dimensional gel electrophoresis, the C_1 light chains resolved into four components; one of them named C_{1d} was present as 3.0 mol/mol of myosin in NRV, but it was nearly absent in HRV. Therefore, it is suggested that the mechanism by which myosin ATPase activity is elevated in early cardiac hypertrophy is reduction of apparent inhibition of enzymatic activity by the C_{1d} light chain, probably because of its slower turnover rate relative to the other myosin light and heavy chains. (Supported by NIH HL 14780.)

316. Thyrotropin (TSH) and Prolactin (HPr) Inhibition by Endogenous Thyroxine (T_4) and Triiodothyronine (T_3) after Sequential Oral Thyrotropin-Releasing Hormone (TRH). J. WILBER, R. UTIGER, AND E. MONTOYA,* North Chicago and Chicago, Ill., and Philadelphia, Pa.

It is now recognized that small changes in serum thyroxine (T_4) and triiodothyronine (T_3) can influence thyrotropin-releasing hormone (TRH)-stimulated thyrotropin (TSH) and prolactin (HPr) secretion. To inquire whether circulating hormones or intrapituitary metabolism of T_3 and/or T_4 is requisite for inhibition of TRH, 80 mg of TRH was given to nine volunteers for 5 days. Serum samples were obtained from 0 to 12 h after daily TRH for determination of T_4, T_3, TSH, HPr, and TRH by radioimmunoassay. Oral TRH (day 1) caused serum TSH to increase to a mean maximum of 17.4 μU/ml at 4–5 h. The maximum serum HPr of 21 ng/ml was attained earlier (1–2 h), in contrast to TSH. Circulating T_4 and T_3 were both elevated on day 1 (base line T_4 8.3 μg/100 ml, maximum 11.62 μg/100 ml; base line T_3 103.5 ng/100 ml, maximum 246 ng/100 ml). Binding changes did not account for these hormone increases. Despite the rise in serum T_4 and T_3 already by day 1, inhibition of TSH and HPr release in response to TRH was not observed until day 3. Mean TRH in blood rose from 280 pg/ml to a maximum of 890 pg/ml at 3–4 h. We conclude the following. (*a*) The latency of inhibition of TRH effects after elevations of endogenous thyroid hormones suggests that intrapituitary turnover of T_4 and/or T_3, and the not steady-state concentration of these hormones, is the determinant of TRH blockade. (*b*) The earlier rise of serum HPr, at a time when blood TRH was lower, suggests that lactotrophs are more sensitive to TRH than thyrotrophs. (*c*) Both T_4 and T_3 can be elevated after a single oral dose of TRH, providing an additional index for evaluation of the hypothalamic-pituitary-thyroid axis in man. (Supported by USPHS Grants 10699, 14997, and 05071.)

317. Deficiency of the Second Component of Complement (C2) in a Patient with Discoid Lupus Erythematosus (DLE). JAMES WILD,* NATHAN ZVAIFLER,** HANS MÜLLER-EBERHARD,** AND CURTIS WILSON,* La Jolla, Calif.

A 37 yr old man (R.B.) with discoid lupus erythematosus was found to have absent serum hemolytic complement activity. Complement components were determined (radial diffusion). C2 was undetectable; Clq, Cls, C4, C5, C6, and C7 were normal. C3 and C9 were elevated. C3 proactivator (C3PA) concentration was normal. The B. family was investigated. Both parents, a daughter, and one of four siblings had approximately 50% C2 hemolytic activity. A sister showed no C2 hemolytic activity and one brother was normal. Discoid lupus erythematosis-(DLE) involved skin was studied by immunofluorescence. In the dermal epidermal junction were granular deposits of IgG and IgM, but no IgA. Clq and C4 were present in a pattern similar to IgG. No other complement components, C3PA, or properdin were noted. R. B. was given intravenous radiolabeled C3 twice. His catabolic rates were 3.03% and 2.48% of the plasma pool per hour. (three normals = mean 1.99%/h). R. B.'s C3 synthetic rates were 2.74 and 2.31 mg/kg per h (three normals = mean 1.22% mg/kg per h). The alternate complement pathway was intact since C3PA was electrophoretically converted after incubation with inulin. C3PA conversion was not accomplished by 250 μg of *E. coli* (O:111 B4) endotoxin (seven or eight normals converted with 5 μg) or 1,000 μg of aggregated IgG, suggesting that an intact classical pathway may be required for these reagents. The association of C2 deficiency with lupus erythematosus is not fortuitous; 3 of 12 reported C2-deficient homozygotes have had SLE or chronic anaphylactoid purpura. Susceptibility to these diseases is thought to be due to deficiency of early C components with tissue injury caused by immune complexes activating the alternate pathway. R. B. shows hypercatabolism and an overcompensated synthesis of C3 consistent with hyperactivity of the alternate pathway, but the inability to activate his bypass with aggregated IgG plus the biopsy findings of IgG, Cl, and C4 without late complement components in involved skin makes it unlikely that these lesions exist because of an intact alternate pathway.

