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Abstracts

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ABSTRACTS

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

1. Characterization of the Hepatitis-Associated Antigen Subtypes by Radioimmunoassay. R. D. AACH,* E. HACKER,* AND C. PARKER,* St. Louis, Mo. (introduced by D. Alpers).

The recent recognition of different antigenic subtypes of hepatitis-associated antigen (HAA) raises questions as to the immunologic contribution of each determinant (a, d, and y) and the efficacy of a given antiserum in detection of HAA. Antibodies were produced in animals by immunization with HAA of different antigenic subtypes. HAA of dissimilar subtypes were radiolabeled with ^{125}I and radioimmunoassays were performed as previously described (1971. *Proc. Nat. Acad. Sci. U.S.A.* **68:** 1056). It was found that these antisera varied widely in their ability to recognize a, y, and d determinants. Circulating HAA in patients ill with hepatitis could be separated into different subgroups and the relative contribution of a, d, or y determinants characterized. 20 patients were screened, 16 patients with HAA-positive acute viral hepatitis and 4 with chronic persistent hepatitis. HAA of 9 of the 16 patients with acute disease were subgroup Y (a^+y^+) and 7 subgroup D (a^+d^+). Three of the four patients with chronic persistent hepatitis had HAA of subgroup D, and one had HAA of subgroup Y. These studies emphasize: (*a*) the ability to readily distinguish antigenic subtypes by radioimmunoassay. This provides a sensitive system which can be used as an epidemiologic tool, and which can be used to relate subtypes with patterns of hepatic disease. (*b*) Antisera raised in animals show wide variability in the recognition of individual determinants. This difference in specificity must be taken into account in the selection of antiserum of antiserum for routine screening of blood donors.

2. Chromosomal Aberrations in Newborns Exposed to Heroin In Utero. CYRIL A. L. ABRAMS* AND PEI-YU LIAO,* New York (introduced by Gerald B. Phillips**).

Equivocal evidence of chromosomal abnormalities has been found upon the administration of various psychotropic drugs. Opiates have received scant attention in this respect, and definitive information concerning their cytogenetic effects is lacking. We examined peripheral blood lymphocytes of 16 newborns aged ½–31 days whose mothers had used heroin during pregnancy, and 14 newborn controls aged 1–13 days whose mothers were not drug users. Both groups of newborns received vitamin K before blood samples were taken. In the heroin group, five newborns had received medication for withdrawal symptoms, and one had had a viral illness before sampling. 10 mothers in the heroin group had received methadone during pregnancy. Cultures were set up in parallel for heroin subjects and controls. Whole blood inoculum (heelstick) was incubated for 72 hr, and colcemide added 2 hr before harvesting. 100 metaphases per subject were analyzed. The heroin group showed 81 chromatid breaks, 29 dicentrics, 28 fragments, 28 gaps, 9 isochromatid breaks, 5 deletions, and 3 bizarre forms in the 1600 mitoses analyzed. The control group showed 20 chromatid breaks and 4 gaps in the 1400 mitoses analyzed. The mean values for damaged chromosomes and for damaged cells in the heroin group were found to be 6 times higher than the corresponding values in the control group ($P < 0.0001$). Although other drugs and various environmental factors must be considered as possible causes of the chromosomal aberrations observed, it is permissible to infer that heroin itself played the major role. (Research supported by NIH grant AM-05531.)

3. Myocardial Damage after Aorto-Coronary Vein Bypass Surgery. STEPHEN ACHUFF,* LAWRENCE GRIFFITH,* J. O'NEAL HUMPHRIES,* C. RICHARD CONTI,* ROBERT BRAWLEY,* VINCENT GOTT,* AND RICHARD ROSS, Baltimore, Md.

60 of the first 153 patients receiving aorto-coronary vein bypass surgery have been reevaluated with coronary arteriography, single-plane left ventriculography, and stress testing (exercise and atrial pacing) an average of 5.3 months after surgery. In this group, electrocardiography (ECG) abnormalities compatible with recent myocardial damage were seen in 35 patients (58%) within 10 days postoperatively. The 60 patients herein reported are comparable with the surgical population as a whole with respect to mean duration of angina (40 months), incidence of previous myocardial infarction (63%), New York Heart Association functional class (95% class III or IV because of angina), and 86% had significant stenosis of two or all three major coronary arteries. Good to excellent symptomatic relief was present in 82% and improved stress tolerance in 85%. Bypass patency for the entire group was 64%. Hemodynamic parameters of left ventricular end diastolic pressure, cardiac index, and ejection fraction showed no significant differences between pre- and postoperative studies. The ECG abnormalities in the above 35 patients were supported by the angiographic findings as follows: coronary arteriography revealed total occlusion of a previously patent major coronary artery in 60%, significant worsening of previously recognized lesions in another 11%, and with ventriculography segmental contractility was judged to be reduced in another 23%. Symptomatic improvement in this group with myocardial damage was present in 86%, stress tolerance improved in 87%, and bypass patency in 53%. These data may be interpreted as suggesting that clinical improvement was related to bypass patency, infarction of ischemic myocardium, or a combination of the two. (Research supported by grants HE 05584, FR 35 from NIH.)

4. Changes in Liver and Skeletal Muscle Transport of Amino Acids Induced by Diets. SIAMAK ADIBI,* SWAMIKKAN NALLATHAMBI,* AND THOMAS MODESTO,* Pittsburgh, Pa. (introduced by I. Arthur Mirsky**).

Present studies were performed to characterize the changes in amino acid transport processes of above tissues in relation to substrate specificity and dietary composition. The in

vivo transport of cycloleucine-^{14}C (CYC) and α-aminoisobutyric acid-^{14}C (AIB) was investigated by determining the in vivo distribution ratio (DR = intracellular concentration/extracellular concentration) of these substrates after i.v. injection in rats. Starvation even for 1 day significantly increased the liver DR of CYC by 32% ($P < 0.01$). Starvation produced an even more dramatic increase in the liver DR of AIB (284%). While the muscle DR of CYC was not affected during the starvation, the DR of AIB was markedly reduced (67%, $P < 0.01$). To distinguish whether the caloric or amino acid deprivation was responsible for the above changes, similar studies were repeated in rats which were force-fed a protein-free diet. Protein deprivation resulted in significant increases in the liver DR of both amino acids, but the muscle DR of neither amino acid was affected. To determine whether the alteration in hormonal milieu accounted for the transport changes, the following studies were performed. (*a*) Liver slices of starved rats, incubated in an ionic media containing only CYC, showed marked increases in the uptake of this amino acid. (*b*) Starvation or protein deprivation for 1 day did not change plasma glucagon levels, but insulin levels were promptly reduced after starvation. It thus appears that caloric restriction is the critical dietary factor for selective amino acid transport changes in the muscle, while liver transport changes for both substrates result from either caloric or protein deprivation.

5. Interrelationship between Cell pH and Metabolism in Potassium Depletion. SHELDON ADLER,* Pittsburgh, Pa. (introduced by Philip Troen**).

In potassium depletion we demonstrated that cell pH (pH_i) in rat diaphragm muscle is unchanged at extracellular pH (pH_e) 7.40, but it is decreased in both extracellular acidosis and alkalosis. Therefore, changes in metabolism in this state could be due either to changes in cell [K] or pH_i. Since citrate decarboxylation in diaphragm is pH dependent, this reaction was employed to determine the relative effect of these variables on metabolism. Intact rat diaphragms were incubated simultaneously for 4 hr in a Krebs-Ringer bicarbonate solution containing 5 or 0 mEq potassium. Hemidiaphragms were then removed and incubated at the same pH_e in a 1 mEq potassium bath. $^{14}CO_2$ production from 1,5-citrate-^{14}C was measured. At pH_e 7.42 differences between normal and potassium-depleted tissue were not significant. At pH_e 6.90, however, citrate decarboxylation was 432 dpm/mg in depleted tissue compared with 372 in normals ($P < 0.001$). pH_i was 6.73 and 6.89, respectively. Similarly, at pH_e 7.65 decarboxylation was 188 and 165 ($P < 0.025$) in potassium depletion and normals, respectively, whereas pH_i was 7.16 and 7.23. When pH_e was 6.94 in potassium depletion and 6.65 in normals thus making pH_i almost identical in both groups, there were no significant differences in decarboxylation. These results indicate that it is pH_i, not pH_e or tissue potassium, which is important in altering muscle metabolism in potassium depletion. Our previous studies indicate that when pH_e is increased in this state, pH_i is closer to that found at a physiologic pH_e of 7.40. It is proposed, therefore, that the lack of clinical symptoms often found in potassium depletion may be due to normal pH_i despite both an extracellular alkalosis and a reduction in muscle potassium.

6. Intracellular Composition and Transepithelial Transport: Inulin as a Potential Source of Experimental Error. Q. AL-AWQATI,* A. LEAF,** A. D. C. MACKNIGHT,* AND M. M. CIVAN,* Boston, Mass.

It has become increasingly apparent that the volume and electrolyte content of epithelial cells is an important determinant of transport activity. Inulin has been widely used in the measurement of the composition of epithelial cells under the assumption that its volume of distribution accurately measures the extracellular space. Recent studies from our laboratory have raised questions concerning the validity of this assumption. Inulin and sucrose spaces were simultaneously measured in cells scraped from the paired halves of urinary bladders from the toad; one hemibladder was incubated for 1 hr and the other for 6, 12, or 20 hr. After 1 hr the nonsucrose space was 3.2±0.1 kg/kg dry weight (dw) and did not change significantly after 6, 12, or 20 hr. The noninulin space after 1 hr was 4.0±0.1 kg/kg dw, and decreased significantly after 6 hr in one or three sets of experiments. After 12 hr, the noninulin space fell by 1.8±0.3 ($P < 0.005$) and after 20 hr, decreased by 0.6±0.2 kg/kg dw ($P < 0.05$). Using sucrose as an extracellular marker, vasopressin increased cell water by 0.38±0.14 kg/kg dw ($P < 0.05$), sodium by 54±12 mEq/kg dw ($P < 0.01$), and chloride by 38±10 ($P < 0.02$). Cell potassium was unchanged. Since the sucrose space is consistently larger than the inulin space and is unchanged with prolonged incubation, sucrose is the more suitable marker. The results indicate that vasopressin increases the permeability for sodium entry into the epithelial cells, consistent with previous data from this laboratory using inulin as an extracellular marker. (Supported by the American Heart Association grant 71-847, NIH grant HE-06664, and John A. Hartford Foundation, Inc.)

7. The Response of the Blood Coagulation System after Acute Thrombic Stroke. NORMA ALKJAERSIG,* BENEDICTE LAURSEN,* AND ANTHONY FLETCHER,** St. Louis, Mo.

20 patients with acute cerebral thrombosis were followed serially for 7 days after the ictus. Plasma fibrinogen chromatography (Fletcher, 1970. *Trans. Ass. Amer. Physicians.* **83**: 159), a method for quantifying fibrinogen complexes, uncomplexed fibrinogen, and other fibrinogen derivatives, detected the presence of blood hypercoagulable/thrombotic states. Such states were diagnosed when fibrinogen complex concentration exceeded 20% of total fibrinogen. Serial assays for plasminogen, fibrinogen, α_1-antitrypsin, α_2-macroglobulin, and antithrombin III were obtained on all samples. Mean plasma fibrinogen rose steadily from 300 mg/100 ml to 350 mg/100 ml over the observation period and mean α_2-macroglobulin approximately 35%, but changes in other component mean values were insignificant. Mean fibrinogen first derivative concentration was elevated throughout the observation period, a finding indicative of enhanced plasma fibrinolysis. 49% of the total plasma samples examined showed blood hypercoagulable/thrombotic patterns. Comparison of plasma samples containing > 20% fibrinogen com-

plexes with those containing $<20\%$ fibrinogen complexes showed antithrombin III, plasminogen, α_1-antitrypsin, and α_2-macroglobulin levels to be significantly depressed ($P < 0.01$ for days 1–3) in plasma exhibiting hypercoagulable/thrombotic chromatographic findings. Also, the concentration of fibrinogen first derivative was significantly decreased ($P < 0.05$) in these latter samples. Depression of antithrombin III concentration in samples showing blood hypercoagulable/thrombotic chromatographic patterns indicated that thrombin had recently been formed in these plasmas. The concomitant decrease in plasminogen and of the plasmin inhibitor α_2-macroglobulin, together with the finding of increased fibrinogen first derivative, is confirmatory of the presence of a secondary plasma fibrinolytic response. The findings demonstrate that a blood hypercoagulable state follows the acute ictus in a high proportion of patients with cerebral thrombosis. Since concomitant activation of the plasminogen system also occurs, it may be inferred that intravascular coagulation complicates the blood hypercoagulable state. (Supported by HE-03745 and NB 06833.)

8. The Isolation of Human Vitamin B_{12}-Binding Proteins Utilizing Affinity Chromatography. ROBERT H. ALLEN* AND PHILIP W. MAJERUS, St. Louis, Mo.

We have developed a method of affinity chromatography which is a potent tool for isolation of the trace B_{12}-binding proteins. The affinity ligand was prepared by partial acid hydrolysis of the amide groups of the propionamide sidechains of the corrin ring of cyanocobalamin (B_{12}). The resultant mixtures of mono-, di-, and tricarboxylic B_{12} derivatives were separated by chromatography on QAE-Sephadex. Monocarboxyl-B_{12} was coupled covalently to the free amino group of 3,3′-diaminodipropylamine-substituted Sepharose using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide thus regenerating native B_{12} stably coupled to Sepharose (yield, 0.7 μmoles B_{12}/ml Sepharose). B_{12}-Sepharose bound ($>95\%$) the B_{12}-binding proteins of human leukocytes, plasma, and gastric juice. After extensive washing the B_{12}-binding protein is eluted in high yield (60–95%) with 7.5 M guanidine-HCl. We have purified the B_{12}-binding protein of chronic myelogenous leukemia granulocytes 10,000-fold using this technique alone. The purified leukocyte binder saturated with B_{12} gives a single band on polyacrylamide disc gel electrophoresis which is red in unstained gels. The molecular weight of the leukocyte binder is 120,000 as determined both by SDS-polyacrylamide gel electrophoresis and Sephadex G-150 chromatography. The protein binds 2.6 moles of B_{12}/120,000 g protein (Lowry) indicating that the leukocyte binder has multiple B_{12}-binding sites. Human serum transcobalamin II (TCII) has been purified 760,000-fold starting with Cohn fraction III from 1000 liters of plasma. This preparation of TCII binds 7.9 μmoles B_{12}/g protein, gives a single red band on disc gel electrophoresis, but contains 50–75% contaminating protein based on the disc gel. Further purification is in progress. (Research supported by grants from NIH and ACS.)

9. Increased Susceptibility to Infection in Type II Essential Hypercatabolism of C3. CHESTER A. ALPER, KURT J. BLOCH, AND FRED S. ROSEN, Boston, Mass.

We have previously described a young man with a lifelong history of infections, hypercatabolism of C3, and multiple defects in complement-mediated functions and deficiencies in plasma proteins affecting C3. The present patient is a 34 yr old woman with a history of meningococcal, β-hemolytic streptococcal, and pneumococcal infections. Humoral antibody production, cellular immunity, serum immunoglobulins, and white cell phagocytic function were normal. All complement components were normal in concentration except for C3 which was 7 mg/100 ml or 5% of normal. Approximately 50% of the immunochemically detected C3 was in the inactivated form, C3i. Metabolic studies with ^{125}I-labeled C3 revealed a 2–3 times elevated fractional catabolic rate of 6% per hr. Complement-mediated functions such as the enhancement of phagocytosis by serum were diminished but were normalized by the addition of C3 in vitro. In the previous patient, GBG (properdin Factor B), C3 proactivator activity, GBGase inhibitor, and C3 inactivator were undetectable, but, in sharp contrast, these proteins were present in normal concentrations in the present patient's serum. C3 added to her serum and incubated at 37°C was converted and inactivated more rapidly than in normal serum. The addition of normal serum increased the rate of C3 breakdown in vitro. The factor in the patient's serum causing C3 breakdown was a 6S heat-labile β-pseudoglobulin requiring Mg^{++} for its action. The cofactor in normal serum had similar physicochemical characteristics but was slightly less heat labile. The abnormality in this patient is thus quite different from that described in the previous patient, but is also associated with undue susceptibility to infection and increased breakdown of C3 in vivo.

10. ABO Blood Group Activity Associated with Human Intestinal Disaccharidases. DAVID H. ALPERS* AND JOHN J. KELLY,* St. Louis, Mo. (introduced by Arthur Z. Eisen.)

Intestinal disaccharidases are glycoproteins resistant to trypsin treatment. We have purified human maltase and sucrase about 300-fold to homegeneity on gel electrophoresis. Carbohydrate content was measured by paper chromatography and specific enzymatic analysis, and by colorimetric techniques. Carbohydrate measured accounted for almost 40% of the isolated protein. Sucrase from a blood type A patient contained per milligram of protein 132 μg hexosamine, 20 μg glucose, 209 μg galactose, 36 μg mannose, 83 μg fucose, and 154 μg xylose, for a total of 634 μg carbohydrate. Maltase from a blood type B patient contained per milligram of protein 56 μg hexosamine, 45 μg glucose, 329 μg galactose, 14 μg mannose, 42 μg fucose, and 125 μg xylose, for a total of 611 μg carbohydrate. Sialic acid could not be detected. Amino acid analysis revealed 17% of residues as threonine and serine. These data suggested the carbohydrate content of blood group substance, also high in galactose, fucose, and hexosamine. Pure disaccharidase inhibited type-specific AB red cell agglutination, at least to a concentration of 1 μg/ml. Group D agglutination was not affected. Inhibition was not due to a small contamination with pure blood group substance for the following reasons: (*a*) disaccharidase protein and activity did not enter a 4% acrylamide gel after incubation with specific antibody; (*b*) preincubation with specific antibody shifted the disaccharidase in sucrose gradients from 11.2 to 14S; and (*c*) periodate treatment rendered the en-

zyme more trypsin-sensitive by 20% while decreasing agglutination inhibition by 50%. We conclude that intestinal disaccharidases have a carbohydrate composition and structure similar to blood group substance, and that this carbohydrate prevents intraluminal hydrolysis of the protein by pancreatic enzymes. (Supported from grant AM-14038 from NIH.)

11. Inhibition of Hemoglobin Synthesis by Cyanate. BLANCHE P. ALTER, YUET WAI KAN, AND DAVID G. NATHAN, Boston, Mass.

Cyanate inhibits sickling and prolongs red cell survival in sickle cell anemia. Since cyanate carbamylates many proteins, several abnormalities in red cell function might be anticipated. We have found that cyanate markedly inhibits hemoglobin synthesis in human and rabbit reticulocytes. Incorporation of radioactive amino acid into hemoglobin by human sickle reticulocytes, bone marrow erythroblasts, or rabbit reticulocytes was inhibited by as little as 5 mM cyanate and abolished by 50 mM. Both α- and β-chains were equally affected. Thrice washing of cells exposed to 50 mM cyanate for 30 min restored 30% of the synthetic activity. Transport of radioactive amino acid (lys or leu) into the cell was evaluated by Sephadex G-25 chromatography of hemolysates prepared from washed cells previously incubated with 50 mM cyanate and amino acid for 2 hr. Free intracellular radioactive amino acid was not decreased and was not carbamylated as shown by iontopheresis at pH 1.9. The influence of cyanate on amino acylation of tRNA was evaluated with leucine-^{14}C, rabbit liver tRNA, and ribosome-free rabbit reticulocyte lysate. Cyanate did not inhibit acylation and the acylated amino acid, released from tRNA by 0.1 N NaOH, was not carbamylated. After incubation of rabbit reticulocytes with methionine-^{35}S and cyanate, polysomes were examined by sucrose density gradients. Cyanate induced degradation of polysomes to monosomes, suggesting inhibition of initiation rather than blockade of termination as the basis of the protein synthetic defect. We conclude that cyanate profoundly inhibits initiation of hemoglobin synthesis. If this effect occurs in other tissues, the chemical may not be suitable for therapy, at least in growing children.

12. Influence of Increased Glucose Availability and Mannitol on Performance of Hypoxic Myocardium. EZRA A. AMSTERDAM,* DUANE FOLEY,* RASHID A. MASSUMI,* ROBERT ZELIS,* AND DEAN T. MASON, Davis, Calif.

Support of the ischemic, failing heart by positive inotropic drugs is limited by the unfavorable alteration in myocardial oxygen supply-demand these agents produce. Other pharmacologic approaches which enhance cardiac performance without accompanying deleterious effects would be advantageous. Therefore, the effects of glucose, insulin, and mannitol on contractile performance of isolated, supported right ventricular cat papillary muscles were studied during normoxia (95% O_2, 5% CO_2) and hypoxia (95% N_2, 5% CO_2). In seven muscles with 100 mg/100 ml glucose, control peak isometric tension fell to 18% (4.8 to 0.8 g/mm^2). During support with 100 mg/100 ml glucose, hypoxia-induced reduction of tension was attenuated ($P < 0.05$) to 27% (4.4 to 1.4). In six additional muscles, augmentation of glucose from 100 mg/100 ml to 1000 mg/100 ml during hypoxia increased tension (0.7 to 1.1, $P < 0.01$). Addition of insulin (200 mU/ml) to six muscles during hypoxia-100 mg/100 ml glucose increased tension from 1.5 to 1.8 ($P < 0.05$). During hypoxia-1000 mg/100 ml glucose, insulin increased tension from 1.4 to 2.2 ($P < 0.01$). Mannitol, equiosmolar to 1000 mg/100 ml glucose, augmented tension during normoxia-100 mg/100 ml glucose (5.4 to 6.4, $P < 0.05$) and improved ($P < 0.05$) hypoxia-induced reduction of tension to 31% of control (6.4 to 2.0) compared to decrease of tension of 18% of control with hypoxia-100 mg/100 ml glucose alone. Therefore, augmented glucose availability and mannitol have protective effects on function of the hypoxic myocardium and may provide additional clinical approaches for support of the ischemic, failing heart. (Supported by NIH Research Grant MH-19489.)

13. Glycolytic Rates and Glycogen Content of Diabetic Rat Jejunal Mucosa. JAMES W. ANDERSON* AND ALBERT L. JONES, San Francisco, Calif. (introduced by Marvin H. Sleisenger**).

Diabetes is associated with significant increases in intestinal transport of sugars, amino acids, and sodium. However, the metabolic alterations in diabetic intestine are less well characterized. Previously we have demonstrated that the activities of certain key glycolytic and pentose phosphate pathway enzymes are significantly increased in jejunum of diabetic rats. In the present investigation, glucose utilization and lactate production as well as glycogen concentrations and localization in jejunal mucosa of nonfasted control and alloxan-diabetic rats and 72-hr fasted rats were studied. Glucose utilization was 4.0±0.2 μmoles/min per g (mean ±SEM) and lactate production was 4.8±0.2 μmoles/min per g in control jejunal homogenates. These values are several-fold higher than rates reported for jejunal supernates and 20-fold higher than values reported for liver. When compared with control values, both glucose utilization and lactate production were significantly reduced in jejunum of fasted rats while both were increased in diabetic rats. Glycogen content and intracellular location have not been well defined in rat jejunal mucosa. Control rats had 444±41 μg glycogen/g mucosa whereas fasted rats had 214±21 μg/g. Diabetic rats, however, had 818±149 μg glycogen/g mucosa. Electron microscopy demonstrated an accumulation of particles of the size and appearance of β-glycogen within jejunal absorptive cells of diabetics. These particles were particularly prominent within the region of the terminal web but were also found scattered at random throughout the apical cytoplasm. The diabetic state, therefore, produces significant alterations in glucose metabolism of intestinal mucosa which include increased glucose utilization, lactate production, and glycogen accumulation. These biochemical alterations undoubtedly are related to reported alterations in intestinal transport in the diabetic animal.

14. Active Role of Blood Cells in Amino Acid Transport in Man. THOMAS T. AOKI,* MURRAY F. BRENNAN,* WALTER A. MULLER,* AND GEORGE F. CAHILL, JR., Boston, Mass.

Studies of amino acid metabolism in human subjects have been predicated on plasma levels, while the potential contribution of the cellular constituents of blood remained unassessed. To ascertain both the role of blood cells in amino acid transport, in particular glutamate, and the ability of insulin to increase glutamate uptake by muscle, five normal human volunteers underwent eight forearm studies in each of which insulin was infused for 90 min into the brachial artery (venous effluent insulin concentration at 90 min = 188 ±58 μU/ml). Enzymatically-determined plasma glutamate arterio-deep venous (A-DV) differences were not significantly increased during the entire procedure, while, in sharp contrast, whole blood glutamate A-DV differences were significantly increased (0 min = 8±3, 60 min = 26±9, 90 min = 25±7, 120 min = 24±9, 150 min = 18±6, 180 min = 13±7, 240 min = 4±8 μmoles/liter of whole blood, mean ±SEM) at 60 and 90 min ($P < 0.05$, paired t test). Using the hematocrit, the glutamate content of the blood cells was calculated. At rest, a net movement of glutamate from arterial plasma into both blood cells and muscle was found. During the infusion, however, glutamate moved out of both plasma and cell compartments into muscle. Insulin thus appears to be capable of increasing glutamate uptake by muscle. Of far greater importance, this change could only be documented when whole blood rather than plasma glutamate determinations were performed. Therefore, insulin affects both the direction and magnitude of the dynamic interplay that exists, with respect to glutamate and perhaps other amino acids as well, between plasma, blood cells, and muscle with the blood cells playing a pivotal role. (Research supported by grants from NIH.)

15. Brain Edema after Rapid Lowering of Plasma Glucose in Normal and Diabetic Rabbits. ALLEN I. ARIEFF* AND CHARLES R. KLEEMAN,** Los Angeles, Calif.

Cerebral edema frequently complicates diabetic coma; the mechanism is unknown. Hyperglycemia (plasma glucose = 1100 mg/100 ml) in normal and diabetic rabbits was induced by glucose (^{14}C-labeled) infusion; measurements were made of Na, K, glucose, Cl, lactate, amino acids, sorbitol, inositol, H_2O, ^{14}C activity, and osmoles (by cryoscopy) in brain, plasma, and CSF. Idiogenic osmoles (Id Osm) were defined as brain osmoles-sum of all measured solutes. In both groups, during 4 hr of hyperglycemia (brain and plasma Osm = 342 mOsm/kg) there was no loss of brain H_2O. In diabetics, brain Na + K increased by 10%, but in normals there was no increase in any of the aforementioned solutes; therefore, the brain generated Id Osm. Brain ^{14}C/plasma ^{14}C activity suggested these Id Osm were not glucose metabolites. When plasma glucose was then rapidly lowered with insulin, cerebral edema developed in both normals and diabetics, with ↑ in brain content (mEq/kg dry wt) of Na (234 → 266), K (406 → 485, H_2O (376 → 454 g/100 g dry wt), Id Osm (32 mM/kg H_2O) and ^{14}C activity (+ 400%) and an osmotic gradient between brain (328 mOsm/kg) and plasma (290 mOsm/kg), but no change in brain lactate, sorbitol, amino acids, or inositol. When plasma glucose was lowered with peritoneal dialysis (PD), brain edema did not occur. These data show: (*a*) during hyperglycemia the brain generates Id Osm in both normals and diabetics but in the latter, 40% of the ↑ in brain Osm is Na + K; (*b*) lowering plasma glucose rapidly with insulin causes brain edema while lowering plasma glucose with PD does not; (*c*) there appears to be a specific action of insulin on brain, affecting K and Na transport and causing Id Osm generation. (Supported by USPHS Grant NS05905.)

16. Antibody Specificity, Immunochemical Heterogeneity, and the Interpretation of Measurements of Plasma Parathyroid Hormone by Radioimmunoassay. CLAUDE ARNAUD, RALPH GOLDSMITH,* JULIANNA BISCHOFF,* GLEN SIZEMORE,* SUSAN OLDHAM,* AND JUDITH LARSEN,* Rochester, Minn.

This investigation was done to explain the discrepancies in the reported results of studies of endogenous plasma PTH measured by radioimmunoassay. 10 anti-PTH antisera from different laboratories were systematically evaluated. We observed large differences in the values for immunoreactive PTH (iPTH) in the same hyperparathyroid (HPT) plasma. Each antiserum was characterized by determining its individual affinity for two preparations of PTH purified from parathyroid tumor culture medium which are immunologically similar if not identical to the 9000 (PTH-9) and the 6–8000 (PTH-7) M.W. species of PTH which are the major components of iPTH in HPT plasma. Two of the antisera had unique properties: in mixtures of varying ratios of PTH-9 and PTH-7, GP 1 M reacted almost exclusively with PTH-7 and CH 14 M with PTH-9. Using these two antisera, we found that PTH-9 had a much shorter half-life and its measurement with CH 14 M more reliably localized parathyroid pathology (during differential venous catheterization) than did measurement of PTH-7 with GP 1 M. On the other hand, greater discrimination between normal and primary HPT was found with GP 1 M (PTH-7) than with CH 14 M (PTH-9). This was even more striking in end-stage renal failure, in which PTH-7 was increased 3 to 20-fold in all patients, whereas PTH-9 was increased in only 70% of such patients. We conclude that (*a*) the differences in the results of iPTH measurements in different laboratories are most likely explained by differences in antiserum specificities for the two major circulating species of PTH, (*b*) assays of PTH-7 more likely reflect the steady state status of circulating PTH, and (*c*) assays of PTH-9 reflect the acute secretory status of the parathyroid gland. (NIH AM12302.)

17. Reduced Catechol-*O*-Methyltransferase (COMT) Activity in the Liver and Increased Pressor Response to Norepinephrine (NE). NUZHET O. ATUK* AND THOMAS C. WESTFALL,* Charlottesville, Va. (introduced by Julian I. Kitay).

The liver is the largest source of COMT, a major enzyme for metabolism of circulating catecholamines. The finding of transient hypertension and increased urinary norepinephrine during episodes of jaundice in a woman with recurrent intrahepatic cholestasis prompted studies to define the mechanism of altered catecholamine metabolism. During icteric (I) and nonicteric (NI) episodes, the following were measured: (*a*) NE metabolism by NE-^{14}C infusion, (*b*) COMT and mono-

amine oxidase (MAO) activity in liver biopsies, and (*c*) pressor response to graded i.v. infusion of NE. After injection of NE-^{14}C urine was collected at intervals and assayed for radioactive NE, *O*-methylated and *O*-methylated-deaminated metabolites. During 0–2 hr total recovery of injected radioactivity in both I and NI phases was less (24 and 25%) than in normals (37±4.9%) and there was decreased formation of *O*-methylated metabolites; during 2–48 hr the recovery of radioactivity was increased (69% I and 79% NI) as compared to normals (61±9.7%). Liver MAO activity was normal, but COMT activity was reduced in both I and NI phases (0.25 and 0.34 nmoles/g per hr). Five control samples contained 1.43–2.65 nmoles/g per hr. Also, the patient showed increased responsiveness to injected NE in both phases. The data suggest that decreased hepatic COMT in this patient was associated with delayed NE metabolism and increased pressor response to NE. The initial decreased excretion of radioactivity may represent a compensatory increase in NE uptake by tissue in the presence of delayed metabolism. Since metabolism of NE was similar in the two phases, and excretion of endogenous NE was increased in the I phase, it is suggested that during the I phase there is increased NE synthesis. Increased production of NE coupled with decreased metabolism may explain the hypertension in the I phase.

18. Uptake of Radioiron-Labeled Hemoglobin and Transferrin by Isolated Hepatic Kupffer and Parenchymal Cells. MICHIYASU AWAI,* BARBARA CHIPMAN,* AND ELMER B. BROWN, St. Louis, Mo.

Although the hepatic uptake of radioiron after injection of hemoglobin or transferrin has been studied, little is known about the initial cellular distribution of these forms of iron in the liver. Recent techniques permit the separation of parenchymal cells from Kupffer cells so that the distribution of iron bound to transferrin or hemoglobin could be localized in specific cells of the liver at various times after injection. Hepatic parenchymal cells were isolated from rats by two methods: by incubation of perfused liver with tetraphenylboron or by mechanical tissue homogenization. Kupffer cells were isolated using a pronase method. Purity of cell separation was estimated by phase microscopy of phagocytosed carbon particles and trypan blue, or by radioisotopic labeled colloidal gold and technetium-sulfur-colloid in Kupffer cells. 7 mg of hemoglobin-^{59}Fe was injected intravenously and was removed 10 to 20 times more effectively by parenchymal than by Kupffer cells of rats killed after 3–24 hr. Blocking Kupffer cell uptake by prior injection of heat-denatured albumin produced no change in total liver or parenchymal cell uptake of hemoglobin-^{59}Fe. Distribution of ^{59}Fe in hepatic parenchymal and Kupffer cells was measured 3–48 hr after injection of transferrin-^{59}Fe into rats with marrow erythroid hypoplasia produced by hypoxic polycythemia. At 3 hr ^{59}Fe was almost equally distributed in parenchymal and Kupffer cells, at 6 hr maximal ^{59}Fe uptake occurred in parenchymal cells, but at 48 hr uptake of ^{59}Fe by Kupffer cells predominated. Extension of these techniques should allow the exploration of various factors influencing iron uptake by the two major hepatic cells types. (Research supported by grants from NIH.)

19. In Vitro Inhibition of Phagocytosis and Disruption of Microfilaments by Cytochalasin B. STANTON G. AXLINE* AND EVE REAVEN,* Palo Alto, Calif. (introduced by Jack Remington).

The role of cytoplasmic microfilaments as a possible mediator of phagocytosis has been investigated using cytochalasin B (CB), an agent that interferes with cell locomotion. Pretreatment of cultivated mouse peritoneal macrophages with CB (5–10 μg/ml) for 2–48 hr reduced the phagocytic uptake of polyvinyl toluene particles by 80%. The inhibitory effect of CB on both the attachment and interiorization stages of phagocytosis was rapidly reversed by removal of CB from the media. Observations by time-lapse photomicroscopy showed that undulating plasma membrane activity was completely inhibited within 10 min of CB application, resulting in irregular cytoplasmic retraction, rounding of the centrosphere region, and shortening of pseudopods at sites of attachment to glass. Electron microscopic examination of macrophages treated for periods from 10 min to 24 hr with CB showed disaggregation of oriented bundles of 40–50 A cytoplasmic microfilaments. In addition, cytoplasm specifically associated with shortened pseudopods and invaginated plasma membranes showed an increased density of the non-oriented subplasmalemmal microfilamentous network. Larger filaments (70–100 A) and other cell organelles were unaffected by CB. The observed drug effects were calcium-independent, and protein synthesis was insensitive to CB. The morphological studies suggest that CB-induced microfilament disorganization may be responsible for membrane immobilization causing inhibition of phagocytosis. (Supported by USPHS Grant AI-10055 and the Veterans Administration.)

20. Effect of Group A Streptococcal Antibody on Heart Valve Tissue Biosynthesis. ELIA M. AYOUB, MICHAEL DENNIS,* AND GLENN D. SWANK,* Gainesville, Fla.

The immunological relationship between antibody to the Group A streptococcus and heart valve tissue was studied. A soluble product which reacted on agar diffusion with antibody to the Group A streptococcus was obtained from human (rheumatic) and porcine mitral valves. Using the radioimmune precipitin technique, the soluble valve antigen inhibited the reaction of purified streptococcal Group A carbohydrate with Group A antiserum. The effect of humoral antibody on the biosynthetic activity of valve tissue was also investigated. Freshly obtained valve tissue was minced, incubated in tissue-culture medium containing radiolabeled precursors (glucosamine-^{14}C and proline-^{3}H). The uptake of these precursors by the tissue, in the presence and absence of antibody to valve tissue and Group A streptococcus, was measured. The data obtained revealed that antibody to valve tissue produced almost complete inhibition of uptake of both precursors. However, while antibody to the Group A streptococcus produced inhibition of the uptake of glucosamine-^{14}C, an enhanced uptake of proline-^{3}H was observed in the presence of this antibody. Similar results were obtained when the tissue was preincubated with the antiserum, then washed before incubation with the labeled precursor. Parallel studies with human mitral valves were performed using proline-^{3}H only and showed enhanced uptake of this precursor in the

presence of streptococcal Group A antibody. The results obtained in this study confirm the presence of a common antigenic component between the Group A streptococcal carbohydrate and heart valve tissue. In addition, studies with labeled precursors suggest that Group A antiserum inhibits synthesis of valvar glycopeptide-mucopolysaccharide, as reflected by inhibition of glucoasmine uptake, and stimulates synthesis of collagen-protein, as reflected by enhanced proline uptake. (Supported by NIH grant AI09645-03 and Florida Heart Association grant 72 AG 16.)

21. Purine Reutilization in Human Lymphocytes. JOHN AYVAZIAN* AND SOLOMON SKUPP,* New York (introduced by Aaron J. Marcus**).

Human lymphocytes in culture, stimulated to transformation by phytohemagglutinin, were used to study the relative importance and degree of reutilization of purines. In the initial study lymphocytes undergoing transformation from the resting stage through initial mitosis were observed. The cells were pulse-labeled early in transformation with hypoxanthine-8-^{14}C. Initially 70% of the incorporated label appeared in the mononucleotides and the remainder in RNA. Portions were taken daily for 3 days thereafter. At the completion of transformation 94% of the labeled purines were found in nucleic acids. Cell survival was 95% as indicated by the increase in DNA. Despite the turnover and interconversion of purine compounds during transformation, the total recovered ^{14}C activity for each portion remained constant with the last having 92% of the activity of the first. In the second study lymphocytes were labeled during the first synthetic phase and cultures followed for 9 days through three to four cellular replications. The cells were doubly-labeled with hypoxanthine-8-^{14}C and thymidine-^{3}H. Excess label was removed and chase media substituted which inhibited reutilization of label from degenerating cells. Correction was made for cell loss by using the decay in the specific activity of DNA-^{3}H to determine the mean number of synthetic phases undergone by the cultures and then determining the theoretical yield of DNA assuming 100% cell survival. The theoretical DNA was compared to the observed and cell loss calculated. During growth and replication the labeled purines shifted from the nucleotides and RNA into DNA but despite this metabolic activity the corrected ^{14}C recoveries demonstrated an efficiency of reutilization of purines that exceeded 95%. (Supported by the Veterans Administration and NIH Grant AM-14059.)

22. Genetic Polymorphism of Parotid Basic (Pb) Proteins. EDWIN A. AZEN* AND WILMA B. BIAS,* Madison, Wis. and Baltimore, Md. (introduced by Fritz Bach).

A frequent polymorphism with three phenotypes showing multi-banded patterns was observed among 90 randomly collected samples from Blacks and was studied by acid-urea starch-gel. Autosomal docominant inheritance was postulated and supported by excellent fit ($P = 0.89$) of observed (64, 23, 3) and expected (63.4, 24.3, 2.3) values for the phenotypes (1-1, 1-2, 2-2, respectively), as well as results of family studies including 32 members. In two families with parents of alternative homozygous types (1-1 × 2-2), all nine children were heterozygotes (1-2). One heterozygous variant, identical electrophoretically to those in Blacks, was found among 101 randomly collected samples from Whites, the rest being type 1-1. There were no variants in 19 samples from Orientals, all being type 1-1. Gene frequencies were: for Blacks, $Pb^1 = 0.84$; $Pb^2 = 0.16$; for Whites, $Pb^1 \sim 0.995$; $Pb^2 \sim 0.005$. A proteolytic degradative process involving these proteins and blocked by protease inhibitors was found in parotid saliva on incubation studies, and this explained, in part, interesting variations in band mobilities and intensities within phenotypic classes. The Pb proteins are distinct from parotid fluid amylase, IgA, lysozyme, and albumin. There was nonidentity between Pb proteins and products of other gene loci tested, including ABO, Rh, MNSs, Kell, Kidd, Lutheran, Duffy, Lewis, Secretor, haptoglobin, transferrin, group-specific component, and hemoglobin (β-chain determinant). This polymorphism should be useful for genetic research since the frequency is high, and it appears virtually restricted to Blacks (like Fv [a–b–] and JS^a). (Research supported by grant AMO-0955-07 from NIH.)

23. Depression of Peripheral Thyroxine Turnover in Heroin Addicts. F. AZIZI,* A. VAGENAKIS,* A. CORMAN,* V. PATCH,* A. SAMARAWEERA,* L. BRAVERMAN, AND S. INGBAR,** Boston, Mass.