318. Influence of Lactic and Hypercarbic Acidosis on the Cardiac Effects of Hyperosmolar Mannitol. JAMES T. WILLERSON,* J. STANLEY CRIE,* ROBERT C. ADCOCK,* GORDON H. TEMPLETON,* AND KERN WILDENTHAL,* Dallas, Tex. (introduced by Jere H. Mitchell).

Hyperosmotic solutions exert potent inotropic effects on heart muscle in vivo and in vitro, and it has been suggested that they may be useful clinically in improving ventricular performance. It has not been established, however, whether their positive effects might be attenuated under some frequently encountered clinical conditions such as acidosis. Accordingly, the interaction of acidosis and hyperosmolality was studied in isolated, isometrically contracting cat right ventricular papillary muscles (12/min, 30°C) and in anesthetized open-chest dogs. Acidosis (pH 6.9–7.0) was produced with lactic acid or 15% CO_2, and hyperosmolality (50 mosmol/kg H_2O above control) with 25% mannitol. In papillary muscles at pH 7.3–7.4, mannitol caused 20 ± 3% (SEM) increase in

developed tension ($P < 0.01$) and a 28 ± 6% increase in the maximal rate of tension development (dT/dt) ($P < 0.01$). At pH 6.9–7.0 induced by addition of lactic acid, mannitol caused no increase in either parameter. In dogs with arterial pH 7.3–7.4, the maximal rate of rise of left ventricular pressure (dp/dt) rose by 35 ± 8% after mannitol ($P < 0.01$), whereas in those with lactic acidosis (pH 6.9–7.0) similar degrees of hyperosmolality did not alter dp/dt significantly. In contrast, acidosis produced by CO_2 (pH 6.9–7.0) did not prevent the inotropic effect of hyperosmolality in isolated papillary muscles (dT/dt rose by 45 ± 8% and developed tension by 36 ± 5%, $P < 0.01$) or in anesthetized dogs (dp/dt increased by 11 ± 3%, $P < 0.01$). The data suggest that there are fundamental differences in the interactions between hyperosmolality and lactic acidosis on the one hand and hyperosmolality and hypercarbic acidosis on the other. Lactic acidosis (pH < 7.0) prevents the inotropic effect of hypertonic mannitol, whereas a similar degree of acidosis produced by CO_2 does not. Whether this differential influence of the two forms of acidosis also applies to other inotropic agents remains to be determined. (Research supported by grants from NIH.)

319. Origin of Urinary Prostaglandins. M. W. WILLIAMS,* T. W. WILSON,* J. A. OATES, A. S. NIES,* AND J. C. FROLICH,* Nashville, Tenn.