Since the activity of hepatic drug-metabolizing enzymes and the rate of peripheral thyroxine turnover seem closely related, and since morphine depresses drug metabolism in some species, we studied thyroxine metabolism in 6 addicts taking "street heroin" (H), 10 undergoing early withdrawal (W), and 10 on prolonged methadone maintenance (M). Compared to serum thyroxine concentration in 21 matched controls (7.6±1.9 μg/ml), values were significantly increased in H (11.3±2.7) and W (9.6±2.1). Fractional thyroxine turnover rates (per cent per day) and clearances (liters/day) were significantly decreased in H (8.0±0.9; 0.9±0.2, respectively) and W (7.6±1.1; 0.9±0.2). Despite individual abnormalities, mean values for serum thyroxine (8.8±2.5), fractional thyroxine turnover (10.7±1.7), and clearance (1.2±0.3) were not significantly altered in M. Despite these differences between groups H and W and group M, thyroxine-binding capacities of TBG in the three groups (28±7; 27±5; 26±5 μg/100 ml, respectively) were equally and significantly increased (normal, 18±4). Values for the per cent of free thyroxine were correspondingly decreased. Lack of the usual inverse relation between increased TBG and retarded thyroxine turnover was evident not only for the three groups as a whole, but also when values in individual patients were examined. Mild abnormalities in liver function were present in many, but not all, patients in the three groups. However, as judged from lack of correlation in individual patients and from previous studies of cirrhosis and hepatitis, disturbances in thyroxine binding and turnover did not seem to result from hepatocellular disease. Conclusions: Abnormalities in thyroxine transport and turnover frequently occur in patients taking heroin or methadone, particularly the former. These probably result from a direct effect of these agents on the cellular, presumably hepatocellular, disposition of the hormone. (Supported by grants AM07917 and AM07953 from NIH.)

24. Stimulation of Adenosine Diphosphate Uptake in Rat Liver Mitochondria by Triiodo-L-Thyronine. BERNARD M. BABIOR,* SIDNEY H. INGBAR,** SUSAN M. CREAGAN,* AND RUBY S. KIPNES,* Boston, Mass.

Entry of ADP into the mitochondrion largely depends upon a carrier-mediated process in which ADP is exchanged for ATP, a process that is specifically inhibited by the complex organic acid, atractyloside. Since the rate of mitochondrial substrate oxidation is limited by the availability of ADP (Lehninger, The Mitochondrion, Chapter 7), we have investigated whether thyroid hormone-induced increase in mitochondrial oxygen consumption could result from stimulation of mitochondrial ADP uptake. Atractyloside-sensitive uptake of ADP-^{14}C and ADP-dependent succinate oxidation by rat liver mitochondria were studied in specimens obtained 20 hr and 3 days after animals had been given a single large dose (20 μg/100 g) of triiodo-L-thyronine (T_3) i.p. No significant effect of T_3 on ADP uptake was observed at 20 hr. By 3 days, however, a time when whole body oxygen consumption response to T_3 reaches a peak, a significant increase in atractyloside-sensitive ADP uptake was seen in T_3-treated animals as compared to saline-treated controls (2.35±0.58 vs. 1.81±0.63 nmoles/min per mg protein; $P < 0.20$). This effect could not be ascribed to increased oxygen consumption, since ADP uptake was measured in the absence of oxidizable substrate. In both control and T_3-treated mitochondria, a significant correlation between ADP-dependent oxygen consumption and atractyloside-sensitive ADP uptake was evident. Moreover regression curves for these functions in the two groups were virtually identical, indicating that T_3, although stimulatory, did not alter the stoichiometric relationship between oxygen consumption and ADP uptake. No increase in ADP uptake was observed when T_3 (10^{-10} to 10^{-6} M) was added to suspensions of mitochondria from normal rats, either 1 hr or immediately before measurements were made. Conclusions: The observations are consistent with the interpretation that the T_3-induced increase in mitochondrial oxygen consumption is secondary to an increase in carrier-mediated ADP uptake. (Research supported by grant AM-09753 from NIH.)

25. *De Novo* Lipid Synthesis as a Repair Mechanism for Membrane Damage in Mammalian Erythroid Cells. SAMIR K. BALLAS* AND EDWARD R. BURKA, Philadelphia, Pa.

Because the mammalian reticulocyte is a nondividing cell, lipid turnover in the cell membrane has been presumed to represent exchange rather than *de novo* synthesis. The importance of the cell membrane in activities other than cell division has prompted investigation of the relationship between erythroid cell lipid metabolism and a functionally intact cell membrane. Membranes of rabbit and human erythroid cells, incubated under various conditions in the presence of labeled lipid precursors, were isolated and the radioactivity in neutral (NL) and phospholipids (PL) was determined. Reticulocytes incorporated acetate, glycerol, fatty acids (FA), and inorganic phosphorus into total cell lipids (TCL). Partial hydrolysis of PL showed glycerol-^{14}C incorporation via α-glycerophosphate into the three carbon backbone of PL, confirming *de novo* synthesis. Glycerol is also incorporated into NL via the Emden-Meyerhof pathway. Acetate-^{14}C appeared in NL and the acyl and 3-C backbone of PL, indicating metabolism to α-glycerophosphate via the Emden-Meyerhof pathway. Since acetate and glycerol are not incorporated into erythrocyte TCL, enzymes necessary for *de novo* lipid synthesis disappear during cell maturation. Puromycin, known only as an inhibitor of protein synthesis, stimulated glycerol incorporation into PL, but not NL, in rabbit and human cells in a concentration-dependent manner (maximum 174±44% at 10^{-3} M) at a step subsequent to formation of α-glycerophosphate. Agents causing transient damage to the cell membrane, such as 10^{-1} M butanol or 1.5×10^{-5} M vincristine, also specifically stimulated the *de novo* synthesis of PL. The data indicate that the mammalian reticulocyte can synthesize total lipids from simple precursors. The pathway for *de novo* PL synthesis is stimulated, at a step subsequent to the formation of α-glycerophosphate, by puromycin by an unknown mechanism and by agents causing membrane damage. The capacity for increased PL synthesis may represent a repair mechanism for the cell membrane under conditions of stress. (Supported by NIH.)

26. Pathogenesis of Alcoholic Hyperlipemia. ENRIQUE BARAONA,* ROMANO C. PIROLA,* AND CHARLES S. LIEBER, New York.

Alcohol produces hyperlipemia, an effect which is much greater in fed than in fasted rats and which we found to be associated with increased lipoprotein production. Recently, because of enhanced lymph lipid output and intestinal fatty acid esterification in vitro, the intestine was incriminated in the development of the hyperlipemia observed in fasted rats 16 hr after ethanol. To assess this mechanism in the postprandial state, rats were given ethanol acutely (3 g/kg intragastrically) or isocaloric carbohydrate in liquid diets containing tripalmitin-^{14}C. In the 1st hr after ethanol, intestinal lymph flow increased by 63% ($P < 0.01$), incorporation of dietary fatty acids into lymph lipids by 165% ($P < 0.01$), and lymph lipid output by 49% ($P < 0.05$), compared to controls. However, no postprandial hyperlipemia ensued and fatty acid esterification by intestinal slices was decreased. By contrast, hyperlipemia developed after administration of a dietary load of fat (with or even without ethanol) in rats given 36% of calories as alcohol for 3–4 wk but not in littermates pair-fed control diets. Lymph flow and lipid output were not increased whereas fatty acid esterification by intestinal slices was enhanced. Thus, esterification in vitro did not correlate with lymph lipid output. Moreover, after lymph diversion, chronic ethanol feeding still produced hyperlipemia (compared to controls), provided adequate and equal intravenous lipid loads (lymph or chylomicrons) were administered; incorporation of i.v. lysine-^{3}H into serum lipoproteins also remained significantly increased. Thus, acute ethanol administration increased lymph lipid output but did not produce postprandial hyperlipemia, whereas in animals fed ethanol chronically, hyperlipemia developed without increased lymph lipid output or despite replacement of intestinal lymph by a constant i.v. fat load; this suggests a nonintestinal, most likely hepatic, mechanism. (Supported by USPHS grants AM 12511, MH 15558, and the Veterans Administration.)

27. Chemical Bonding in Sputum Gels. A. D. BARTON,* J. V. POWERS,* AND R. V. LOURENÇO,* Chicago, Ill. (introduced by Morton D. Bogdonoff**).

We investigated the types of chemical bonding responsible for the gel structure of thick sputum because abnormal bronchial secretions may impair mucociliary clearance, obstruct the airways, and facilitate the persistence of chronic infection. We studied the dispersal of sputum samples ranging from mucoid to grossly purulent, by several agents that cleave secondary bonds; the material not dispersed was separated by sedimentation. Extraction with 30% propylene glycol (PG) dispersed mucoid sputum almost completely. Mucopurulent sputum contains deoxyribonucleoprotein (DPN) fibrils that constitute part of the gel structure; this material resisted dispersal by PG, but was dispersed by extraction with *N*-acetylcysteine (NAC). Part of the mucopurulent sputum gel resisted dispersal by all of the secondary bond agents tested, including urea, sodium dodecylsulfate, concentrated salt solutions, etc. The residue was rich in a γ-glutamyl transpeptidase (GGT) whose level of activity may indicate the existing inflammatory reaction, since it parallels the degree of purulence. The gel structure of mucoid sputum appears to be due to secondary bonding since PG dispersed it. In mucopurulent sputum the DPN fibril material may be cross-linked with S-S-bonds, since it is dispersed by NAC but not by PG. Part of the mucopurulent sputum gel appears to be cross-linked by other more stable covalent bonds since it resists dispersal by all of the secondary bond agents tested. The presence of a GGT suggests that transpeptidation may play a role in such cross-linking. (Research supported by NIH grant HE13824 and Veterans Administration funds.)

28. Molecular Basis of Glucocorticoid Response: Specific DNA Binding of a Steroid-Receptor Complex. JOHN D. BAXTER,* GUY G. ROUSSEAU,* STEPHEN J. HIGGINS,* AND GORDON M. TOMKINS,** San Francisco, Calif.

The mechanism of glucocorticoid hormone action has been studied in two systems: induction of specific protein synthesis in cultured rat hepatoma (HTC) cells, and lysis of cultured mouse lymphoma cells. In HTC cells, inducer steroids (S) bind reversibly to specific cytosol receptor protein (B). This interaction leads to an initial conformational change in the receptor (SB*) followed by a second, temperature-dependent alteration in its properties (SB**). The resulting complex (SB**): (*a*) binds in the nucleus with high affinity to a fixed number ($\sim$ 20,000 per nucleus, 1 pmole/mg DNA) of DNA-containing sites; (*b*) binds with a similar affinity to a fixed number of sites (7 pmoles/mg DNA) on purified HTC cell (but not *Micrococcus lysodeikticus*) DNA. Thus binding to DNA simulates binding to nuclei, although in the latter chromosomal proteins may restrict the number of available sites. Progesterone, an anti-inducer in HTC cells, reversibly binds to the cytosol receptors but does not promote the conformational changes required for nuclear binding in intact cells, or for high-affinity binding to purified DNA. Development of steroid resistance in lymphoma cells is associated with a marked reduction (by 90%) in cytosol receptors and further alterations in their properties. Nuclei from resistant cells retain the capacity for binding steroid-receptor complexes from sensitive cells. These studies support the following hypotheses: (*a*) glucocorticoids are allosteric effectors which induce conformational changes in specific cytoplasmic receptors; (*b*) the steroid-receptor complex then binds to specific sites on nuclear DNA to affect gene transcription and thereby protein synthesis; and (*c*) steroid unresponsiveness may result from alterations in the cytoplasmic receptor proteins. (Research supported by NIH and ACS.)

29. Specificity of the Change in the Amino Acid Composition of the Diabetic Glomerular Basement Membrane. PAUL J. BEISSWENGER,* Philadelphia, Pa. (introduced by Albert I. Winegrad).

The human glomerular basement membrane (GBM) is a glycoprotein of the collagen family containing 7% carbohydrate. In previous studies GBM from patients with long-standing diabetes was found to differ from that of age-matched controls in that they contained increased hydroxylysine and decreased lysine although the total lysine plus hydroxylysine was unaltered. This suggested that a greater fraction of the lysine residues was hydroxylated after peptide synthesis in the diabetic. The diabetic GBM also contained an increase in glucosyl-galactose covalently linked to hydroxylysine residues (P. J. Beisswenger and R. G. Spiro. 1970. *Science* (*Washington*). **168:** 596). To determine the specificity of these changes the amino acid composition of GBM was determined in nondiabetics with well characterized renal diseases associated with proteinuria. The amino acid composition of GBM from patients with accelerated essential hypertension, systemic lupus erythematosus, severe nephrosclerosis, rapidly progressive glomerulonephritis, and postpartum necrotizing renal arteriolitis was determined and compared with GBM from age-matched nondiabetic controls without renal disease. In contrast to diabetic GBM, the amino acid composition of the GBM of nondiabetics with renal disease and proteinuria did not differ from that of age-matched normal controls. In all patients the ratio of lysine/hydroxylysine in the GBM was greater than 1.0; in the GBM of diabetics the ratio averaged 0.65. In the nondiabetic patients with renal disease more than 20% of the residues are glycine; hydroxyproline and proline were present in nearly equal quantities; and there was a significant amount of half-cystine as in the normal human GBM. These observations suggest that the changes observed in the composition of the diabetic GBM may be unique to diabetes mellitus. (Research supported by the American Heart Association.)

30. Serum Inhibitors of Chemotactic Factors. JEFFREY L. BERENBERG* AND PETER A. WARD,* Washington, D. C. and Farmington, Conn. (introduced by Irwin H. Lepow**).

The present studies demonstrate the existence in human serum of an inhibitor of chemotactic factors. Using in vitro micropore filter techniques and human or rabbit neutrophils as the indicator cells, chemotactic responses of leukocytes to the complement-derived chemotactic factors (C3a, C5a, C$\overline{567}$) and a chemotactic factor derived from *Escherichia coli* have been studied. The chemotactic inhibitor isolated from human serum blocks the activity of all chemotactic

factors tested, is soluble in ammonium sulfate (at 45% saturation), and in sucrose density gradient ultracentrifugation as well as gel filtration appears in a biphasic distribution with activity near the IgG and albumin markers. The inhibitor acts directly on the chemotactic factor rather than on the leukocyte. Evidence for binding of inhibitor to chemotactic factor (C3a) has been negative. Demonstration of the inhibitor in normal human serum requires fractionation and/or concentration of serum, whereas serum from most patients with agammaglobulinemia (Bruton type) or Hodgkins disease is rich in inhibitor activity. In these pathological sera inhibitor activity is at least fourfold increased over the level in normal serum. To date all studies suggest the inhibitor present in pathological sera is qualitatively similar to, or identical with, the inhibitor present in normal human serum. The amount of chemotactic activity generated in serum by addition of zymosan is significantly less in inhibitor-rich serum compared to normal (inhibitor-poor) serum. The existence in serum of inhibitors of chemotactic factors may represent a control mechanism of these inhibitors. While the chemotactic factor inhibitor(s) differs from the anaphylatoxin inactivator in many respects, evidence does not yet permit a conclusive statement about the relationship of these two inhibitors (inactivators). (Supported in part by NIH Grant AI-09651.)

31. Defective Bromsulphalein Metabolism in Patients with Gilbert's Syndrome (GS). PAUL D BERK* AND TERRENCE F. BLASCHKE,* Bethesda, Md. (introduced by Nathaniel I. Berlin**).

Although the defect in bilirubin metabolism in GS has been extensively studied, hepatic transport of other organic anions in this condition has received little attention. We have examined the metabolism of bromsulphalein (BSP) (5 mg/kg) and indocyanine green (ICG, 0.5 mg/kg) in 26 patients in whom GS was diagnosed on clinical grounds, confirmed by studies of bilirubin kinetics (24), and supported by liver biopsy findings (18). Plasma BSP concentration at 45 min was abnormal (0.52;1.52 mg/100 ml; nl < 0.50) in 9 of the 26 patients. Complete BSP disappearance curves were obtained in 18 of the patients and 12 normal volunteers (NV's) and analyzed by digital computer. In all instances, the curve was resolvable into a sum of two exponentials. In eight patients (group I), curves were indistinguishable from normal, and BSP clearance (C_{BSP}) was 4.3±0.6 ml/min per kg (nl 4.4±0.7). 10 of the patients, including all 9 with increased 45 min retention, had abnormal curves. In four of these (group II), initial disappearance rate from the plasma was normal, but increased 45 min retention resulted from an increased intercept of the second exponential. In six (group III), initial disappearance rate was reduced. C_{BSP} in group II (3.1±0.6 ml/min per kg) and group III (2.6±0.4 ml/min per kg) were both significantly reduced compared to NV's ($P < 0.01$, $P < 0.001$, respectively). Compartmental analysis indicates that the curves in group III result from defective hepatic BSP uptake, while those of group II are due to an abnormality at a later stage of the BSP transport process. There were no significant correlations between abnormalities of specific parameters of BSP metabolism and corresponding parameters of bilirubin metabolism in these studies. In contrast to the results with BSP, C_{ICG} in 16 patients with GS (8.6±2.4 ml/min per kg) was not significantly different ($P > 0.6$) from the value in 17 NV's (8.9±1.6). The data indicate that the hepatic defect in GS may involve organic anions other than bilirubin, and illustrate the heterogeneity of the patients classified under that diagnosis.

32. The Regulation of Intracellular Glutathione Levels. E. BEUTLER, N. V. PANIKER,* AND K. G. BLUME,* Duarte, Calif.

Red cell reduced glutathione (GSH) levels are increased in some patients with myeloproliferative disorders. The administration of drugs such as diaminodiphenylsulfone (DDS) or methylene blue (MB) also increases GSH levels. At one time it was believed that the increase of GSH found under these conditions might be due to reduction of intracellular GSSG to GSH. However, with the development of an improved method for GSSG determination we were able to demonstrate that about 99.75% of the glutathione in red cells was already in the reduced state. The synthesis of red cell glutathione is accomplished in two steps. First a peptide bond is formed between glutamic acid and cysteine by γ-glutamylcysteine synthetase (GC-S) and then the product, γ-glutamylcysteine, forms a bond with glycine through the action of GSH synthetase (GSH-S). The activity of these two enzymes was measured in a group of patients with myeloproliferative disorders, including chronic granulocytic leukemia, myelfibrosis, and polycythemia vera. Patients with elevated red cell GSH levels were found to have increased levels of GSH-S, but normal GC-S activity. No change in the kinetic properties of the enzyme was observed. In studies of rabbits we found MB and DDS administration increased the GSH content of red cells both in vivo and in vitro. MB and DDS failed to affect GC-S, but produced a kinetic change in the second enzyme, GSH-S, lowering its K_m for γ-glutamylcysteine. Since the levels of this peptide in red cells is much lower than the K_m this increases the rate of the GSH-S reaction without affecting the amount of enzyme present. These studies indicate that the level of GSH in red cells is regulated by the activity of GSH-S. Pathophysiological alterations of the activity of this enzyme alter the level of GSH in erythrocytes.

33. Glucose Stimulation of Active Sodium Transport in the Human Jejunum. HENRY J. BINDER,* New Haven, Conn. (introduced by H. M. Spiro**).

Alternate theories have been advanced to explain the mechanism of the stimulation of sodium absorption in the human jejunum by glucose. One proposes active Na transport based on the Na gradient hypothesis associated with carrier-mediated Na entry into the epithelial cell, and the other suggests that glucose-stimulated Na movement in the absence of HCO_3 is not an active process but secondary to bulk water flow. To test these hypotheses 10 pieces of normal jejunum at laparotomy from four patients were placed in an Ussing chamber. Identical HCO_3-free, Cl-free 140 mM Na isethionate solutions bathed both the mucosa and serosa. Spontaneous transmural potential difference (PD)

and short-circuit current (I_{sc}) were monitored before and after the addition of 10 mM glucose. Without glucose mean (±SE) PD and I_{sc} were -1.4 ± 0.2 mv (mucosa negative) and 14 ± 2 $\mu a/cm^2$, respectively. Glucose caused an immediate and sustained increase of both PD and I_{sc} to -7.1 ± 0.5 mv and 81 ± 7 $\mu a/cm^2$. Simultaneous unidirectional Na fluxes (J^{Na}_{net}) with ^{22}Na and ^{24}Na under short-circuit conditions were determined on seven pieces of tissue from two patients. Before glucose addition J^{Na}_{net} was 0.6 ± 0.2 $\mu Eq/hr \cdot cm^2$ when the I_{sc} was 0.5 ± 0.1 $\mu Eq/hr \cdot cm^2$. Glucose increased both J^{Na}_{net} (2.5 ± 0.3 $\mu Eq/hr \cdot cm^2$) and I_{sc} (3.0 ± 0.2 $\mu Eq/hr \cdot cm^2$). These studies provide convincing evidence that active glucose-stimulated electrogenic Na transport is present in the human jejunum although they do not exclude the existence of other possible mechanisms. (Supported by grants from NIH and John A. Hartford Foundation.)

34. Control of Embryonic Cartilage Growth by Endogenous Cyclic Adenosine-3'5' Monophosphate. B. M. BIRCH,* H. K. DELCHER,* J. L. RENDALL,* AND H. E. LEBOVITZ,* Durham, N. C. (introduced by S. H. Appel).