We have identified prostaglandins E_2 and $F_{2\alpha}$ (PGE_2 and $PGE_{2\alpha}$) in human urine by mass spectrometry. The present studies were undertaken to ascertain the renal origin of urinary prostaglandins (PG's) and to determine the site at which they enter the tubule. Angiotensin (20 ng/kg per min) was infused into a single renal artery in five dogs and urine collected from each ureter. Creatinine clearance decreased on the infused side only by an average of 35%. PGF was determined by radioimmunoassay and PGE by a competitive protein binding assay utilizing a high affinity binding site (dissociation constant 10^{-9} M) with specificity for PGE. Excretion rate of PGE increased silgnificantly on the infused side from 1.79 ± 0.1 to 3.2 ± 0.79 ng/min ($n = 5$, $\bar{x} \pm$ SE, $P < 0.05$) and for PGF from 0.78 ± 0.4 to 2.1 ± 0.67 ng/min ($n = 3$) but did not change on the contralateral side. The site of entry of PG's into the tubular fluid was investigated by stop-flow experiments in four dogs. The peak urinary concentration of PG occurred in the distal half of the nephron but proximal to the peak inulin concentration. The peak PG levels were 8.5 times basal levels, while the peak inulin levels were less than twice basal levels. During the indomethacin infusion the PG peak disappeared. These findings indicate that urinary PG's reflect renal PG synthesis. The site of entry appears to be either the loop of Henle or the distal tubule, and before the collecting duct. This point of entry into the tubular fluid provides the PG's access to all distal sites in the nephron, including its juxtaposition with glomerular arterioles. (Supported by GM 15431.)

320. Enhancement by Cell Surface Alterations of the Activity of a Granule-Associated L-Thyroxine Deiodinating System in the Human Neutrophil. KENNETH A. WOEBER, San Francisco, Calif.

Phagocytosing neutrophils metabolize L-thyroxine (T_4) much more actively than do resting neutrophils. To clarify the mechanism underlying this phenomenon, the deiodinative activity of cell fractions isolated from human neutrophils by differential centrifugation in 0.34 M sucrose was studied. Of the several fractions obtained, the granule fraction was found to possess the greatest T_4-deiodinative activity per unit mass of protein, as little as 10 μg effecting significant deiodination of added labeled hormone. Little deiodinative activity was found in the microsome fraction and none in the cytosol fraction. Prior exposure of intact neutrophils to opsonized zymosan particles to induce phagocytosis resulted in a selective increase in the T_4-deiodinative activity of the subsequently isolated granule fraction. Since phagocytosis is accompanied by significant perturbations at the cell surface, the influence of a surface-active agent was also examined. Prior exposure of intact neutrophils to saponin also resulted in a selective increase in the T_4-deiodinative activity of the subsequently isolated granule fraction. By contrast, direct exposure of the isolated granule fraction to saponin did not increase its T_4-deiodinative activity. Moreover, as has been shown to be the case with phagocytosing neutrophils, neutrophils accumulated added T_4 much more actively in the presence of saponin, the increase in T_4 accumulated being associated with the granule and cytosol fractions. These data indicate that events taking place at the surface of the neutrophil exert a profound influence on intracellular sites of T_4 uptake and metabolism. (Research supported by NIH Grant AM-16497.)

321. Testosterone (T) Modulation of Pituitary Response to LRH: Differential Effects on Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). FLEMMING WOLLESEN,* RONALD S. SWERDLOFF,* M. PETERSON,* AND WILLIAM D. ODELL. Torrance, Calif.

Under clinical and experimental conditions LH/FSH secretion may be >1 or <1. If a single LRH-FSHRH exists, then modulation of pituitary response must occur. We have evaluated the possibility that T exerts such modulation using a rat model. In preliminary studies the peak LH and FSH response to IP LRH was shown to occur at 30 min in castrate rats. Groups of adult male rats were castrated and then treated for 21 days with T given intraperitoneally in oil in doses of 1, 5, 10, 50, 100, 500, 1,000, 5,000, and 10,000 ng/100 g body weight. LRH was administered in doses of 10–1,000 ng/100 g body weight so that groups receiving each dose of T received every dose of LRH. LH and FSH was quantified at 30 min. In non-LRH-treated animals T suppressed serum LH FSH: serum LH was first lowered by 10 ng and suppressed to intact levels at 500 ng; for FSH 100 and 1,000 ng were required. Serum LH and FSH response to LRH was inhibited by T in a dose-related relationship, i.e., the highest dose of T (1,000 ng) completely blocked increases of LH to all doses of LRH. The lowest dose of T (10 ng) *partially* inhibited the response of LH to all but the highest dose of LRH. T was less effective in inhibiting FSH response to LRH: the highest dose of T (1000 ng) only partially blocked FSH increase; 10, 50, and 100 ng *had no effect* on FSH response to LRH. In summary, T is more effective in suppressing LRH stimulation of LH than FSH; thus, T is capable of differentially modulating the LRH stimulation of FSH and LH secretion by the pituitary.