We have shown that serum sulfation factor (SSF)-stimulated $^{35}SO_4$ incorporation into embryonic chick cartilage is partially inhibited by exogenous cyclic AMP (CAMP) in a dose-related manner. SSF stimulation of cartilage had no effect on tissue CAMP levels. The present study was designed to determine whether endogenous CAMP levels alter the response of chick embryonic cartilage to SSF. $^{35}SO_4$ incorporation into pelvic cartilage from 12- to 14-day chick embryos was determined using a modification of Hall's technique. Cartilage CAMP was assayed by the competitive protein-binding technique of Gilman. Glucagon and insulin did not effect $^{35}SO_4$ incorporation by cartilage, nor tissue CAMP levels in the presence or absence of SSF. Theophylline at 2.5 and 5 mM caused a dose-related inhibition of SSF-stimulated $^{35}SO_4$ incorporation (15 and 46%, respectively). Corresponding cartilage CAMP levels increased 150 and 292% above control values. A positive correlation existed between tissue CAMP levels and inhibition of $^{35}SO_4$ uptake. ATP, 5'AMP, and adenosine at 1 mM all significantly inhibited SSF-stimulated $^{35}SO_4$ incorporation into embryonic chick cartilage (29, 44, and 50%). Tissue CAMP levels rose in cartilages incubated with 1 mM ATP, 5'AMP, and adenosine (41, 52, and 62%). These adenosine compounds at 1 mM do not cross-react with our CAMP-specific protein kinase. Measurements of chromatographically isolated CAMP from cartilages incubated with the various agents confirmed the elevations noted in tissue extracts. This data supports the thesis that CAMP acts as a modulator of SSF action on cartilage. It also demonstrates that incubation with ATP, 5'AMP, and adenosine leads to elevated cartilage CAMP levels. (Research supported by grants from the NIH and VA.)

35. Pyrogen Production In Vitro by Lymphoid Tissue in Malignant Lymphoma. PHYLLIS BODEL, New Haven, Conn.

The mechanism of fever in malignant lymphoma is unknown. Endogenous pyrogen (EP) production by blood or tissue leukocytes has been clearly implicated as the cause of experimental fever in several animal models. Therefore blood, spleen, and lymph node cells from patients with lymphomas were examined for their capacity to release EP. Cell suspensions prepared from operative specimens were incubated for successive 24-hr periods in sterile, pyrogen-free tissue culture medium; blood leukocytes were incubated overnight. Supernates were cultured and injected into rabbits for pyrogen assay. Blood leukocytes from febrile or afebrile patients, like those from normal individuals, rarely produced EP spontaneously, although pyrogen release always occurred after a phagocytic stimulus. However, 5 of 10 preparations of spleen cells and 4 of 5 lymph node cell suspensions from patients with Hodgkin's disease released EP spontaneously. By contrast, no pyrogen was detected after comparable incubations of eight preparations of spleen cells from patients with splenomegaly resulting from various nonmalignant disorders. EP was produced in all instances after addition of heat-killed staphylococci. Pyrogen from tissues was clearly different from endotoxin, and appeared identical with leukocyte pyrogen. However, unlike blood leukocytes, spontaneously active spleens and lymph nodes from patients with lymphoma usually continued to produce EP for 48–72 hr. Production was suppressed by inhibitors of protein synthesis. Spontaneous pyrogen release did not correlate with (*a*) presence of fever in the patient, (*b*) pathological diagnosis of tissue infiltration, or (*c*) numbers of granulocytes in the tissues. Mononuclear leukocytes were clearly responsible for EP production by lymph node cell suspensions. These studies demonstrate for the first time spontaneous and prolonged production of EP by lymphoid tissue from patients with malignant disease.

36. Fractionation of Human Lymphocytes: Isolation of Two Populations Differing in Their Response to Concanavalin-A. DAVID BOLDT,* SR. ANN MARIE SKINNER,* AND STUART KORNFELD, St. Louis, Mo.

Lymphocytes represent a functionally heterogeneous population of cells despite their similar morphology. Techniques for separation of lymphocyte subclasses have been identified for several laboratory species, yet in man, fractionation of lymphocytes into functionally distinct subpopulations has proved to be more difficult. We have identified two human lymphocyte subpopulations differing in responsiveness to the plant mitogen concanavalin-A (Con-A). When peripheral blood lymphocytes (responsive to Con-A) are passed through a nylon column, a subpopulation of lymphocytes responsive to Con-A is removed and another subpopulation of cells relatively unresponsive to Con-A emerges from the column. Con-A-stimulated DNA synthesis is 6.5-fold greater and the percentage blast formation is 4- to 5-fold greater in the responsive vs. the unresponsive population. Mixing responsive with unresponsive cells fails to induce responsiveness in the latter indicating that a "helper" cell is not involved. The failure to respond to Con-A is specific for that mitogen since both populations respond equally to phytohemagglutinin and the pokeweed mitogen. Fluorescein-conjugated Con-A binds to all cells in both populations, the predominant pattern being homogeneous staining. Cap formation occurred in 10% of the responsive population vs. 2% in the unresponsive population. Binding studies with ^{131}I-labeled Con-A demonstrate that the responsive population possesses four times as many Con-A

receptors per cell while both cell populations bind the mitogen with the same affinity (Ka of 10–20 μg/ml). From these data we conclude that (*a*) nylon columns fractionate lymphocytes into distinct subpopulations, characterized by different abilities to respond to various plant mitogens; and (*b*) binding of a plant mitogen to the lymphocyte cell surface is necessary but not sufficient to induce blast transformation. (Research supported by grants from NIH.)

37. Starvation: Dissociation of Renin and Aldosterone and the Insensitive Nephron. PHILIP BOULTER,* RICHARD SPARK,* AND RONALD ARKY, Boston, Mass.

Early starvation is characterized by natriuresis, and subsequent carbohydrate refeeding by antinatriuresis. To investigate these phenomena, sequential analyses of plasma renin activity (PRA) and aldosterone secretory rate (ASR) were performed in eight healthy obese subjects, after equilibration, while undergoing 10- and 15-day periods of fasting with carbohydrate refeeding on a fixed 50 mEq Na — 87 mEq K intake. During early fasting, urinary sodium excretion (U_{Na}) increased from a mean control of 43.2 to a peak of 83.7 mEq/24° while mean ASR increased from 393 to 636 μg/24°. PRA was dissociated from the rising ASR, decreasing from a mean control of 2.6–1.4 ng/ml per hr. Carbohydrate refeeding induced an abrupt antinatriuresis, mean U_{Na} of 1.4 mEq/24°, and a fall in mean ASR from 686 to 323 μg/24°. With refeeding, PRA was again dissociated from the falling ASR by increasing from 2.71 to 7.68 ng/ml per hr. Persistent natriuresis occurring despite rising ASR suggested tubular insensitivity to mineralo-corticoid in early fasting. Administration of fludrocortisone (0.6 mg/day) for 2 days during early fasting failed to prevent either the natriuresis or the rise in ASR, whereas a similar dose of fludrocortisone administered on days 11 and 12 of fasting resulted in prompt antinatriuresis and suppression of endogenous aldosterone secretion. The natriuresis of early starvation and the antinatriuresis of carbohydrate refeeding are characterized by dissociations of the renin-aldosterone system. ASR rises while PRA falls during natriuresis; ARS falls and PRA rises with antinatriuresis. Early starvation is further characterized by renal tubular insensitivity to endogenous and exogenous mineralo-corticoids. Restoration of tubular sensitivity to mineralo-corticoid is demonstrable as fasting progresses. These data suggest that renal tubular utilization of available metabolic substrates is a major determinant of mineralocorticoid action. (Research supported by NIH.)

38. On the Nature of the Circulating Natriuretic Factor in Chronic Uremia. JACQUES BOURGOIGNIE,* R. WILLIAM SCHMIDT,* KUO HWANG,* TEWFIK NAWAR,* SAULO KLAHR, LEWIS CHASE,* AND NEAL S. BRICKER, St. Louis, Mo.

The striking and progressive increase in natriuresis per nephron that occurs in patients with advancing renal disease on a normal salt intake suggests that natriuretic forces are mobilized increasingly as GFR falls. Thus, if a natriuretic hormone is among these forces, the opportunity for its detection might be enhanced greatly in patients with advanced uremia. We have described a gel filtration fraction of uremic serum that inhibits sodum transport in vitro and is natriuretic in the rat. It is unclear, however, whether this factor is a physiologic mediator of sodium excretion or a uremic toxin retained in blood because of failure of excretion. The present studies were performed to further characterize this material. The natriuretic effects of the fraction could not be reproduced by the uremic "toxins" guanidinosuccinic acid or methylguanidine. On the other hand, incubation of the fraction with leucine aminopeptidase abolished its natriuretic activity suggesting that the active factor might be a peptide. In addition, the active fraction increased 3'5' cyclic AMP excretion. Phosphate excretion also increased; however, the active material does not appear to be related to parathyroid hormone. Nor does it appear to be a prostaglandin, angiotensin, thyrocalcitonin, or vasopressin. The relationship of the fraction to sodium balance is suggested by the observation that no natriuretic activity could be detected in the serum fraction from severely uremic patients with marked edema formation. Finally, in uremic dogs in which natriuresis per nephron was prevented by decreasing sodium intake in proportion to GFR, no natriuretic activity was found. The observations thus are consistent with the view that the natriuretic factor of uremia may be a peptide hormone with a physiologic role.

39. Cyclic Adenosine Monophosphate (cAMP) and Leukocyte Function: Effects of Choleragen. H. R. BOURNE,* L. M. LICHTENSTEIN, AND C. S. HENNEY,* San Francisco, Calif., and Baltimore, Md.

Choleragen, the exotoxin of *Vibrio cholerae,* increases cAMP content of gut and other tissues. We tested the effect of choleragen on two leukocyte functions thought to be regulated by cAMP in vitro: (*a*) IgE-mediated release of histamine from leukocytes of allergic donors (H-release); (*b*) Cytolysis of allogeneic mast cells by splenic lymphocytes obtained from specifically immunized mice (lymphocyte cytolytic activity, LCA). Choleragen, 10 ng/ml, inhibited both H-release and LCA by 75–90% and caused a parallel four- to sevenfold rise in cAMP content of human leukocytes and mouse splenic lymphocytes. These effects of choleragen were competitively blocked by a specific cholera antitoxin and choleragenoid. Choleragen's effects were undetectable before 30 min, but increased thereafter to a plateau at 60–90 min. The rise in cAMP was associated with increased basal adenyl cyclase activity, and was not prevented by cycloheximide, 1×10^{-3} M. Antitoxin and choleragenoid completely prevented the rise in cAMP, measured at 60–90 min, only if added to cells simultaneously with choleragen; the block was undetectable if the antagonists were added at 10 min or later. Also, repeated washing of leukocytes after 10 min of exposure to choleragen (at either 0° or 37°C) failed to prevent the subsequent cAMP accumulation, the increase in adenyl cyclase activity, or the inhibition of histamine release. The choleragen effect on leukocyte cAMP contrasted with those of prostaglandins, catecholamines, and histamine, which were rapid (maximal at 10 min) and easily reversed by washing. These results suggest that choleragen stimulates cAMP production by a unique mechanism involving rapid and irreversible binding to the leukocyte, followed by a delayed stimulation of adenyl cyclase. Further, they confirm previous reports implicating leukocyte cAMP as an inhibitor

of both immediate (H-release) and delayed (LCA) hypersensitivity in vitro. (Research supported by NIH grants.)

40. Clinical Studies on the Luteinizing Hormone and Follicular-Stimulating Hormone Releasing Activity of pGLU-HIS-TRP-SER-TYR-GLY-ARG-LEU-PRO-GLY-NH_2. C. Y. Bowers,* J. Malacara,* F. Gomez,* S. Hernandez,* J. Chang,* and K. Folkers,* New Orleans, La., Leon, Mexico, and Austin, Tex. (introduced by G. E. Burch).

To study the problem of whether DP is the physiological regulator of both luteinizing hormone (LH) and follicular-stimulating hormone (FSH) or only LH, serum LH and FSH levels were measured before and 15, 30, 45, and 60 min after a single intravenous injection of 50 or 200 μg of synthetic DP in 30 normal men (NM), 30 normal women (NW), 8 postmenopausal women (PW) before and during oral medication with 5 mg diethylstilbesterol (DES) daily, 4 NM before and during medication with 100 mg testosterone (T) given intramuscularly five times daily, and 5 women with amenorrhea (A). The greatest mean Δ mIU/ml rises in NW studied three times at monthly intervals were: LH 90, 62, 67, and FSH 18, 3.5, 5.8. Largest Δ FSH rises (mean 37) were obtained in 13 of 89 tests performed in these NW and the associated mean Δ LH rise was >100. Nine of these 13 NW were tested near the midcycle. The other Δ mean rise results were: NM LH 55 and FSH 3.5; PW LH 126 and FSH 30 and after 5 wk of DES Rx LH was 36 and FSH 4.4. The three daily mean values of the NM before T were LH 41, 50, 50, and FSH 3.6, 4.9, 2.9, and during T Rx were LH 24, 34, 34, 54, 95, and FSH 0.6, 1.2, 0.9, 0, 0. The mean Δ LH rise in the women with A was 109 and Δ FSH rise 8.4. In conclusion, DP releases both LH and FSH in NW and NM. The LH and FSH surge at ovulation could be explained solely by the LH-FSH activity of the DP; thus, it appears to be the hypothalamic-ovulation-hormone. However, because of the consistently high LH and low FSH activity of the DP, it is unlikely that DP regulates both LH and FSH except at ovulation. Thus the DP seems to be physiologically only LH-RH.

41. Identification of Puberty by Synchronization of Luteinizing Hormone Release with Sleep. Robert M. Boyar,* Jordan Finkelstein,* Howard Roffwarg,* and Leon Hellman,** New York.

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured in plasma by radioimmunoassay every 20 min for 24 hr in 12 normal children (ages 8–15) in various stages of sexual maturation and 5 normal adult men (ages 21–40). Polygraphic monitoring was carried out simultaneously to precisely identify sleep onset, wakefulness, and sleep stages. Four prepubertal children showed LH concentrations in the prepubertal range (2.0–4.0 mIU/ml) throughout the 24 hr period. Four boys in early puberty showed LH secretory patterns during the day that were indistinguishable from prepubertal children (2.5–4.5 mIU/ml); however, with onset of night sleep, there was an immediate increase of LH secretory activity that resulted in mean LH concentrations two- to fourfold higher (7.1–10.1 mIU/ml) than during wakefulness ($P<0.001$). By experimentally delaying sleep onset, synchronization of this LH secretory "program" with actual sleep was clearly demonstrated. The number of LH secretory episodes during either night or day sleep corresponded to the number of sleep cycles. Sleep LH secretory activity was independent of FSH which did not show any sleep synchronization. Two children with complete sexual maturation showed LH secretory episodes indistinguishable from the 24-hr LH patterns of five normal adult men with no significant difference between sleep and awake periods. These data show that (*a*) in early puberty, LH secretory activity becomes synchronized with sleep; (*b*) this CNS "program" for LH release is lost with advancing sexual maturation; (*c*) there is absence of concurrence between LH and FSH release throughout puberty suggesting different control mechanisms. This finding may constitute a new biological index for early detection of puberty.

42. Intracellular Potassium: the Regulator of Aldosterone Production. John E. Boyd* and Patrick J. Mulrow, New Haven, Conn.

Small acute increases in plasma [K^+] stimulate aldosterone secretion, while chronic K loading of rats increases secretion, although plasma [K^+], ACTH, and renin activity may be unchanged (Boyd et al. 1971. *Endocrinology.* **88:** 556). Using the in vitro rat adrenal gland technique, we have investigated this problem further and have shown that an increase in [K^+] of 0.35 mEq/liter (3.85–4.20) in the incubation media stimulated aldosterone production 43% ($P<0.01$) without affecting gluco-corticoid production (B). It seems unlikely that such small changes in E.C.F. [K^+] altered membrane potential and hence steroid secretion, rather, the data suggest intracellular K^+ is the regulator of aldosterone production. The effect of K^+ requires Na-K-ATPase activity. Procedures which inhibit Na-K-ATPase reduce aldosterone production. The absence of Na^+ or marked increase in [K^+] to 24 mEq/liter in the incubation media reduce aldosterone production. The addition of ouabain (5×10^{-4} M), which is known to reduce rat adrenal cell K^+, blocks the stimulation of aldosterone production by increased [K^+], 1 vs. 8 mEq [K^+], % Δ aldo 90, with ouabain 13, $P<0.01$. Increasing the [K^+] further partially overcomes the ouabain inhibition. Thallium, which has a higher affinity than K^+ for Na-K-ATPase, also inhibits aldosterone production. None of these manipulations have a consistent effect on B. Furthermore, the activity of the Na-K-ATPase is greater in the outer portion (containing the zona glomerulosa cells) than the inner portion of the adrenal gland (1.0 ± 0.14 SE vs. 0.44 ± 0.16 μM Pi/mg protein per hr $P<0.01$). Addition of ACTH in vitro stimulates aldosterone and B production; ouabain inhibits the aldosterone but not the B stimulation (% Δ aldo and B, 24 and 716, with ouabain -32, 573). Similar results were found when cyclic 3′5′ AMP was added to the media (% Δ aldo and B, 21, 432, with ouabain -36, 403). Conclusion: aldosterone production in the zona glomerulosa cell is sensitive to changes in intracellular K^+ while steroidogenesis by the fasciculata-reticularis cells is not. The site of action of intracellular K^+ appears to be beyond 3′5′ cyclic AMP formation.

43. The Influence of Inorganic Phosphate (Pi) on Red Cell (RBC) Metabolism: In Vitro and In Vivo Studies. MICHAEL C. BRAIN, ROBERT T. CARD,* AND NORMAN L. JONES,* Hamilton, Ontario, Canada.

Pathological changes in plasma Pi are known to affect RBC adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) levels. The effects of Pi concentration near to the physiological range have been studied. Normal RBC were incubated at 20% hematocrit in isotonic bicarbonate-glucose buffer containing 0.5–5.0 mM Pi in air and 5% CO_2 at controlled pH 7.40±0.01 for 5 hr. Levels of RBC glucose-6-phosphate (G-6-P), fructose-1, 6-diphosphate (FDP), triose, DPG, 3-phosphoglycerate (3-PG), glucose, lactate, and ATP were measured every 60 min. After 3 hr incubation at concentrations of Pi between 1.5 and 5.0 mM, total triose (FDP and triose) rose 10-fold, (correlation to Pi concentration; $r = 0.9$), glucose consumption twofold, and DPG less than 5%. Pyruvate (0.2 mM) addition led to a rapid sustained fall in total triose and a rise in DPG (mean 0.7 mM/liter RBC), and transient rises in 3-PG and G-6-P. The intravenous infusion of 20–75 mmoles of isotonic phosphate (pH 7.4) into male volunteers resulted in a two- to threefold rise in plasma Pi (control 1.0±0.3 mM), a 10–20% rise in DPG, but no rise in total triose. Sustained muscular exercise at 80% of $\dot{V}O_{2\,max}$ on a bicycle ergometer resulted in an 80% rise in plasma Pi and a 10% rise in DPG. A rise in Pi concentration above 1.5 mM influences RBC metabolism and DPG levels, probably through phosphofructokinase, and may enhance tissue oxygen delivery and, by shifting hemoglobin-oxygen dissociation, may account for the "anemia of childhood" when lowered levels of hemoglobin coincide with the hyperphosphatemia of growing children. (Research supported by a grant from M.R.C. Canada.)

44. Weight Gain and Work Efficiency in Normal Volunteers. GEORGE A. BRAY, BRIAN J. WHIPP,* AND SANKAR KOYAL,* Torrance, Calif.

This study was designed to investigate overeating and the efficiency of coupling food energy to muscular work. Four normal males were studied while eating 1800 cal/m² and again after a 28 day supplement of 4000 cal/day. Subjects performed cycle ergometer exercise to steady state at "0," 25, 50, 75, and 100 w and also a step test. Signals from a pneumotachograph and rapidly responding O_2 and CO_2 analyzers were processed by a digital minicomputer. Minute ventilation ($\dot{V}_E$), CO_2 production ($\dot{V}CO_2$), O_2 uptake ($\dot{V}O_2$), respiratory exchange ratio (R), and alveolar O_2 and CO_2 tensions were calculated and displayed breath-by-breath. Weight gain ranged from 3 to 10.8 kg. Assuming that half the added weight was fat, only 12–44% of total caloric intake was stored. Distance walked daily did not increase during the weight gain. Fecal mass rose 50–100% (106–173 g/day and 117–275 g/day). $\dot{V}O_2$ at rest increased slightly. $\dot{V}O_2$ was higher at each work rate on the cycle ergometer following weight gain in proportion to the reduced R. Resulting work efficiency (Whipp and Wasserman. 1969. *J. Appl. Physiol.* **26**: 644.) approximated 30% before and after weight gain. This efficiency was unaltered by ingestion of a 1000 cal breakfast 30 min before cycling. $\dot{V}_E$ increased in proportion to $\dot{V}CO_2$. Changes in these functions after weight gain were attributable to changes of R. Efficiency during the step test did not deteriorate with weight gain. We conclude that the ingested calories, which cannot be accounted for by accumulated weight, were not dissipated by decreased efficiency of coupling oxidation to muscular work.

45. Direct Assessment of Pressures, Flows, and Resistances Across Single Glomerular Units of the Rat Kidney. B. M. BRENNER,* J. TROY,* R. MACINNES,* AND T. DAUGHARTY,* San Francisco, Calif. (introduced by T. B. Bradley, Jr.).

Pressures, flows, and resistances were determined for accessible glomerular capillaries and single afferent (AA) and efferent (EA) arterioles on the renal surface of adult Munich-Wistar rats. Using a servo-null device, mean pressures were recorded in glomerular ($\overline{P_{GC}}$) and peritubular (P_C) capillaries. From measurements of nephron GFR and surface filtration fraction (SFF), glomerular (GPF = GFR/SFF) and EA plasma flows (EAPF = GPF − GFR) were calculated. From knowledge of the pressure drop (ΔP) across AA (mean arterial presure, [$\overline{AP}$], $-\overline{P_{GC}}$) and EA ($\overline{P_{GC}} - P_C$) and blood flow through these single vessels (GPF or EAPF/1-Hct), resistances to flow through AA (R_A) and EA (R_E) were calculated. In 11 hydropenic rats ($AP > 150$ cmH_2O), $\overline{P_{GC}}$ and $\overline{P_{GC}}/\overline{AP}$ averaged 62 cmH_2O ±2 SE and 0.38±0.01, respectively, indicating a large ΔP across AA. GPF, and therefore AAPF, averaged 71 nl/min ±6. R_A averaged $4.5 \pm 0.4 \times 10^{10}$ dynes · sec · cm^{-5}. P_C and EAPF averaged 9.4 cmH_2O±0.6 and 47 nl/min±6. At these flows since ΔP across EA was less than across AA, R_E was likewise less than R_A, averaging $2.9 \pm 0.2 \times 10^{10}$ dynes · sec · cm^{-5}, or 40% of the total arteriolar resistance ($R_T = R_A + R_E$). Graded reductions in renal perfusion pressure (to $\overline{AP}$ 125, 100, < 85 cmH_2O) resulted in progressive declines in $\overline{P_{GC}}$ (59, 52, 46 cmH_2O) which were disproportionately less than in AP; hence $\overline{P_{GC}}/\overline{AP}$ increased (0.44, 0.49, 0.59). This tendency to preserve $\overline{P_{GC}}$ (and therefore GFR) resulted from progressive reductions in R_A (3.2, 2.4, 2.1×10^{10} dynes · sec · cm^{-5}) while R_E remained nearly constant (2.6, 2.4, 2.9). Thus, the AA serves as the principal variable resistor in maintaining $\overline{P_{GC}}$ in the face of large reductions in perfusion pressure. (Research supported by NIH Grant AM13888 and VA Grant 01/1073.1.)

46. Effects of Elevation of Plasma Sodium Concentration on Tubular Reabsorption of Sodium during Meralluride or Ethacrynic Acid Diuresis. EMANUEL H. BRESLER* AND KRISTIN T. NIELSEN,* New Orleans, La. (introduced by George E. Burch**).

Clearance studies at varying levels of P_{Na} in the hypernatremic range have shown that as P_{Na} is elevated T_{Na} is proportionately elevated. Recently, however, dogs given ethacrynic acid and chlorothiazide have been reported not to display this relationship, suggesting that this type of glomerulotubular balance does not hold for the proximal tubule. In this study, 19 hydropenic anesthetized dogs were given meralluride or ethacrynic acid after varying degrees of

stable hypernatremia had been produced by saline infusions. Urine collections were obtained via surgically-introduced ureteral catheters. Glomerular filtration (GFR) was determined by either exogenous creatinine or inulin clearance. Clearance periods were selected during the height of diuresis. The known covariance of T_{Na} and GFR was factored out by plotting T_{Na}/GFR against P_{Na}. Although the variability in diuretic response tended to obscure the previously reported relationship between T_{Na} and P_{Na} found in nondiuretic animals, a significant linear relationship between T_{Na}/GFR and P_{Na} remains ($P < 0.001$). In addition, when T_{Na} was plotted against GFR using different symbols for points obtained at $P_{Na} < 162$ mEq/liter and those obtained at $P_{Na} > 171$ mEq/liter two regressions were obtained which were found to be significantly different ($P < 0.001$). When a similar plot was made for T_{H_2O} against GFR these two regressions were not found to be significantly different. These results show that T_{Na} remains proportional to P_{Na} even during strong meralluride or ethacrynic acid diuresis.

47. Biologic Action of 1,25-Dihydroxycholecalciferol in Uremic Man. ARNOLD S. BRICKMAN,* JACK W. COBURN,* AND ANTHONY W. NORMAN,* Los Angeles and Riverside, Calif. (introduced by Seymour Dayton).

Considerable evidence indicates that 1,25-dihydroxycholecalciferol (1,25-diOHCC) is the active form of cholecalciferol (vitamin D_3). The kidney is the only known organ capable of producing this hormone from its precurser, 25-hydroxycholecalciferol. Thus, destruction of renal parenchyma in chronic renal disease might impair the production of 1,25-diOHCC and be responsible for "vitamin D resistance" in uremia. The possibility that exogenously administered 1,25-diOHCC may bypass this defect and reverse certain abnormalities of calcium metabolism in uremia prompted a study of its effects in uremic man. 1,25-diOHCC was given to three uremic patients in doses of 50–100 U/day (1 U = 0.025 μg) for 3–7 days. During metabolic balance study, one anephric patient, dialyzed thrice weekly and with overt secondary hyperparathyroidism, received 100 U/day for 6 days. Serum calcium increased by 5 mg/100 ml and phosphorus by 1.5 mg/100 ml during treatment. Absorption of ^{47}Ca increased from 24 to 47% (normal range for his dietary calcium intake, 17–37%), and mean fecal calcium fell from 740 to 550 mg/day. Another patient, with stable chronic uremia, received 100 U/day for 7 days; serum calcium increased from 6.4 to 8.5 mg/100 ml and phosphorus from 6.6 to 7.5 mg/100 ml; ^{47}Ca absorption was 35%, compared with 18±6% (mean ± SD) in all uremics. A third patient, undergoing regular hemodialysis, showed no response to 50 U/day for 3 days. Thus, extremely small amounts of 1,25-diOHCC may augment calcium absorption and raise serum calcium level in uremic man. The latter may occur, in part, due to mobilization of bone mineral. These results are consistent with the theory that impaired production of 1,25-diOHCC in uremia may account for certain abnormalities of calcium homeostasis. This agent may hold future promise in the management of disordered calcium metabolism in uremia. (Research supported by USPHS grant AM14750 and Contract PH 43-68-1040).

48. Factors Influencing Gelation of Hemoglobin S. ROBIN W. BRIEHL, Bronx, N. Y.

Factors which inhibit or enhance gelation of hemoglobin S may reflect precipitating causes of sickle crises and may also offer information on gel structure and specific intermolecular interactions. Hemolysates of blood from homozygous S patients were stripped of 2,3-diphosphoglycerate (DPG) on Sephadex G-25, dialyzed into bis Tris [bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane] or potassium phosphate buffer, deoxygenated in tonometers with absorption cells and viscometers attached, and studied for viscosity as a function of increasing temperature up to the gelation temperature. Deoxygenation was carried out with nitrogen followed by anaerobic addition of small amounts of sodium dithionite to ensure total absence of oxyhemoglobin. Hemoglobin concentrations were 10–18 mM (heme). End points were sharp, the sol-gel transition occurring wholly within less than 1°. The solutions were then subject to change in DPG concentration, pH, ionic strength, or buffer concentration anaerobically and end points determined again. Increase in DPG from 0 to 6 mM lowered end points by 4° to 9° in 0.05 M bis Tris with 0.01 or 0.1 M NaCl. Decrease in pH lowered and increase raised the temperature end points. In potassium phosphate increase in buffer from 0.1 to 1 M lowered end points. However, increase in NaCl from 0.1 to 1 M raised end points. Therefore, DPG, inorganic phosphate, and increasing acidity favor gelation whereas increasing ionic strength alone inhibits it. Thus the region on the molecular dyad where DPG binds may be concerned in intermolecular interactions; and the presence of charge interactions is supported (in addition to hydrophobic interactions). If symmetry is retained in the helical or rod-like structures of the gel, the molecular dyads lie perpendicular to the cylinder axis; such symmetry restricts the possible locations of DPG in the gel helix or rod. (Research supported by a grant from the American Heart Association.)

49. Neural Control of the Metabolic and Hormonal Responses to Glucopenic Stress in Man. ROBERT G. BRODOWS,* F. XAVIER PI-SUNYER,* DONALD S. SCHALCH,* AND ROBERT G. CAMPBELL,* Rochester, N. Y. and New York. (introduced by Christine Waterhouse**).

The effect of autonomic denervation on the metabolic and hormonal responses during cellular glucopenic-induced stress has been examined. 2-Deoxy-glucose (2 DG), a competitive inhibitor of glucose metabolism, was administered intravenously to normal volunteers (N = 9) and to two patients, one with complete cervical cord transection (C-6) and another with idiopathic orthostatic hypotension, anhidrosis, and mild carbohydrate intolerance. Before, during, and following a 20 min infusion of 2 DG (50 mg/kg) plasma concentrations of free fatty acids (FFA), glycerol, lactate, total catecholamines, glucose, immunoreactive insulin (IRI), human growth hormone (HGH), and cortisol were determined for periods up to 150 min. In control subjects, the initial elevation of FFA, glycerol, glucose, HGH, and cortisol corresponded with the rise in total catecholamines, with maximal levels attained at 40 min postinfusion; lactate rose at a slower rate, reaching peak levels at 160 min. No change in IRI was noted. In contrast, 2 DG-induced glucopenic stress in the autonomic

denervated subjects was characterized by no detectable catecholamine release or rise in FFA, glycerol, glucose, lactate, or IRI. However, a twofold increase of HGH release above the mean maximal response of controls was noted in the cord-sectioned patient, while no significant rise of HGH was seen in the patient with idiopathic orthostatic hypotension. Cortisol responsiveness in both patients was no different than controls. Conclusion: In man, the integrity of the autonomic nervous system is essential for both the lipolytic and glycogenolytic response to glucopenic-induced stress. It is unlikely that HGH and cortisol together or independently play a significant immediate counter-regulatory role during periods of sudden, carbohydrate deprivation. (Research supported by grants from NIH and Nutrition Foundation.)

50. Augmentation of Leukemic Lymphocyte Reactivity to Phytohemagglutinin by Salivary Receptor. JEROME I. BRODY, Philadelphia, Pa.

The purpose of this investigation was to determine whether the response of the leukemic lymphocyte to phytohemagglutinin (PHA), restricted because of membrane receptor deficit, could be augmented by providing additional binding sites for the mitogen from an external source. To delineate the feasibility of such a maneuver, normal peripheral blood lymphocytes were incubated with serial concentrations of Pronase, a sialoglycoprotein-releasing enzyme, until, under controlled culture conditions, their Tdr-^{14}C incorporation after PHA stimulation dropped to 50% of that obtained before enzyme treatment, but with cell viability maintained. Lyophilized saliva (LS), an acknowledged source of PHA binder, with potency standardized by hemagglutination inhibition assay, was added to a portion of enzyme-altered lymphocytes for a set period, the cells washed, and these and the portion not incubated with LS placed in culture with PHA. Duplicate cultures of leukemic lymphocytes from patients with chronic lymphocytic leukemia were also cultured with PHA, one vial holding plain cells and the other lymphocytes previously incubated with lyophilate as described. Leukemic lymphocytes never were exposed to Pronase. The diminution of Tdr-^{14}C incorporation by normal, enzyme-treated cells was reversed by incubation of these lymphocytes with lyophilized saliva. More significantly, salivary receptor doubled PHA reactivity of leukemic lymphocytes in terms of their ability to synthesize DNA. These observations indicate that, for the first time, a deficient number of PHA receptors on the neoplastic lymphocyte may be augmented by an external substance with sufficient complementary surface binding to permit increased synthetic activity of the leukemic cell. This modification of the anomalous leukemic cell membrane may provide a logical base for further attempts at making this inherently immunoincompetent cell more amenable to immunogenic stimuli and a better participant in host defense. (Research supported by NIH grants.)

51. Hepatic and Splenic Involvement in Lymphoma. S. FRED BRUNK,* HRAIR P. GULESSERIAN,* ROBERT L. GIVLER,* AND ROBERT T. GUTHRIE,* Iowa City, Iowa (introduced by William R. Wilson**).

Attempted cure of lymphoma by radiation therapy requires accurate staging. This study was designed to determine the incidence of hepatic and splenic involvement in lymphomas. 130 patients with lymphomas had exploratory laparotomy, splenectomy, and biopsy of liver, bone marrow, and lymph nodes. 88 patients had Hodgkin's disease whereas 42 patients had non-Hodgkin's lymphoma. Splenic involvement occurred in 4 of 23 patients with clinical stage I Hodgkin's disease, in 13 of 27 with stage II disease, 22 of 35 with stage III disease, and in 1 of 3 patients with stage IV disease. The liver was not involved in stage I or II Hodgkin's patients; in stage III, 11 of 35 patients had involvement, and in stage IV, 1 of 3 patients had hepatic involvement. In non-Hodgkin's lymphoma none of the 12 patients with stage I or II disease had hepatic or splenic involvement. In stage III, 18 of 28 had involvement of the spleen and 7 of 28 had involvement of the liver. In stage IV, 1 of 2 patients had involvement of the liver and spleen. 32% of Hodgkin's patients and 16% of non-Hodgkin's lymphoma patients had their stage changed as a result of exploratory laparotomy. Whether staging by laparotomy is of benefit to patients with stage I or II disease is uncertain. If the spleen is not removed surgically in patients with stage I or II disease, the spleen should be irradiated along with the nodes in the upper abdomen. In stage III disease, exploratory laparotomy provides valuable information about areas of involvement and permits more carefully planned radiation therapy. (Research supported by grants from ACS, Iowa Division, Inc., and NIH grant 5T01 HE5577-10.)

52. Evidence for Multiphasic Release of Postheparin Lipolytic Activity. JOHN D. BRUNZELL,* NEALE D. SMITH,* DANIEL PORTE, JR., AND EDWIN L. BIERMAN, Seattle, Wash.

A group of hypertriglyceridemic subjects with untreated fasting hyperglycemia has been characterized by normal postheparin lipolytic activity (PHLA) after a standard dose of heparin (380 U/m^2), but an inability to maintain PHLA levels during a maximal heparin infusion (4560 U/m^2 per hr). PHLA levels during the early phase (0–150′) of the infusion were similar to those of nondiabetic hypertriglyceridemic subjects (60′ peak: diabetic: 0.647±0.206, n = 13; nondiabetic: 0.621±0.198, n = 17; μEq FFA/ml per min, $\bar{x}\pm$SD). After 150′ there was a dissociation of PHLA levels between the two groups with a lower steady-state PHLA attained in diabetics (per cent decrease from 60′: diabetics: 27±17; nondiabetics: 12±11; $P < 0.02$). The per cent decrease was directly related to fasting glucose in diabetics ($r = 0.77$, $P < 0.001$). Diabetics recently treated with insulin (1–3 wk, n = 10) had no change in the degree of PHLA decrease (37%±10) or triglyceride levels, despite improved glucose levels. When treated for longer periods of time, their TG levels approached normal. In four such subjects the PHLA response was then identical with that of the nondiabetics (per cent decrease: 12±6), suggesting that deficit in later phase of PHLA release was due to insulin deficiency. To investigate the difference between PHLA released early and late, paired heparin infusions were given to dogs (n = 3) with and without pretreatment with cycloheximide. No consistent difference in PHLA was noted between control and cycloheximide-treated dogs during the early phase of heparin infusion (0–150′), but during the later phase, all cycloheximide-treated dogs reached a lower steady-state PHLA com-

pared with controls (mean per cent decrease from control: 37%). Thus, there appear to be two phases of PHLA release: (*a*) a late phase presumably dependent on continued protein synthesis which is impaired in untreated hypertriglyceridemic, hyperglycemic subjects, and (*b*) an early phase which is normal in these subjects and independent of new protein synthesis. (Research supported by VA and NIH.)

53. Antibody Response to Gonococcal Pili in Patients with Gonorrhea. T. M. BUCHANAN,* J. SWANSON,* AND E. C. GOTSCHLICH, New York.

Gonococcal pili are filamentous structures present on the surface of colonial variants T1 and T2 of *Neisseria gonorrhoeae*. Variants T1 and T2 have produced experimental infections in human volunteers, whereas colonial variants T3 and T4 devoid of pili have not. Using chick embryos as an animal model, we found the same correlation between colonial type and infectivity. To delineate their immunochemistry and function, gonococcal pili were purified by repeated precipitation with 0.1 M $MgCl_2$ and dissolution in 0.1 M Tris buffer pH 8 containing 0.01 M NaN_3. This product contained only pili when examined by electron microscopy and migrated as a single band in urea polyacrylamide gel electrophoresis. Fluorescein conjugated rabbit antibody against purified pili stained the surface of variants T1 and T2 in all of 18 strains tested. No fluorescence was observed with variants T3 and T4 of gonococci, or with strains of pilated meningococci, nonpathogenic *Neisseria spp.*, and *Escherichia coli*. An antigen-binding assay using ^{125}I-labeled gonococcal pili was developed to detect anti-pili antibody. Sera were tested from 12 persons with no history of gonorrhea and from 41 patients with the following conditions: gonorrhea (28), nasopharyngeal carriage of meningococci (5), meningococcal meningitis (3), nonspecific urethritis (2), and acute illnesses other than gonorrhea (3). The mean antigen binding by serum from the patients with gonorrhea was significantly higher than the mean for the controls ($P < 0.001$) over a wide range of dilutions. The amount of antigen bound by serum from 22 of 28 patients with gonorrhea was higher than the highest amount bound by any of the controls ($P < 0.001$). This test may prove useful for diagnosing gonorrhea and detecting asymptomatic gonococcal infections. (Research supported by grants from the NIH.)

54. Renal Tubule Calcium Reabsorption after Parathyroidectomy. JOHN BUERKERT,* DANIEL MARCUS,* AND REX JAMISON,* Stanford, Calif. (introduced by John Leutscher**).

Previous studies have shown that parathyroid extract (PTE) enhances calcium reabsorption in the distal nephron. Uncertainty about the nature of PTE caused us to examine parathyroid influence by studying tubule function in 15 normal rats and 17 rats 24–48 hr after parathyroidectomy. Samples were obtained by micropuncture: calcium was measured by helium-glow photometry and inulin-^{14}C by scintillation counting. After parathyroidectomy, plasma ultrafilterable calcium decreased from 3.02±0.06 to 2.00±0.06 mEq/liter, GFR remained unchanged, and urinary excretion of calcium and sodium increased significantly. In normal rats, the proximal tubule fluid-to-ultrafilterable (TF/UF) calcium was 1.06±0.024 (significantly > 1, $P = 0.025$); TF/UF calcium in the distal tubule was 0.31±0.038. Fractional calcium reabsorption by the proximal tubule was 53.7±2.8% and in the distal tubule, 93.7±0.05%. After parathyroidectomy, TF/UF calcium was unchanged in the proximal but below normal in the distal tubule, 0.21±0.02, ($P < 0.025$). Unexpectedly, fractional calcium reabsorption by the proximal tubule was strikingly reduced to 29.3±5.4% ($P < 0.001$). Sodium reabsorption was similarly diminished from 57.1±0.22% to 32.7±0.49% ($P < 0.005$), although dietary intake had not increased. Since the filtered load of sodium, F_{Na}, unlike F_{Ca}, did not decrease, the calcium-sodium-linked reabsorptive mechanism in the proximal tubule was partially uncoupled. The increment in calcium delivered to Henle's loop in parathyroidectomized rats was completely reabsorbed, as indicated by normal fractional reabsorption in the distal tubule, 95.7±0.6%. These experiments complement previous work by suggesting the enhanced urinary calcium excretion which occurs in the absence of parathyroid hormone reflects diminished calcium reabsorption by the collecting tubule. However they also reveal profound suppression in absolute reabsorption of calcium and sodium by the proximal tubule, and probable enhancement in absolute reabsorption of calcium by Henle's loop. (Research supported by grants from AHA and NIH.)

55. Inhibition of Renin Secretion by Propranolol: a Specific Treatment for Renal Hypertension? FRITZ R. BÜHLER,* LESLIE BAER,* EDWIN D. VAUGHAN,* HANS R. BRUNNER,* AND JOHN H. LARAGH, New York.