322. Bacteriologic Study of Lower Respiratory Tract Secretions/Exudates Obtained with the Flexible Fiberoptic Bronchoscope. GORDON A. WONG,* PAUL D. HOEPRICH,** ARTHUR L. BARRY,* THOMAS A. PEIRCE,* AND DAVID C. RAUSCH,* Davis, Calif.

Optimal management of lower respiratory tract infections requires etiologic diagnosis based on specimens certifiable as tracheobroncho-pulmonary secretions/exudates (TBSE). Percutaneous transtracheal aspiration (TTA) can yield valid specimens, but is contraindicated when there is a bleeding diathesis; the flexible fiberoptic bronchoscope (FFB) can be used when bleeding is a hazard. If authentic specimens can be obtained using FFB, other advantages would accrue: (*a*) collection of specimens under direct vision; and (*b*) sampling from sites

distal to the carina. To validate FFB collection of TBSE, bronchoscopy was carried out (*a*) immediately after TTA in 10 patients with chronic bronchitis (CB); and (*b*) in five volunteers without respiratory tract disease. In addition, TBSE were obtained from 20 patients with CB who had not received antimicrobics for at least 2 wk. Aerobic and anaerobic cultures of each specimen were set up qualitatively and quantitatively. The results with FFB and TTA specimens were generally in agreement with regard to aerobic bacteria; bronchoscopy yielded more kinds and greater number of anaerobes than TTA. FFB caused less discomfort than TTA. Specimens collected from volunteers were either sterile or contained fewer than 10^4 bacteria/ml (total). Patients with CB yielded various aerobes and anaerobes normally found in saliva: *viridans* streptococci were present in all specimens (range, 10^4–10^8/ml); *Hemophilus influenzae* were present in 12/20 specimens (range 10^3–10^8/ml). Several genera and species of anaerobes were recovered in densities ranging from 10^4 to 10^8/ml. Serious complications from FFB were not observed. It appears that authentic TBSE virtually uncontaminated with upper respiratory tract secretions can be collected using FFB with proper precautions.

323. The Sezary Syndrome Lymphoid Cell: Surface Ultrastructure and Properties and Abnormal Mitogenic Responsiveness. STANLEY YACHNIN, RAUL BRAYLAN,* CAROL REESE,* HARVEY GOLOMB,* AND DAINA VARIAKOJIS,* Chicago, Ill.

Sezary's Syndrome (SS) is a variant of mycosis fungoides (MF) associated with erythroderma, in which the peripheral blood is invaded by large numbers of lymphoid cells possessing indented and convoluted nuclei. We have studied the blood lymphocytes of five patients with SS whose WBC ranged from 20 to 140 × 10^3/mm^3 (50–>90% lymphoid cells). Nonphagocytic lymphoid cells were isolated by nylon column filtration. Such cells from patients with SS showed markedly defective mitogenic response ([2-^{14}C]thymidine incorporation) to a broad dose range of phytohemagglutinin, pokeweed mitogen, concanavalin A, and a rabbit antihuman lymphocyte serum, when compared with normal human lymphocytes. Ficoll-hypaque mononuclear lymphoid cells from patients with SS were analyzed for their ability to form E-rosettes with unsensitized sheep erythrocytes. Three patients studied showed 3, 10, and 28% E rosettes, respectively (nl = 57.4 ± 12.6%). The proportion of such lymphoid cells bearing surface immunoglobulin determinants by direct immunofluorescence was found to be 3, 1, and 1% in three patients studied (nl = 24.8 ± 7.4%). By way of contrast three patients with MF having normal peripheral WBC showed normal lymphocyte responses to mitogens as well as normal proportions of E-rosette-forming and surface Ig-bearing lymphocytes. The Sezary cells from one patient were examined by scanning electron microscopy. The majority of cells resembled "nonsmooth" T cells possessing surface microvilli in moderate numbers. These studies do not allow the Sezary cell to be classified as either a B or T lymphocyte, but indicate that whatever its origin it lacks surface properties of both B and T lymphocytes and lacks as well the ability to respond to a variety of mitogenic stimuli. Our studies suggest that some SS lymphoid cells may best be placed in the "null" lymphocyte category.

324. Cyclic Nucleotide-Microtubule Interaction in the Regulation of Erythrocyte Shape and Survival. YOSHIHITO YAWATA,* NOBORU MATSUMOTO,* AND HARRY JACOB, Minneapolis, Minn.

In cultured malignant cells microtubular proteins regulate cell shape, growth, and contact inhibition. Abnormal analogous proteins possibly underlie hereditary spherocytosis, since exposure of normal erythrocytes to vinblastine or colchicine, agents which interfere with microtubule assembly, reproduces all characteristics of this disease. In cultured cells, cyclic nucleotides are required for proper microtubule function. We report that analogous interaction of cyclic nucleotides with vinblastine/colchicine-reactive membrane protein regulates erythrocyte shape, permeability, and survival. Cyclic 3′5′GMP (cGMP) or 3′5′AMP (cAMP), but not their cyclic 2′3 congeners, stabilize these proteins to provide normal erythrocyte shape, resistant to vinblastine or colchicine alteration. Thus, 5 mM cGMP or cAMP prevents vinblastine or colchicine-induced sphering, as assayed by scanning electron microscopy and osmotic fragility. By double-reciprocal plots, cyclic 3′5′-nucleotides inhibit, with ideal competitive kinetics, vinblastine-induced sodium leakiness, cGMP (K_i = 4.9 ± 0.5 mM) more efficiently than cAMP (K_i = 6.9 ± 0.7 mM). Similarly, cyclic nucleotides prevent vinblastine's manyfold acceleration of splenic destruction of reinjected [^{51}Cr]erythrocytes. Subcellularly, cyclic nucleotides also stabilize membrane proteins, preventing vinblastine-induced precipitation of solubilized ghost proteins. We conclude that cyclic nucleotides, through modulation of microtubule-like proteins, regulate erythrocyte shape and plasticity. Although human erythrocytes generally synthesize cyclic nucleotides poorly, cAMP inducers (isoproterenol, prostaglandin E_1) alter their plasticity significantly, implicating endogenous, as well as plasma, nucleotides in erythrocyte conformation. Cyclic nucleotides probably sustain normal cell shape by stimulating protein kinase-ATP phosphorylation of (? microtubule) membrane proteins. We predict that abnormal cyclic nucleotide-microtubule interaction underlies some hemolytic diseases with aberrant erythrocyte shape and plasticity. A recently reported deficiency in cAMP-catalyzed phosphorylation of membrane proteins in hereditary spherocytosis supports this suggestion.

325. Effects of Thyroxine on the Enzymatic Properties of Cardiac Myosin: Possible Mechanism of Enhancement of Cardiac Contractility. YOSHIO YAZAKI* AND MAURICE S. RABEN,** Boston, Mass.