Renin secretion may be stimulated through an autonomic pathway involving intrarenal beta-receptors. Propranolol has been shown to suppress renin secretion. In this study, propranolol was administered to 32 patients (28 with essential and 4 with renovascular hypertension), characterized into low (n = 7), normal (n = 20), and high renin (n = 6) categories. Their mean arterial pressures were 154±18, 134±16 and 151±20 mm Hg (mean ±SD). 40 to 540 mg propranolol were given daily in 15 studies under metabolic ward conditions for 4–6 days and in 19 other studies for up to 10 months. In all studies, propranolol lowered plasma renin activity markedly and often to well below the normal range. The greatest fall was observed in high renin patients (84%) as compared with 60 and 47% in the other groups. Mean pressure fell by 22% in high as compared with only 12% in normal and 7% in low renin patients. The mean pressure dropped by more than 15 mm Hg in 5 of 6 high renin patients as opposed to 11 of 20, and 3 of 7 in the other groups. Irrespective of control renin (two high, two normal) large fall in pressure (mean = 23 mm Hg) occurred in all renovascular hypertensive patients. Propranolol did not consistently produce commensurate reductions in aldosterone. The data indicate a hyperkalemic effect of the drug, operating directly to raise aldosterone and suppress renin secretion. The results suggest a relationship between pre-existing renin levels and the hypotensive effect of propranolol. It may constitute a diagnostic indicator and an effective treatment for hypertensive disorders which involve increased renin recretion. Lack of response suggests low renin or a prerenal type of

hypertension. (Research supported by USPHS Grant 1-P17-HL-14148-01.)

56. Enterobacterial Common Antigen-Induced Lymphocyte Reactivity in Inflammatory Bowel Disease. DAVID M. BULL* AND THOMAS F. IGNACZAK,* Columbus, Ohio (introduced by Norton J. Greenberger).

Certain ubiquitous environmental antigens possess antigenic cross-reactivity with host constituents, possibly leading to autoimmune tissue damage. Using hemagglutination-inhibition, we demonstrated an antigenic relationship between enterobacterial common antigen (ECA) and colonic mucosal extracts. Using ECA as antigen in the macrophage migration inhibition test, we then sought the presence of cellular immunity to this bacterial component in the inflammatory bowel diseases (IBD), ulcerative colitis (UC), and regional enteritis (RE). ECA from *Salmonella typhimurium* was incubated in TC199 with purified lymphocytes from IBD patients, patients with other intestinal diseases, and healthy subjects. Supernates from these cultures were placed in migration chambers with capillary tubes packed with guinea pig peritoneal macrophages. Migration inhibitory factor (MIF) activity was expressed as the migration index (MI) where MI = migration in antigen-stimulated supernate/migration in unstimulated supernate. In 15 patients with moderately severe IBD (8 UC, 7 RE) the MI was 0.48±0.09 (range 0.32–0.61). In eight healthy subjects and in seven of eight patients with other intestinal diseases, the MI was 0.93 ±0.10 (range 0.73–1.09). Control lymphocyte cultures not stimulated by ECA, from three patients with severe, life-threatening IBD (two UC, one RE) yielded inhibitory supernate, suggesting in vivo induction of an inhibitor, possibly MIF. Migration in TC199 with ECA or in unstimulated supernates with ECA added during the migration phase was not inhibited. ECA-stimulated IBD lymphocytes did not elaborate soluble cytotoxic factors capable of damaging labeled-Cr^{51} chicken erythrocytes. We conclude that ECA-induced cellular autoimmunity may induce or perpetuate IBD, with soluble lymphotoxins being less likely as a mechanism of tissue damage than one involving direct cell-cell contact.

57. Disturbance of Lymphocyte Circulation by Granulomatous Infection. WARD E. BULLOCK,* Lexington, Ky. (introduced by J. W. Hollingsworth).

In this study, the circulation of thoracic duct lymphocytes (TDL) from normal Lewis rats was traced through lymphoid tissues of rats infected with *Mycobacterium lepraemurium*. Results suggest impaired TDL recirculation in rats with granulomatous infection, possibly due to entry-block at lymph nodes and splenic white pulp. A mean of 1.3×10^6 uridine-^{3}H-labeled TDL/g body weight was given intravenously to four matched pairs of infected and normal rats. Lymphocytes were then collected from thoracic duct fistulas (TDF) every 8 hr for 3 days. The mean peak (16–24 hr) lymphocyte output (LO) of infected rats was decreased (17.7×10^6/hr) as compared with normals (22.0×10^6/hr) and was below normal at all other time intervals. By radioautography, the ratio of labeled lymphocyte output (LLO) from infected vs. normal rats at 24 hr was 0.56. Four pairs of rats were depleted of lymphocytes by TDF 3 days before intravenous injection of labeled TDL. Lymph was collected every 4 hr thereafter for 48 hr. The mean LO of normals was 5.4×10^6/hr at 0 hr with a peak of 17.4×10^6/hr at 16 hr. LO of infected rats at 0 hr was 4.4×10^6/hr and increased only to 7.1×10^6/hr at 16 hr. The ratio of LLO from infected vs. normal rats at 16 hr was 0.41. Two pairs of lymphocyte-depleted rats were sacrificed at 12 and 24 hr after injection of ^{51}chromium-labeled TDL. Mean radioactivity/100 mg of infected vs. normal spleen at 12 and 24 hr was 28.8 and 38.6%, respectively. Radioactivity of infected lymph nodes was 41.1 and 35.4% of normal at these times. These findings demonstrate a mechanism by which granulomatous involvement of lymphoid tissue may interfere with the delayed immune response. (Research supported by NIH Grant AI-10094.)

58. Stimulatory Effects of Induced Phagocytosis on Isolated Thyroid Cell Function. G. BURKE,* K. KOWALSKI,* D. BABIARZ,* AND S. SATO,* Chicago, Ill. (introduced by Eric Reiss**).

Stimulation of endocytosis is a very early effect of thyrotropin (TSH) on thyroid. However, the relationship between endocytosis and the many other TSH effects on thyroid is not clearly defined. Since phagocytosis in isolated thyroid cells (ITC) is a model for in vivo endocytosis of colloid, we induced phagocytosis in ITC by incubating them at 37°C with 0.109 μ-diameter polystyrene microbeads; phagocytosis was confirmed in each experiment by electron microscopy and/or spectrophotometric analysis of dioxane cell extracts. ITC incubated with 50–100 μ-diameter polystyrene macrobeads (too large to ingest) served as controls. Microbead-induced phagocytosis in ITC was consistently accompanied by increases in: (*a*) cyclic AMP-^{14}C formation from adenine-8-^{14}C (66%); (*b*) iodide-^{131}I trapping (40%); (*c*) protein and RNA synthesis (30%); (*d*) phospholipogenesis (50%); (*e*) α-aminoisobutyric acid-1-^{14}C uptake (15%). In all instances, save the last, the magnitude of increase was related to the concentration of microbeads added. Polystyrene macrobeads did not influence ITC function in any of these experiments. Aminotriazole, 5×10^{-3} M, a peroxidase inhibitor, blocked the stimulatory effect of microbead-induced phagocytosis on phospholipogenesis only. These studies indicate that in ITC, phagocytosis, per se, may alter membrane-bound adenyl cyclase activity. The resultant increase in cyclic AMP may be a triggering mechanism for subsequent metabolic changes occurring during phagocytosis. Since these changes mimic those induced by TSH, it is suggested that a variety of thyrotropin effects on thyroid may be secondary to stimulation of colloid resorption. Although increased cyclic AMP formation is believed to precede, and be requisite to, all other TSH effects on thyroid, it would appear that in this in vitro ITC system induction of phagocytosis may be the primary event. (Research supported by NIH Grant AM 11136.)

59. Effect of High Doses of Methylprednisolone on Immunity in Man. WILLIAM T. BUTLER, ROGER D. ROSSEN,* AND EVAN M. HERSH,* Houston, Tex.

To study the effects of methylprednisolone (MP) on immune mechanisms in the absence of other immunosup-

pressive agents or immunologically mediated diseases, we gave 10 normal adult male volunteers 96 mg of MP daily for 5 days. IgG concentrations began to decrease shortly after MP treatment in nine of the volunteers, reached a low value between 2 and 4 wk, and then began to rise gradually. Mean concentration of IgG before MP was 12.4 mg/ml; by 4 wk, the mean had fallen by 21.8% to 9.7 mg/ml. In four cases, the IgG decreased by more than 30%. No significant changes occurred in 10 untreated normal controls studied simultaneously. Plasma survival of IgG-^{125}I was normal during MP treatment; a slightly increased catabolic rate occurred for 1–2 wk after MP but was not sufficient to account for the decreased serum IgG. Despite this decreased IgG synthesis, antibody responses to keyhole limpet hemocyanin and diphtheria toxoid given at the start of MP were normal. Circulating lymphocytes decreased slightly during MP treatment; however, the blastogenic response of lymphocytes in vitro was markedly suppressed to phytohemagglutinin and to antigens. For example, the mean response to streptolysin-*O* before MP was 15,500 cpm/10^6 cells; 48 hr after starting MP the mean was 1200 cpm/10^6 cells. Recovery of responsiveness began by the 4th treatment day; normal responses reappeared within 2 days after stopping MP. A short course of high doses of MP has profound effects on the immune system; suppression of lymphocyte reactivity appears early during treatment whereas the net effect of decreased immunoglobulin synthesis is delayed in appearance and persists for prolonged periods.

60. A Defect in Lipid Mobilization from Adipose Tissue in Diphtheritic Guinea Pigs. DAVID R. CHALLONER AND MARILYNN MCKEE,* Indianapolis, Ind.

During metabolic studies on the effects of diphtheria toxin on myocardium we noted that, despite a loss in body weight, the adipose tissue mass in diphtheritic guinea pigs appeared grossly to be as abundant as that in control animals which had gained weight on ad lib. feeding. To investigate this 35, 250-g guinea pigs were injected with 2 LD_{50}'s of diphtheria toxin and placed on a modified paired feeding regimen so that the food intake in diphtheritic and control groups was equal. Over 3 days diphtheritic animals went from 265±6 to 218±4 g while control animals went from 272±6 to 237±4 g. Despite this, the epididymal fat pad weights were: diphtheritic, 285±15 vs. control, 220±12 mg, $P = 0.002$; and fat pad weight (milligrams)/final body weight (grams)= diphtheritic, 1.32±0.006 vs. control, 0.93±0.04, $P < 0.001$. Greater differences were found on ad lib. feeding. Fat pads from control animals yielded 2962±181 cells/mg of fat pad digested vs. 1790±182 for diphtheritic animals, $P = 0.002$, demonstrating that the fat cells were larger in diphtheritic animals. Blood glucose values remained unchanged while blood FFA levels decreased to 65% of those in control animals, $P = 0.002$. Fat cells were isolated, incubated in vitro with increasing doses of epinephrine, and glycerol release assayed. The response of diphtheritic cells was not different from normal cells. These results suggest a defect in lipid mobilization in vivo in diphtheritic guinea pigs despite a catabolic state. The decrease in blood FFA despite a previously demonstrated decrease in FFA oxidation by isolated hearts and whole animals also indicates a defect of mobilization into the blood FFA pool. The defect does not appear to be in the lipolytic enzymes of the fat cell, suggesting the possibility of a defect in sympathetic nervous control (neuropathy?). This defect in lipid mobilization in the face of caloric need could be of pathophysiologic significance in the diphtheritic syndrome and could be subject to therapeutic manipulation. Whether primary or secondary, the carcass weight loss in diphtheritic animals is at the expense of components other than fat, most likely muscle. (Support from the NIH, HE 06308 and AM 11805, and the Indiana Heart Association is gratefully acknowledged.)

61. Alterations in the Adenylate Cyclase-Cyclic 3′5′-Adenosine Monophosphate (cAMP) System in Rat Hepatoma and Human Carcinoma of the Colon. REUBEN CHAYOTH,* SHELDON EPSTEIN,* AND JAMES B. FIELD, Pittsburgh, Pa.

Since cAMP may be involved in malignant transformation of cells, the adenylate cyclase system was evaluated in human colon carcinoma and ethionine-induced rat hepatoma. Each rat, when possible, provided tissue from malignant nodules, premalignant nodules, and normal liver. In humans, tissue was obtained from the carcinoma and adjacent normal mucosa. Tissues were assayed for cAMP, adenylate cyclase, and phosphodiesterase activities and adenine-^{3}H incorporation into cAMP-^{3}H. Hepatoma cAMP was significantly higher than normal liver or premalignant nodules (15.2±2.7, 3.2±0.3, and 3.3±0.2 nmoles/g protein, respectively). Malignant nodules incorporated more adenine-^{3}H into cAMP-^{3}H (180±8% of normal liver) than premalignant nodules (106±12%). Malignant nodule adenylate cyclase (395±70 cmp/mg protein) was also significantly higher than normal liver (226±15). Phosphodiesterase was similar in all three tissues. Although cAMP in colon carcinoma (3.3±0.6 nmoles/g protein) and normal mucosa (4.7±1.3) were similar before incubation, after 20 min incubation in Krebs-Ringer bicarbonate buffer, normal mucosa cAMP (40±15) was significantly higher than in carcinoma (7±2). Adenylate cyclase in carcinoma and adenine-^{3}H incorporation were significantly reduced to 38 and 40% of that in normal mucosa, respectively. A benign polyp in a patient with carcinoma gave results similar to normal mucosa. Prostaglandin A_1, B_1, E_1, and $F_{1\alpha}$ increased cAMP and adenine-^{3}H incorporation into cAMP-^{3}H in slices of carcinoma and normal mucosa with a greater increase in normals. These studies indicate that differences in the adenylate cyclase system exist between malignant and normal tissues but they are sometimes in opposite directions. (Research supported by grant from NIH.)

62. Increased Number of Stem Cells in Blood of Patients with Myelofibrosis. PAUL A. CHERVENICK,* Pittsburgh, Pa. (introduced by Dane R. Boggs).

Colonies of granulocytes and mononuclear cells can be grown in vitro from blood and marrow of normal individuals and from patients with various diseases. This report describes the growth of increased numbers of colonies from the blood of patients with myelofibrosis. Blood leukocytes from five patients with idiopathic myelofibrosis were suspended in 1.4% methylcellulose, McCoys 5A culture medium,

and 15% fetal calf serum. Colony growth was stimulated by a feeder layer of peripheral leukocytes. After incubation for 18 days at 37°C in 7.5% CO_2, colonies were counted and removed for morphological identification. Increased numbers of colonies grew from four of five patients and ranged from 80 to 560 per 10^6 nucleated blood cells plated. Normal subjects gave rise to 1–22 colonies, and blood from 15 patients with nonmalignant hematologic diseases gave rise to 1–50 colonies per 10^6 nucleated cells. Colony size was similar to that observed from normal individuals (50–1500 cells) and consistently larger than colonies from blood of patients with acute myeloblastic leukemia (maximum 300–400 cells) and most patients with chronic myelocytic leukemia. Morphologically colonies consisted of either eosinophils, neutrophils, monocytes, or macrophages. Blood leukocyte counts ranged between 3,600 and 32,000/mm^3. Granulocytes capable of proliferation (myeloblasts, promyelocytes, myelocytes) ranged between 3 and 37%. No direct correlation was observed between the blood leukocyte count or the number of potentially proliferating granulocytes and the number of colonies. This study indicates that increased numbers of stem cells circulate in the blood of patients with myelofibrosis. Colonies arising from these patients have growth characteristics similar to those from normal individuals and are larger than colonies from leukemic cells. This suggests that idiopathic myelofibrosis may not be intimately related to myeloid leukemia.

63. Heavy Incorporation of Mevalonic Acid-2-^{14}C into Cholesterol Precursors by Human Platelets In Vitro. PHIN COHEN AND ARIE DERKSEN,* Boston, Mass.

Pure human platelet suspensions in TES or Krebs-Ringer buffer, or autologous plasma, were incubated with mevalonic acid-2-^{14}C. Lipids were extracted and subjected to thin-layer chromatography (TLC) on silica H (hexanes : ether : acetic acid/55 : 45 : 1) and $AgNO_3$-impregnated silica H (chloroform : acetone/99 : 1) along with authentic references of commercially available sterols and quinones. As shown by radioautography of TLC separations, quinones were not labeled, but 1.5–2% of total radioactivity was incorporated into eight well-defined bands. More than half of the uptake appeared in lanosterol and squalene, both identified by TLC and, after purification, by GLC-mass spectrometry. Lanosterol, and two other compounds, putatively sterols, were recovered in extracts of digitonin precipitates, as shown by $AgNO_3$ TLC-radioautography. However, only trace amounts of label appeared in the cholesterol region in the two TLC systems. Approximately 10% of incorporated radioactivity migrated with the fatty acids, suggesting prenoic acid labeling. Other compounds, including one heavily labeled with R_F immediately subjacent to lanosterol or unimpregnated silica H, are presently unidentified. Labeling of squalene and the compound subjacent to lanosterol was considerably increased in an oxygen-poor environment. Disruption of platelets by sonication, freeze thawing, or detergents, sharply reduced the number and intensity of all labeled bands. By contrast, exposing platelets to hypoosmolar media strongly stimulated uptake of the label, particularly into lanosterol's subjacent neighbor, suggesting an architectural requirement for optimal mevalonic acid incorporation. All known stimulants for cholesterol biosynthesis—oxygen, energy substrates, cations, redox intermediates—in various combinations, did not increase labeling in the cholesterol region, but did affect labeling of some other compounds. (Supported by grant HE-13584 from NIH and grant DADA-17-70-C-0083 from the U. S. Army.)

64. Relative Importance of Preload and Afterload as Determinants of Left Ventricular Performance in Acute Myocardial Infarction. JAY N. COHN, JOSEPH A. FRANCIOSA,* MARTIN I. BRODER,* NABIL GUIHA,* AND CONSTANTINOS J. LIMAS,* Washington, D. C.

Acute myocardial infarction (AMI) is characterized by increased left ventricular filling pressure (LVFP), reduced stroke volume (SV), and low, normal, or elevated aortic pressure (AP). SV may be influenced by changes in diastolic filling (preload) and AP, which is an important determinant of left ventricular systolic wall tension (afterload). Hemodynamic effects of primary reduction in preload (tourniquets or phlebotomy) and primary reduction in afterload (nitroprusside infusion) was studied in 30 patients with AMI. During acute reduction in preload LVFP fell from an average of 23.6 to 15.6 mm Hg ($P < 0.01$) while SV was reduced from 32.6 to 29.2 ml/M^2 ($P < 0.01$) and heart rate (HR) was unchanged (87 to 84 beats/min). Mean AP fell from 100.0 to 91.5 mm Hg ($P < 0.01$). During nitroprusside infusion LVFP fell from an average of 23.8 to 12.4 mm Hg ($P < 0.01$) while SV rose from 24.5 to 28.4 ml/M^2 ($P < 0.02$) and HR was unchanged (95.4 to 96.6 beats/min). Mean AP fell from 102.9 to 88.4 mm Hg ($P < 0.01$). Therefore primary decrease in venous return and primary vasodilatation both reduced LVFP and AP, but SV rose only with the latter. These data indicate that pharmacological vasodilatation is hemodynamically more favorable than reduction in venous return in patients with AMI. Furthermore, greater reduction in LV wall tension during ejection may indicate lower myocardial oxygen consumption during vasodilator infusion. (Supported in part by NIH grant HE 09785.)

65. Mechanism of Steroid Hormone Action: Proof of the Messenger RNA Hypothesis. JOHN COMSTOCK,* GARY ROSENFELD,* ANTHONY MEANS,* AND BERT O'MALLEY, Nashville, Tenn.

Steroid hormones stimulate growth and protein synthesis in target tissues. It has been suggested that these effects are mediated by messenger RNA (mRNA). However, much controversy has centered on whether steroids act primarily at the transcriptional or translational level due to lack of definitive evidence on the existence of these hypothetical mRNA's in animal cells. In the chick model system, estrogen causes quantitative and qualitative changes in oviduct RNA and subsequent synthesis of cell specific proteins such as ovalbumin. We now report the isolation of ovalbumin mRNA from oviduct. The 12S–17S RNA was extracted from polysomes of estrogen-stimulated chick oviduct and tested for mRNA activity in a cell-free system containing ribosomes and translational factors from rabbit reticulocytes. Before addition of the chick RNA, valine-^{14}C is incorporated almost entirely into hemoglobin. Addition of chick

RNA results in appearance of a ^{14}C protein peak coincident with authentic ovalbumin in SDS-acrylamide gel electrophoresis. Synthesis of ovalbumin-^{14}C showed linear dependence on exogenous oviduct mRNA. Oviduct ribosomal RNA or RNA from liver demonstrated no capacity to direct in vitro synthesis of ovalbumin-^{14}C. Hen oviduct contained large quantities of ovalbumin mRNA, but RNA from immature chicks contained no mRNA for ovalbumin; however, mRNA could be induced in immature chicks with estrogen. Finally, estrogen withdrawal resulted in disappearance of chick ovalbumin mRNA. Estrogen readministration induced marked accumulation (> 10-fold) of ovalbumin mRNA. These data represent an initial isolation and translation of specific mRNA from eucaryotic hormone-dependent tissue and document its presence to be directly dependent on prior hormonal stimulation. It also substantiates the hypothesis that steroid hormones act in target cells to induce protein synthesis by first promoting accumulation of mRNA.