The effect of thyroxine on myosin ATPase was examined in rat and rabbit. Rat cardiac myosin has 3 times ATPase activity of rabbit in 10 mM Ca^{++}, though equal activity in 0.6 M KCl-EDTA, and is also distinctive with respect to other enzymatic properties—lower activating energy, lower rate of inactivation at alkaline pH, lower sensitivity to the inhibitory effect of KCl in Ca^{++}, and no activation by *N*-ethylmaleimide. These findings suggest a difference in the myosin molecule at or near the active site. After thyroxine treatment of rabbit (300 μg/day for 3 wk), Ca^{++}-activated ATPase activity of cardiac myosin was increased from 0.29 ± 0.02 to 0.68 ± 0.05 μmol Pi/mg min^{-1} ($P < 0.01$), with no change in activity in KCl-EDTA, and showed a pattern of activity similar to rat cardiac myosin. Conversely, thyroidectomy in rat decreased cardiac myosin ATPase activity from 1.16 ± 0.04 to 0.41 ± 0.04 μmol Pi/mg min^{-1} ($P < 0.001$) and changed the enzymatic properties to the rabbit type showing lability at alkaline pH and activation by *N*-ethylmaleimide. These changes were specific for the heart, since no change was observed in skeletal myosin. Isoproterenol treatment and aortic constriction in rabbit, which also increase cardiac contractility, failed to change the enzymatic properties of cardiac myosin. Thus, the known enhancement of cardiac contractility in normal rat may be mediated by the distinctive enzymatic properties of cardiac myosin maintained by thyroid hormone, and thyroxine-induced inotropy in rabbit may result from a structural change in the cardiac myosin molecule inducing enzymatic properties similar to rat cardiac myosin. (Supported by NIH Grants AM01567 and HL06924.)

326. Uptake of Maltose and Other Sugars by Rat Diaphragm. ELEANOR A. YOUNG* AND ELLIOT WESER,* San Antonio, Tex. (introduced by George W. Frimpter).

Maltose is metabolized as efficiently as glucose after infusion in man and animals. On a molar basis, maltose yields twice the calories as glucose and therefore may be exceedingly useful in parenteral alimentation. Intestinal maltase plays no role in this metabolism. In the present study, the transport of maltose into muscle tissue of rat diaphragm (selected because of absent maltase activity) was compared with that of other sugars. Nonfasting male rats were sacrificed, and the diaphragms were removed and divided into three equal parts to determine percent water, [^{14}C]inulin space, and intracellular sugar concentration. Diaphragms were incubated in buffer containing insulin and one of the following radiolabeled sugars: maltose, sucrose, lactose, galactose, fructose, or glucose. After incubation, diaphragms were homogenized, the sugar radioactivity determined in the supernatant, and sugar transport into intracellular water calculated. All sugars entered cells by diffusion. At all equimolar concentrations, disaccharide transport into intracellular water was equal but only 50% that of monosaccharides. However, when intracellular sugar was calculated on a weight basis (mg/ml), transport of all disaccharides and monosaccharides was equal. These studies suggest that disaccharides, like monosaccharides, enter rat diaphragm cells by diffusion. In cells that possess intracellular disaccharidase activity, hydrolysis of disaccharides to monosaccharides would account for their subsequent metabolism. Efficient metabolism of maltose after infusion occurs because tissues other than small intestinal mucosa contain maltase activity. (Supported by NIH Grant R01 AM 12484-05.)

327. Characteristics of Chloroma Cells in Permanent Suspension Culture. ADEL A. YUNIS, GRACE K. ARIMURA,* R. JUDITH RATZAN,* AND HAROLD J. HAINES,* Miami, Fla.

Shay chloroma of rat has many similarities to human myelogenous leukemia and has been used as an experimental model for the study of this disease. In vitro culture of cells from these tumors may provide a useful system for investigating regulatory mechanisms in leukemic myeloid proliferation. Chloroma tumor cells grown in agar form distinct colonies. Maximal colony formation requires an exogenous source of colony-stimulating factor indicating that growth is not altogether autonomous. Chloroma cell lines have been established in permanent suspension cultures. They are grown in Dulbecco's Modified Eagle medium with 10% fetal calf serum and 2.5% horse serum. Cells which have been maintained for over a year have a doubling time of 12 h and will uniformly produce chloroma tumors when injected into newborn rats. Preliminary experiments suggest that these cells secrete both growth-stimulating and growth-inhibiting activity into the medium at certain stages of proliferation. They maintain a diploid complement with a modal number of 42 chromosomes. Histochemical staining shows a strongly positive reaction for peroxidase and alkaline phosphatase. Cells obtained from tumors which, upon repeated transfer, lost their alkaline phosphatase activity regain full activity in culture. Electron microscopic examination of tumors and cells derived from them reveal the presence of budding C-type virus particles on the cell membranes and intracisternal A-type particles. The availability of a permanent chloroma cell line in culture which maintains the biological properties of the parent tumor provides an unusual opportunity for investigating regulatory mechanisms in myeloid leukemia. (Supported by NIH Grant AM 09001-10 and by Howard Hughes Medical Institute.)

328. Increased Plasma Renin Activity (PRA) in Prolonged Bed Rest. P. G. ZAGER,* G. A. MELADA,* R. H. GOLDMAN,* C. M. GONZALES,* AND J. A. LUETSCHER,** Stanford, Calif.

The circadian rhythm of PRA in normals rises episodically during early morning, falling during the afternoon and evening in recumbent normals. Standing evokes a rise in PRA, which falls with recumbency. We studied the diurnal variation of PRA in five normals on constant diet (Na 130, K 90 meq/day) during normal daily activity and after 3–12 days of continuous bed rest. Unexpectedly, bed rest was followed by a marked rise in PRA during the morning. The lowest PRA was found at 8 a.m. and 11 p.m. during the ambulatory period, and the maximum occurred at noon. After 3–12 days of continuous bed rest, PRA at 8 a.m. was 3 times the control and remained as high at noon as after a morning of standing. Neither 5 p.m. nor 11 p.m. PRA values during bed rest differed from the ambulatory controls. Renal blood flow (PAH) and glomerular filtration rate (inulin) were unaffected by bed rest. Plasma angiotensinogen levels were also unchanged. Although plasma volume may fall during bed rest, extracellular fluid volume was increased by an average of 2 liters. Cardiac output and blood pressure were not altered. With resumption of normal activity, standing evoked exaggerated rise in PRA. Marked increases in PRA were observed in hypertensive patients after 32 h of bed rest. We conclude that prolonged bed rest enhances the circadian peak of renin during the early morning hours, and increases the rise of PRA when upright posture is resumed. This effect must be considered in evaluating PRA in normal men and in hospitalized hypertensive patients.

329. Effect of Different Lobar Resistances on the Pleural Surface Pressure. ARNOLD ZIDULKA,* STEVEN NADLER,* BRIAN MURPHY,* AND NICHOLAS R. ANTHONISEN, Montreal, Canada.

To determine whether pleural surface pressure differences occur as a result of different lobar time constants, we measured intrapleural pressure changes by flat balloons inserted via thoracotomy over the right upper lobe (RUL) and right caudal lobes (RCL) in seven supine anesthetized and paralyzed dogs. With the left lung obstructed, a constant volume respirator ventilated the right lung through a high resistance pathway to the RCL and a low resistance pathway to the RUL. The tidal volumes (Vt) of each were determined by integrating flow measured by a pneumotachygraph in each pathway. We found that with increasing respiratory frequencies (5 to 48.5 breaths per min) Vt increased to the RUL and decreased to the RCL. RCL lagged RUL progressively. The pleural pressures were more negative over the RCL so that a greater inflating pressure was applied to this lobe compared to the RUL. These changes did not occur when resistances to both lobes were similar. We conclude that differences in pleural pressure swings and phase occur as a result of different lobar time constants and act in a direction to cause greater uniformity of ventilation. (Research supported by grants from the Medical Research Council and Canadian Thoracic Society.)

330. Familial Aggregation of Urinary Kallikrein and Blood Pressure in Children. STEPHEN H. ZINNER,* HARRY S. MARGOLIUS,* BERNARD ROSNER,* HARRY R. KEISER,* AND EDWARD H. KASS,** Providence, R.I., Bethesda, Md., and Boston, Mass.