66. The Role of Sialic Acid in the Phagocytic Activity of Blood Neutrophils. ANDREAS CONSTANTOPOULOS* AND VICTOR A. NAJJAR,** Boston, Mass.

Stimulation of the phagocytic activity of blood neutrophils has been obtained by an autologous cytophilic γ-globulin fraction that binds specifically to polymorphonuclear leukocytes (1967. *Biochemistry.* **6:** 3386). The whole stimulatory activity resides in a small peptide, tuftsin. Tuftsin is cleaved off the parent γ-globulin molecule by a specific enzyme leukokininase present on the outer surface of the cell membrane (1970. *Nature* [*London*]. **228:** 672). It has been identified as Thr-Lys-Pro-Arg and synthesized. Its activity in vitro is expressed in hormone-like quantities and is composed in the process. It appears to be synthesized in the spleen since it is completely absent in splenectomized dogs and humans. Sialic acid is indispensible for its biological activity. Treatment of the cells with neuraminidase from either *Vibrio cholerae* or *C. perfringens* purified by affinity chromatography (1971. *Biochem. Biophys. Res. Commun.* **44:** 178) inhibits completely the stimulatory effect exerted by tuftsin and its parent molecule, the specific leukophilic γ-globulin. Treatment of the cells with influenza virus neuraminidase resulted only in a partial inhibition. The latter enzyme is specific for the 2–3′ and two bacterial enzymes cleave both 2–3′ and 2–6′ linkages. It can be assumed therefore, that membrane-bound sialic acid of neutrophils contains both linkages and is concerned with receptor site binding of tuftsin, whether free as the tetrapeptide or bound to the parent carrier specific γ-globulin. (Supported by USPHS grant AI-09116.)

67. Definition of the Essential Red Cell Membrane. RICHARD A. COOPER,* Philadelphia, Pa. (introduced by A. S. Relman**).

Red cell membrane free-cholesterol (FC) exchanges with plasma lipoprotein FC. It increases under certain pathologic conditions in vivo, particularly liver disease, and decreases when lipoprotein FC is esterified in vitro. Yet no simple relationship exists between plasma FC concentration and membrane FC content. However, FC exists with phospholipids (PL) and protein (Prot) in membranes and plasma lipoproteins. Interrelationships between these constituents were therefore studied. Membrane FC/PL mole ratios were increased to 0.92–1.41 in 22 patients with severe liver disease (14 with "target" and 8 with "spur" cells), but were 0.87 ±0.02 in 13 normals. FC/PL and FC/Prot of low density lipoprotein (LDL) and high density lipoprotein (HDL) were also increased in patients. Membrane FC/PL correlated well with the FC/PL of whole serum ($r = 0.78$) and of LDL ($r = 0.86$), and it correlated closely with the FC/Prot of LDL ($r = 0.94$). Lipoproteins and red cells were more saturated with FC in patients with spur than with target cells; however, these data formed a continuum yielding similar regression lines. Extrapolation of these regression lines to zero values for the FC of serum and LDL intercepted membrane FC/PL mole ratios of 0.63, 0.48, and 0.62. Increases in normal membrane FC/PL induced by incubation in FC-enriched normal serum corresponded to values predicted from these regression analyses. Decreases due to FC esterification in vitro followed a regression line which intercepted a membrane FC/PL mole ratio of 0.50 at zero serum FC. Thus, the FC saturation of plasma lipoproteins, as defined by their FC/PL or FC/Prot ratios, relates directly to the FC/PL mole ratio of red cell membranes. Moreover, a membrane FC/PL mole ratio of approximately 0.55 defines the lipid composition of the "essential membrane"; values greater than this reflect the FC saturation of plasma lipoproteins.

68. A "Proliferation Inhibitory Factor" (PIF) Produced by Human Blood Lymphocytes. S. R. COOPERBAND, J. R. GREEN,* AND A. M. BADGER,* Boston, Mass.

Circulating lymphocytes are involved in the production of delayed hypersensitivity, graft rejection, and immune surveillance against the growth of neoplastic cells. It is probable that these effects are mediated, at least partly, by a number of humoral effector factors. We have observed the production of a new factor (PIF) by human blood lymphocytes in culture which acts to inhibit the growth of nonlymphoid cells and may be involved in immune surveillance. PIF is produced by mitogen-(PHA, Concanavalin A) and antigen-(mixed leukocyte cultures, PPD) stimulated cells. It may be produced by purified lymphocytes, and is detectable 3 hr after stimulation, although it accumulates during 2–3 days in culture. The factor upon a variety of human cell types, in culture, to inhibit DNA synthesis, mitotic index, new cell production, and replication of individually cloned cells without cytotoxicity. We generally assay PIF in unfractionated culture supernatants at dilutions of 1:20–1:100, but we have observed activity in dilutions of 1:1000. Different cells demonstrate different sensitivity to PIF—embryonic and tumor cells generally are most sensitive, while adult primary cells (i.e., kidney) are insensitive. The PIF appears to be species specific, and human PIF is not active against chick or murine cells. It did not appear to inhibit proliferation of autologous lymphocytes stimulated by PHA. We have examined the mechanism of action of PIF on cloned HeLa cells which are in synchronous division. The PIF does not influence cells when added after the onset of the G1 phase of cell division and the next cell division occurs normally. When PIF is present during mitosis, cells become arrested in

the following G1 phase. This effect occurs even when PIF is added for only a few hours during mitosis and early G1, and then removed. The PIF is heat stable up to 85°C for 30 min, nondialyzable, nonsedimentable at 90,000 *g* and is destroyed by trypsin. (Research supported by grants from NIH.)

69. Thrombokinetic Studies in Acute Leukemia. DALE H. COWAN,* Cleveland, Ohio (introduced by John W. Harris**).

Thrombocytopenia commonly occurs during the course of acute leukemia and is generally attributed to decreased platelet production resulting from marrow invasion by leukemic cells. No studies using quantitative techniques have been published to substantiate this contention. To evaluate thrombokinetics in acute leukemia, platelet production and survival were measured in untreated patients with this disorder. In nine patients (platelets: 14,000–325,000/μl) the survival of autologous ^{51}Cr-labeled platelets ranged from 0.5 to 5.9 days, recovery of labeled platelets was 22–45%, and platelet turnover was 13,900–334,800 platelets/μl per day (normal: 8.5±0.2 days, 62±3%, 49,200±3600 platelets/μl per day, respectively). In five of the nine, turnover was greater than normal. In all nine patients, labeled platelets disappeared exponentially; in normals the pattern of disappearance is linear. Using Harker's method, total megakaryocyte mass in the patients with leukemia ranged from 0.4 to 61.3×10^{10} μ^3/kg (median 9.3×10^{10} μ^3/kg) (mean of nine normals: $5.1 \pm 1.0 \times 10^{10}$ μ^3/kg). In six patients, the total mass exceeded normal. In five patients, the numbers of megakaryocytes were increased, ranging from 35 to 408×10^6/kg (normals: $13.0 \pm 4.3 \times 10^6$/kg) and cell volumes were decreased, ranging from 1503 to 2637 μ^3 (normal: 3982±253 μ^3). These changes are similar to those seen in megaloblastic anemias. In four of these five and in one other patient, total production, determined from total megakaryocyte mass, exceeded effective production, determined from platelet turnover. Serum B12 and erythrocyte folate levels were normal in each. The data indicate that total thrombopoiesis is generally not subnormal in patients with acute leukemia. Rather, it often exceeds normal. Thrombocytolysis is present and is often uncompensated despite increased total thrombopoiesis. One mechanism for the uncompensated thrombocytolysis is ineffective thrombopoiesis occurring in the absence of cellular folate or B12 deficiency. (Research supported by Grant T-548 from ACS and Grant CA 12399 from NIH.)

70. Communication between Normal and Enzyme-Deficient Cells in Tissue Culture. RODY P. COX, MARJORIE R. KRAUSS,* M. E. BALIS,* AND JOSEPH DANCIS,* New York.

Correction of certain mutant phenotypes by intimate contact with normal cells—i.e. "metabolic cooperation"—is an easily studied form of cell communication. Metabolic cooperation between normal cells and mutant cells deficient in hypoxanthine-guanine or adenine phosphoribosyl transferase (HGPRTase and APRTase, respectively) appears to be the result of transfer of the enzyme product, nucleotide, or nucleotide derivative, from normal to mutant cells. This process shows selectivity in that mutant derivatives of mouse L cells are unable to function as recipients of HGPRTase or APRTase products, while hamster and human fibroblasts with these enzyme deficiencies exhibit correction of the mutant phenotype, when in contact with normal donor cells. There is also selectivity with respect to substances transferred, since other mutant phenotypes, i.e. G-6-PD deficiency, are not corrected by contact with normal cells. Species specificities do not appear to influence metabolic cooperation, therefore heterospecific cell mixtures provide an opportunity to cytologically distinguish cells and study individual cell interactions. Transfer of nucleotide from normal to mutant cells is less dependent on energy production than is the incorporation of radioactive purines into cellular material. The nucleotide translocation mechanism is not susceptible to sulfhydryl blocking agents. (Research supported by grants from NIH.)

71. Partial Gonadal Mosaicism and Genetic Counseling in X-Linked Lethal Recessives. DANIEL CRAMER,* R. J. KRYSCIOR,* E. R. PIERCE,* AND EDMOND A. MURPHY,** Bethesda and Baltimore, Md.

Well-known theory shows that one-third of all X-linked recessive lethals are new mutations. Since mutations can arise wherever copying of DNA occurs, the proportion of affected ova may vary randomly as a branching process. A convenient model is perhaps that proposed and solved approximately by Lea and Coulson (*J. Genet.* 1949. **49:** 264) for the propagation of mutant bacterial strains on a culture medium. More exact methods are here described for finding (*a*) the individual probabilities and (*b*) the moment-generating function and hence the moments of the distribution of the number of mutant cells. These probabilities are the prior distribution of the individual proportions and are modified in the usual way by the posterior information to give a posterior distribution of proportions of mutant cells. From these it is easy to compute the risk that the next son will be affected. Biological data suggest that the ova are a randon sample of about 300,000 cells from a total of about 5,000,000. A mutation probability per mitosis is chosen such that the mean number of mutant cells accords with population estimates of the mutation rate per generation. From these values it is shown that partial gonadal mosaicism has only a small effect on the classical risk of recurrence (*Hum. Heredity.* 1969. **19:** 126). The methods are illustrated from actual data.

72. Differential Effects of Exogenous Free Fatty Acids (FFA) on Catecholamine-Induced Changes in Lipolysis and Glycogenolysis in the Perfused Working Rat Heart. M. F. CRASS III* AND J. C. SHIPP, Omaha, Nebr.

The purpose of this study was to examine interactions of catecholamines and exogenous substrates (glucose, FFA) on cardiac lipolysis and glycogenolysis. Hearts of fed male rats were labeled in vivo with palmitate-1-^{14}C, and preperfused for 5 min followed by a 30 min recirculated perfusion in a closed working system at 10 cm H_2O left atrial filling pressure. Perfusate was oxygenated Krebs-Henseleit bicarbonate buffer containing 5.5 mM glucose with or without palmitate (3% albumin). Epinephrine (E) or norepinephrine (NE) was added after 2 min of recirculated perfusion. Tension and rate changes were monitored. Tissue glycogen, content and ^{14}C labeling of triglycerides, and $^{14}CO_2$ were determined. In the absence of added catecholamines, exogenous FFA, but not glucose, inhibited triglyceride fatty acid oxidation and glycogenolysis. E and NE, 8.2×10^{-8} to 1.3×10^{-6} M, pro-

duced concentration-dependent increases in triglyceride mobilization, triglyceride fatty acid (TGFA) oxidation, and glycogenolysis, without affecting content or labeling of tissue phospholipids in hearts perfused with glucose alone. Maximal stimulation with E and NE was observed at 4×10^{-7} M. With added palmitate (0.6 mM), the E-stimulated lipolysis and TGFA oxidation was abolished, though the full glycogenolytic effect of E persisted. These findings, under conditions which approximate those in vivo, indicated that catecholamines and exogenous FFA exert different regulatory effects on triglyceride and glycogen metabolism of heart muscle. (Supported by grants from NIH [No. AM-14986] and Nebraska Heart Association.)

73. α-Galactosidase Defect in Fabry's Disease. Specific Inhibition of One Enzyme by Myoinositol. JOHN C. CRAWHALL AND MARIANNA BANFAVLI,* Montreal, Canada.

Fabry's disease has been characterized by a deficiency of the enzyme ceramide trihexosidase. α-Galactosidase activity in leukocytes and cultured skin fibroblasts is also reduced to about 20% of normal control values. This residual activity could be accounted for either by reduction of the normal enzyme quantity, a structural enzyme variant, or as a residual isoenzyme. We have studied these possibilities by use of myoinositol as a specific inhibitor of α-galactosidase using 4-methylumbelliferyl-α-galactoside as substrate. Initial experiment showed that myoinositol was an effective competitive inhibitor of the normally occurring enzyme but was not inhibitory to the enzyme found in mutant cells. It is known that there are differences between the properties of mutant and normal cell enzymes in that the former has a higher K_m and is not heat labile compared with the latter. We have combined this information together in an experimental design to measure myoinositol inhibition of normal cell α-galactosidase before and after heat inactivation at 51°C for 75 min and the following changes were observed. The specific activity of the enzyme was rapidly reduced at first but the last 10% of activity remained relatively thermostable. The K_m of the enzyme changed from 3 mM to 19 mM and myoinositol which was originally an inhibitor of the enzyme became noninhibitory. This data indicated that two α-galactosidase isoenzymes are present in normal cultured fibroblasts. The more active of these has the lower K_m, increased heat lability, is inhibited by myoinositol, and is deficient in the cells obtained from patients with Fabry's disease. The other, which is still present in cells obtained from patients with Fabry's disease has a higher K_m, minimal heat lability, and is not inhibited by myoinositol.

74. Role of Growth Hormone (GH) in the Alterations of Carbohydrate Metabolism Induced by Oral Contraceptive Agents (OCA). MAYER B. DAVIDSON* AND GERALD B. HOLZMAN,* Los Angeles, Calif. (introduced by J. Brown).

The mechanism whereby OCA cause glucose intolerance is unknown. Metabolic responses to tolbutamide were utilized to study this problem since glucose, GH, insulin, and free fatty acids (FFA) are all affected by this single agent. 13 normal subjects and 8 hypopituitary patients on replacement medication were tested before starting and 3 and 6 months after OCA were begun. In normal subjects after OCA, not only were fasting GH levels elevated, but after tolbutamide, there was a significantly blunted fall of plasma glucose, an increased GH response, and a heightened rebound of FFA. As expected, plasma cortisol was markedly elevated by OCA. Although unchanged by OCA, insulin responses during the three tests were significantly correlated in the same subject. Therefore, insofar as the tolbutamide-stimulated response reflects release of stored hormone, the quickly releasable insulin pool remains constant in a single individual and is unaffected by OCA. In contrast to normals, the glucose, FFA, and insulin responses to tolbutamide were unchanged by OCA in the hypopituitary patients. Fasting GH levels were mostly undetectable by our assay and there were only minimal hypoglycemia-induced rises of GH in any of the tests. Plasma cortisol was also markedly elevated by OCA in these pituitary-deficient patients. Five additional normal females on OCA had an increased GH (mean±SEM) response to a moderate amount of exercise (two flights of stairs) compared to five women not on OCA (16.4±3.5 vs. 5.0±1.9, $P < 0.025$). This suggests that subjects on OCA are exposed to high levels of GH throughout the day. It is concluded that estrogen stimulation of GH secretion is the mechanism whereby OCA causes glucose intolerance.

75. Complement Abnormalities and Renal Vascular Injury. N. K. DAY,* H. GEIGER,* R. MCLEAN,* A. MICHAEL, AND R. A. GOOD,** Minneapolis, Minn.

Complement component deficiencies have been associated with renal-vascular disease. The presumption has been that the C abnormalities and renal-vascular pathology are related because C components are being utilized in immunological processes injurious to the glomerulus (e.g., serum sickness). Early we encountered three distinct selective deficiencies of C components associated with progressive renal disease and we can now link hereditary deficiency of C1r, C1s, C2 to increased susceptibility to infection, to renal-vascular disease, and to lupus erythematosus. An additional experience with a patient suffering from recurrent hematuria and glomerulitis after infection seems most provocative in this perspective. Histologically the patient's renal lesion comprised focal glomerulonephritis with hyalinization and glomerular deposition of β1C. The serum showed depression of hemolytic C3, properdin, and conversion of C3 at 0°C; C3PA (Müller-Eberhard) was reduced in fresh and incubated serum. Total hemolytic C in fresh serum is high and drops sharply at 0°C. C activation is dependent on Mg^{++} and not on Ca^{++} and appears to involve primarily activation of C3 but to a lesser extent C5 and C7. C1, C4, C2, C6, C8, C9, as well as Clq and Cls, remain normal by immunochemical and/or functional assay. The vigorous activation of the alternate C pathway observed in vitro at low temperatures is probably occurring in vivo as well (cf. C3PA) to account for the episodes of renal vascular disease. Taken together, these several clinical associations suggest that the integrity of the classical C pathway may provide not only a defense against infection but a major defense against excessive activation of the alternate or properdin pathway which when activated excessively may lead to serious renal-vascular injury. (Aided by NIH.)

76. Cyanate-^{14}C: a New Method for Red Cell Survivals. FRANK G. DE FURIA,* JOSEPH H. GRAZIANO,* AND ANTHONY CERAMI,* New York (introduced by Zanvil Cohn**).

The reversible binding of ^{51}Cr and the hydrolysis of the phosphate bond formed with ^{3}H- or ^{32}P-labeled diisopropylfluorophosphate (DFP) has led to difficulties in determining the in vivo survival time of erythrocytes. The recent finding that low concentrations of cyanate (^{-}NCO) react specifically with the amino terminal valine of the hemoglobin molecule in an irreversible carbamylation reaction has led us to develop a cyanate-^{14}C method for determining red cell survival. 30 ml of blood anticoagulated with acid citrate dextrose solution B was divided into two portions and incubated for 1 hr at 37°C with either 25 μCi of $Na_2{}^{51}CrO_4$ (SA 30.2 mCi/g) (Chromitope) or $KN^{14}CO$ (SA 80 mCi/g). The cells were washed three times, recombined, suspended in saline, and returned to the donor animal. The blood sample taken 24 hr after reinfusion was considered to be day O for both methods. The average apparent half-survival time ($t_{\frac{1}{2}}$) by ^{51}Cr and ^{14}C methods, respectively, were: four adult female rhesus monkeys, 15.6 and 19.3 days; four adult female beagles, 19.2 and 27.5 days; an anemic Basenji dog (hereditary pyruvate kinase deficiency), 1.5 and 3.4 days. In contrast ^{51}Cr data, the ^{14}C data yielded a straight line when plotted on linear coordinates, further emphasizing the irreversible nature of the carbamylation. Since ^{-}NCO reacts selectively with RBC's in vivo, intravenous administration of the label is also feasible. 100 μCi of cyanate was administered intravenously to an adult female beagle and the anemic Basenji with a resultant $t_{\frac{1}{2}}$ of 22.3 and 5.2 days, respectively. The greater $t_{\frac{1}{2}}$ of the Basenji observed when cyanate-^{14}C was administered in vivo probably reflects the advantage of labeling fragile RBC in vivo. (Supported in part by NIH Contract Grant 71-2371; USPHS Grant AM-14691, and grants from the Rockefeller Foundation and the Children's Blood Foundation.)

77. The Prognostic Implication of Glomerular Deposits in Systemic Lupus Erythematosus. MARTIN DILLARD,* ISABELLE DUJOVNE,* CONRAD L. PIRANI,* AND VICTOR E. POLLAK, Chicago, Ill.