Recently familial aggregation of blood pressure was demonstrated in children aged 2–14 yr similar to that seen in adults. Also, the children with higher pressures tended to maintain higher pressures when examined 4 yr later. Urinary kallikrein (UKal) excretion is decreased in adults with essential hypertension. To determine if there is a relation between UKal

concentration and blood pressure in children, urine specimens and blood pressures were obtained in the homes of 327 children in 79 families whose blood pressures had been studied 4 yr earlier. Blood pressures were adjusted for age and sex and expressed in standard deviation units. Familial aggregation of urinary kallikrein concentration as well as blood pressure was demonstrated by analysis of variance ($F = 3.21, P < 0.001$ for UKal; $F = 1.91, P < 0.001$ for BP). Moreover, there was a highly significant inverse relation of systolic pressure and log UKal concentration ($b = -0.195, P = 0.002$). Urinary kallikrein concentration was significantly lower in black children than in white children (logs = 3.93 for blacks; 4.56 for whites, $P < 0.001$). These data suggest a familial influence on blood pressure and urinary kallikrein concentration in children and that lower urinary kallikrein is associated with higher blood pressures in childhood. (Supported by AHA Grant in Aid 72-930.)

331. Specific IgG and IgM Antibody in Gram-Negative Bacteremia. STEPHEN H. ZINNER* AND WILLIAM R. MCCABE, Providence, R. I., and Boston, Mass.

Previous studies of gram-negative bacteremia failed to demonstrate any correlation between increasing antibody titers in acute serum specimens against homologous gram-negative bacilli and protection against shock or fatal outcome. Since these investigations utilized a hemagglutination technique which preferentially measures 19S antibody, the present studies were undertaken to evaluate the protective effect of specific IgG and IgM antibody in acute serum specimens obtained in 170 episodes of gram-negative bacteremia. Titers of specific IgG and IgM antibody reacting with the patient's infecting organism were determined by immunofluorescent staining with fluorescein-labeled goat antihuman IgG and IgM. IgG and IgM titers were correlated with titers of hemagglutinating antibody before and after treatment with 2-mercaptoethanol (2-ME), titers of antibody to core glycolipid, the patient's underlying disease, and the frequency of occurrence of shock and fatal outcome. Significant correlations were observed between titers of IgM against the homologous bacilli and levels of hemagglutinating antibody and between IgG levels and titers of 2-ME-resistant hemagglutinating antibody ($P < 0.01$). Examination of the relation of the frequency of shock and death with specific immunoglobulin levels by point biserial correlation demonstrated a significant correlation between increasing IgG titers and a decreasing frequency of shock and death. In contrast, no correlation could be detected between levels of IgM and the occurrence of shock and death. The apparent protective effect of core glycolipid, previously demonstrated, was reconfirmed and appeared to be independent of type specific IgG antibody.

332. Increased Hb:Cell-Water Interaction in Sickle Cells After Anoxia. A. ZIPP,* I. D. KUNTZ,* S. J. REHFELD,* AND S. B. SHOHET, San Francisco, Calif. (introduced by L. K. Diamond**).

Proton magnetic resonance spectra of water in erythrocytes from patients with sickle cell disease (SS) were studied before and after anoxia. Upon sickling the water line-width of packed SS cells broadened markedly from 6 Hz to 18 Hz. Control cells did not change (5 Hz). In erythrocytes from both heterozygotes and patients, broadening was linearly related to the percent Hb-S in the cell and decreased when Hb-F was present. The SS line-width increased further to 20 Hz with a shift in spectrometer frequency from 60 to 100 mHz. This suggested that changes in both Hb:water binding and cell shape might contribute to the broadening. However, persistence of the bulk of the anoxic change after freeze-lysis mitigated against a major effect of cell shape. Moreover, spin-lattice relaxation times of SS cells showed a 2- to 5-fold decrease when anoxic, further suggesting that a large reduction in cell-water mobility had occurred. Finally, mild hypertonic shrinkage (7%) of both SS and normal cells increased line-width less than 0.5 Hz. This indicated that the change induced by anoxia was not due to any slight cell dehydration secondary to membrane permeability changes and cation shifts associated with sickling. These observations indicate that major increases in hemoglobin:cell-water interactions occur during sickling. (Supported by NIH Grants AM16095, AM37237, and GM19267.)

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