In patients with systemic lupus erythematosus (SLE) it is now thought that glomerular lesions result from deposition of circulating immune complexes. Deposits are assumed to occur mainly in the subepithelial position, and γ-globulin, with anti-DNA activity, has been eluted presumably from this site. To assess the relationship and character of deposits, and severity and activity of renal disease, 40 renal biopsies from 24 patients were investigated. The extent and site of deposits was estimated semiquantitatively (0–4+) in low power electron micrographs of glomeruli. The plasma proteins in the deposits was assessed in 0.5 μ frozen substituted sections, using monospecific antisera against heavy chains (γ, α, μ), light chains (κ, λ), β_1C globulin, fibrinogen, albumin, and transferrin. Later, sections were stained with periodic acid-Schiff to facilitate precise localization. Deposits were not distributed randomly in the glomeruli. There was a positive relationship between: (*a*) the extent of subendothelial and mesangial deposits, (*b*) the extent of subepithelial and intramembranous deposits. There was a striking inverse relationship between the extent of subendothelial and subepithelial deposits. Subepithelial deposits contained IgG, IgM, and β_1C globulin. With extensive subepithelial and intramembranous deposits, the clinical course was benign, and serum β_1C globulin levels were normal. Serial studies revealed persistence of these deposits for years. Subendothelial deposits contained all plasma proteins including immune globulins, β_1 globulin, fibrinogen, albumin, and transferrin. In the presence of subendothelial deposits serum β_1C globulin levels were low. Extensive subendothelial deposits occurred only with histologically active lesions, and were predictive of death within weeks. Subendothelial deposits disappeared with treatment in survivors. These studies indicate an important predictive role for electron microscopy and immunohistologic observations in patients with lupus nephritis. (Supported by NIH Grant AM-10314.)

78. Sensitivity of Methods for the Detection of Anti-DNA Antibodies. CAROLE DORSCH* AND EUGENE BARNETT, Los Angeles, Calif.

The relative sensitivity of four methods for the detection of antinuclear antibodies has been examined. 41 heat-inactivated sera were selected on the basis of positive indirect immunofluorescent antinuclear antibody tests (ANA). The diagnoses included systemic lupus erythematosus (SLE) (29), rheumatoid arthritis (6), and miscellaneous diseases (6). 23 sera had precipitating antibodies to double strand (ds) and/or heat-denatured, single strand (ss) calf thymus DNA by counterimmunoelectrophoresis (CIE) in 1% agarose in barbital buffer, pH 8.2. 8 of these 23 sera also precipitated with DNA by Ouchterlony double immunodiffusion in 0.4% agarose in phosphate-buffered saline (PBS), pH 7.4. 32 of the 41 sera showed positive DNA binding (>25%) by the ammonium sulfate precipitation technique of Farr, using ds-calf thymus DNA-labeled with actinomycin D-^{3}H. These included 21 of the 23 sera containing precipitins by CIE. Of the 32 positive-binding sera, all 10 with values >60% had precipitating antibodies in CIE. 22 sera had DNA-binding values between 25% and 60%; only 11 of these were positive by CIE, while 11 contained no demonstrable precipitins to ss- or ds-DNA. To explain differences in precipitability with DNA of sera with comparable DNA-binding values, differences in immunoglobulin class, quantity, affinity, and specificity were evaluated. CIE represents a method for detecting substances precipitating with DNA which is more rapid, requires less serum, and is more sensitive than the more commonly employed double immunodiffusion in PBS. Neither of these methods detects DNA antibodies in as many sera as do primary binding assays with radio-labeled DNA. Neither has the same clinical discriminatory value as ANA tests which, when negative, essentially exclude the diagnosis of SLE. (Supported in part by USPHS Grant GM 15759.)

79. In Vitro Synthesis of DNA Components of Human Genes for Globin: Use as a Probe for Messenger RNA (mRNA). L. W. DOW,* D. KACIAN,* M. TERADA,* S. METAFORA,* S. SPIEGELMAN,* P. A. MARKS, AND A. BANK, New York.

Purification of human mRNA from polyribosomes of hemolytic anemia reticulocytes (retics) and purified RNA-dependent DNA polymerase (reverse transcriptase) from avian myeloblastosis virus permitted the in vitro synthesis of DNA-^{3}H. This DNA-^{3}H product has a size corresponding to 8.3S (neutral sucrose gradient), and hybridizes rapidly with human globin mRNA, but not with ribosomal or viral RNA. The DNA-^{3}H-human globin mRNA hybrid bands at 1.55 g/cm^3 in Cs_2SO_4, the expected density for a true DNA-RNA hybrid in which both polynucleotides are of about the same length. Purified DNA-^{3}H product bands at 1.47 g/cm^3 in Cs_2SO_4: the density of a DNA strand with approximately the mol per cent G + C (50%) content expected for globin mRNA. These data suggest that the DNA synthesized in vitro approaches the size predicted for a human globin structural gene and has a base sequence complementary to human globin mRNA. The human 10S isolated from polyribosomes of normal retics directs synthesis of equal amounts of normal α- and β-globin in a Krebs cell-free system. β-thal retic mRNA directs synthesis of α-chains fivefold that of β-chains in the Krebs cell-free system. The decreased formation of β-chains in thal could be due to deficient or defective mRNA for β-chains. The in vitro synthesized DNA-^{3}H provides a probe to distinguish these alternatives. The DNA-^{3}H product of normal human globin mRNA hybridizes with thal globin mRNA. If there is decreased mRNA for β-globin in thal, higher concentrations of thal globin mRNA will be required for saturation of the DNA compared to that with a given concentration of normal globin mRNA. (Supported by NIH, National Science Foundation, ACS, and Cooley's Foundation grants.)

80. Ultrastructural Changes Associated with Platelet Nucleotide and Calcium Release. MICHAEL J. DROLLER,* Bethesda, Md. (introduced by Robert S. Gordon, Jr.).

Platelet activity is thought to be mediated by the secretion, or "release," of calcium and nucleotides from platelets in response to various aggregating agents. The present study describes the morphological events that occur during such nucleotide/Ca^{++} release. Washed human platelets were incubated at 37°C for 10 min with either thrombin, collagen, ADP, or epinephrine, and fixed in 3% glutaraldehyde and 1% OsO_4 for electron microscopy. At 37°C, where maximal release of nucleotides/Ca^{++} occurs in response to thrombin and collagen, alpha granules, thought to contain the substances released, almost completely disappear. A complex lamellar-vacuolar system, continuous with the cell membrane and probably the pathway for outward movement of nucleotides, now dominates platelet ultrastructure. Wisps of electron-dense material, comprising fibers 50–80 A in diameter with an occasional 180–220 A periodicity, are conspicuous in the lamellar spaces. The wisps are morphologically similar to fibrin precipitated either in vivo or in vitro. They are seen even after only 5 sec of incubation, when many alpha granules have not yet been released. Epinephrine and ADP are less effective in stimulating release and its associated morphological changes. Prostaglandin E_1 and theophylline, inhibitors of aggregation, inhibit nucleotide/Ca^{++} release and many of the accompanying ultrastructural events. Release is not observed in platelets incubated at 4°C, or at 37°C without any of these agents; such platelets undergo none of the morphological changes that characterize release. The differences thus observed between the effects of various agents on the platelet release reaction establish a pattern of events that undoubtedly play an integral role in subsequent clot formation.

81. Interaction of Glycoprotein Hormones with Agarose-Concanavalin A. MARIA L. DUFAU,* TSUNEO TSURUHARA,* AND KEVIN J. CATT,* Bethesda, Md. (introduced by M. B. Lipsett**).

The ability of agarose-coupled Concanavalin A to bind carbohydrate and glycoprotein molecules has been applied to chromatography of glycoprotein hormones. The carbohydrate binding activity of Concanavalin A is mainly directed toward D-gluco- and D-mannopyranosyl residues, and interaction with polysaccharides has been ascribed to the terminal α-linked nonreducing portion of the molecule. However, HCG and subunits bearing predominantly sialic acid terminal residues were bound strongly by Sepharose-Concanavalin A, and could be displaced by 0.2 M methyl α-D-glucopyranoside. Desialylated HCG was eluted more slowly than the intact hormone, and asialo-agalacto-HCG was not readily dissociable from combination with Concanavalin A. While the binding of unmodified HCG by Concanavalin A is largely attributable to the substantial mannose content of the molecule (16 residues/mole), the presence of terminal sialic acid and galactose residues significantly influences the binding affinity of HCG for Concanavalin A. Human luteinizing hormone and follicular-stimulating hormone were also absorbed by Sepharose-Concanavalin A columns, and eluted with methyl α-D-glucopyranoside. Prior combination with specific antibody did not prevent HCG-^{125}I binding to Concanavalin A, and subsequent elution with methyl glucopyranoside released the antigen-antibody complex in high yield. Chromatography of HCG on Sepharose-Concanavalin A was applied to: (*a*) preparation of ^{125}I-labeled HCG with improved binding affinity for antibody and gonadal receptor sites, (*b*) purification and isolation of HCG from crude urine extracts, (*c*) comparison of plasma and urinary HCG, revealing an elution profile consistent with partial desialylation of the urinary hormone. These experiments have shown that the group-specific interactions of glycoprotein hormones with agarose-Concanavalin A are of considerable value for purification and isolation of such hormones and their derivatives, and for the preparation of radioiodinated glycoprotein hormones of improved binding characteristics.

82. Experimental *Streptococcus viridans* Endocarditis: Alternatives to Penicillin in Prophylaxis. DAVID DURACK* AND ROBERT PETERSDORF, Oxford, England.

Antimicrobials other than penicillin are often prescribed for patients with valvular heart disease undergoing dental or other surgical procedures in an attempt to prevent bacterial endocarditis (BE). If patients cannot tolerate penicillin or if bacteremia with resistant organisms is anticipated, a variety of antibiotics has been recommended. This approach is empiric at best because there is no clinical or experimental evidence to document its efficacy. The development of a model in which BE regularly follows one

intravenous injection of *Streptococcus viridans* into rabbits with pre-existing sterile endocardial lesions permitted a systematic examination of this problem. Antibiotics were given 30 min before or after an intravenous injection of approximately 10^8 *S. viridans* into rabbits with sterile endocarditis in doses calculated to correspond with those used in man. After 24 hr the animals were killed and the vegetations cultured quantitatively. The organism was fully sensitive to all drugs and serum levels greatly exceeded minimum inhibitory concentrations. Persisting infection with *S. viridans* was found despite administration of cephaloridine, cephalexin, erythromycin, tetracycline, and cotrimoxazole. However, vancomycin was successful in sterilizing the vegetations in all animals. If treatment was delayed for 6 hr after induction of bacteremia, the vegetations were not sterilized by any drug except vancomycin which was only partially successful. A patient under penicillin treatment for streptococcal endocarditis was studied during the course of full-mouth dental extractions. Early operative blood cultures were positive for oral microorganisms while cultures subsequent to infusion of vancomycin were sterile. The results indicate that vancomycin is an effective alternative to penicillin for preventing BE in susceptible hosts. Moreover, they suggest that this model provides an excellent system for the study of antimicrobials and their mechanisms of action.

83. Tumor Antigen (Carcinoembryonic Antigen—CEA) in Gastric Adenocarcinoma Masked by a Binding Substance, Likely an Antibody. BARBARA J. DYCE* AND BERNARD J. HAVERBACK,** Los Angeles, Calif.

We have found at least two tumor-specific antigens in gastric, pancreatic, colonic, and rectal adenocarcinoma and their metastases. It was considered unusual when perchloric acid extract from liver metastases of a gastric adenocarcinoma, purified by Sepharose 4B gel column chromatography and repeated polyacrylamide gel electrophoresis, contained no detectable CEA using highly specific antiserum with the Ouchterlony gel double diffusion technique. Accordingly, an explanation for this negative finding was sought and specifically the masking of antigen by naturally occurring antibody was investigated. As CEA is soluble and immunoglobulins or CEA bound to goat antibody are insoluble in 0.6 N perchloric acid, the precipitate from this extraction was treated with 15% NaCl or acidified to pH 3 to dissociate the glycoprotein antigen from antibody. Antibody was then precipitated by 50% saturated ammonium sulfate. After exhaustive dialysis and concentration, CEA was then found in the supernatant in both instances showing two precipitin bands with highly specific antiserum. Also, treatment of an initial isotonic saline homogenate of the tumor with 15% NaCl or controlled acidification to pH 3 with HCl, followed by 50% NH_4SO_4 precipitation, resulted in the presence of two antigens in the supernatant. Subsequent purification of these antigens by Sepharose 4B gel column chromatography and repeated polyacrylamide gel electrophoresis, showed them to have similar chemical characteristics, as well as lines of identity with antigens extracted by Gold's method from hepatic metastases of colonic adenocarcinoma. It is clear from these studies that (*a*) the presence of CEA in this cancer was masked by a naturally occurring substance, likely an antibody, and (*b*) perchloric acid extraction did not dissociate CEA from this substance. Also, it is possible that CEA may be masked by a naturally occurring substance, likely an antibody, in serum giving negative or falsely low values, and dissociation procedures should be included in methodology.

84. Mechanisms of Hyperoxaluria in Regional Enteritis (RE). DAVID EARNEST,* HIBBARD WILLIAMS, AND WILLIAM ADMIRAND,* San Francisco, Calif.

The mechanisms of hyperoxaluria and recurrent calcium oxalate nephrolithiasis were investigated in 20 patients with RE (8 ileal disease, 12 ileal resection). This hyperoxaluria has been attributed to increased supply of the oxalate precursor, glyoxylate, from bacterial degradation of glycine-conjugated bile salts (GBS). Oral taurine (8 g/day) resulted in a consistent reduction in oxalate excretion (mean 51%) by substitution of taurine for glycine in bile salt conjugation and thereby decreasing the quantity of GBS in the gut. Similarly, oral tetracycline (3 patients) reduced oxalate excretion (mean 57%) by decreasing glyoxylate synthesis from GBS by intestinal bacteria. Accumulation of glyoxylate, as in primary hyperoxaluria type I, results in increased urinary excretion of both oxalate and glycolate. In contrast, urinary glycolate excretion was normal in all 20 hyperoxaluric RE patients. Furthermore, intravenous glyoxylate-^{14}C administration (five patients) demonstrated decreased glycolate synthesis in the presence of increased formation of oxalate-^{14}C (mean 19.2%, normal 12% of dose). These findings suggested increased conversion of glyoxylate to oxalate by LDH-NAD. Indeed, the elevated serum lactate: pyruvate ratio (mean 35, normal < 15) in nine hyperoxaluric RE patients supported this concept. The discovery of hyperoxaluria in three cholecystectomy patients with bile diversion by T-tube drainage confirms that this enhanced oxalate synthesis may be related to increased NADH: NAD-linked bile salt synthesis which is accelerated in many patients with ileal disease. These findings demonstrate that a dual mechanism may be responsible for the hyperoxaluria that occurs in patients with RE: (*a*) expansion of the glyoxylate pool, and (*b*) increased NAD: NADH-linked conversion of glyoxylate to oxalate. (Research supported by grants from NIH.)

85. Oxygen Transport after Blood Loss. MILES J. EDWARDS,* AND BELLE CANON,* Portland, Oreg. (introduced by J. David Bristow).

We studied the effect of mild blood loss on blood's affinity for oxygen, designated by its inverse expression, the P_{50} (Po_2, at 50% oxygen saturation of hemoglobin). A high P_{50} would improve oxygen unloading in the tissues. We also correlated the P_{50} with two of its known determinants; (*a*) red cell 2,3-diphosphoglycerate (DPG) and (*b*) the proportion of red cells which are young, indicated by the activity of red cell glutamic oxaloacetic transaminase (GOT), an enzyme present only in young red cells. We removed 250–750 ml blood over 1–3 wk from normal young adult nonsmoking males, taking blood samples for study 1–3 wk later. Hemoglobin concentrations decreased

(mean 16.0–14.8 g/100 ml) but remained within the normal range. The P_{50} increased significantly (mean 28.2–30.1 torr) and correlated with increased red cell GOT, indicating the presence of greater numbers of young red cells. DPG did not change significantly (mean 13.1–13.0 μM/g hemoglobin). The increased P_{50} which followed blood removal is presumably the result of increased erythropoietic activity, thereby increasing the relative numbers of young red cells in blood. Young red cells have a high P_{50}. We have calculated that the improved oxygen unloading afforded by the increased P_{50} more than compensated for the decreased hemoglobin concentration so that cardiac output could decrease and still maintain an unchanged normal mixed venous P_{O_2}. This may help explain the previously reported decrease in frequency of angina pain in patients with angina pectoris after phlebotomy (Burch and dePasquale. 1963. *Arch. Intern. Med.* 111: 687). (Research supported in part by NIH Grant HE-12249.)

86. Effect of Water Loading on Pulmonary Extravascular Water Volume (PEWV) with and without Norepinephrine Infusion. JAMES H. ELLIS* AND JOHN F. MURRAY, San Francisco, Calif.

We tested the hypothesis that water-loading animals with peripheral vasoconstriction would cause increases in PEWV owing to redistribution into the lungs of the infused volume. PEWV was measured by the multiple indicator dilution technique in 16 anesthetized (pentobarbital) dogs using albumin-^{125}I, THO, and indocyanine green. Initial measurements of PEWV were made in all animals and were repeated immediately before and after water loading (20 ml/kg over 10 min). Vasoconstriction was induced in nine dogs by intravenous norepinephrine infusion (0.1–0.4 μg/kg per min) that was continued during water loading. Seven animals were water loaded without norepinephrine administration. Control animals had a mean PEWV of 4.64±0.85 (SD) ml/kg initially, 4.89±0.69 ml/kg before and 5.22±1.16 ml/kg after water infusion. The increase in PEWV after water infusion was not statistically significant ($P > 0.20$). Cardiac index increased 56% from 160±30.2 to 249±39.5 ml/kg per min ($P < 0.001$) after water infusion; mean pulmonary artery and left atrial pressures increased 5.7±1.7 mm Hg and 2.8 ±0.4 mm Hg, respectively. Animals given norepinephrine had a PEWV of 4.70±0.75 ml/kg initially, 4.94±0.92 ml/kg before, and 5.34±0.92 ml/kg after water infusion. The increase in lung water after water loading was statistically significant ($P < 0.01$). Water loading produced an 87% increase in cardiac index from 166±31.6 to 311±88.5 ml/kg per min ($P < 0.001$) without significant differences in pulmonary artery or left atrial pressures compared to those of controls. We conclude that virtually none of an acutely administered water load is detectable in the PEWV of control animals, but in the presence of vasoconstriction, lung water increases significantly. These finding may have clinical relevance to fluid administration in critically ill patients. (Supported by research grants HE-06285, HE-05705, and HE-14201-SCOR from NIH.)

87. Ouabain-Calcium Interaction in Myocardial Microsomal Membranes. MARK L. ENTMAN,* JULIUS C. ALLEN,* AND ARNOLD SCHWARTZ,* Houston, Tex. (introduced by Rubin Bressler).

Cardiac glycosides are thought to exert their cardiotonic effect by increasing myocardial calcium concentration in systole. It has been suggested that this action depends on digitalis binding to a specific binding site on the membrane, Na^+, K^+-ATPase. A microsomal membrane fraction was isolated from dog heart which had the ability to bind calcium and also contained ouabain-inhibitable Na^+, K^+-ATPase (12–20, μmoles/mg per hr). Several interactions between calcium and ouabain were observed. (*a*) Ouabain (10^{-6} M) stimulated ATP-induced calcium exchange (29.9 vs. 21.1 nmoles/mg) but not ATP-dependent calcium binding (38.2 vs. 36.3). (*b*) Calcium (10–100 μM) stimulated specific ouabain-^{3}H binding (50–100%) to the preparation at pH 6.8 (which is optimal for calcium binding). The basic characteristics of this binding were the same as those described for "purified" Na^+, K^+-ATPase in the presence of standard ligand conditions (ATP, Mg^{++}, and Na^+). (*c*) Endogenous calcium (10 μM) did not affect the N^+, K^+-ATPase but it prevented its inhibition by ouabain (10^{-6}–10^{-3} M). Removal of the calcium by EGTA chelation restored the ability of ouabain to inhibit the Na^+, K^+-ATPase activity. The coexistence of Na^+, K^+-ATPase and calcium-binding activity in the preparation was required for these interactions; i.e., ouabain does not affect calcium exchange in fractions lacking Na^+, K^+-ATPase and calcium does not alter the ouabain inhibition of "purified" Na^+, K^+-ATPase (the latter lacks calcium-binding activity). This represents in vitro evidence that cardiac glycosides may act by binding to Na^+, K^+-ATPase and effecting augmented myocardial calcium flux. While the exact cellular location of the membrane preparation is unknown, the presence of sarcolemma is probable. (Research support by grants from NIH and American Heart Association.)

88. The Induction of Interferon by Vaccinia Antigen In Vitro: Lymphocyte-Macrophage Interaction and Effect of Reimmunization with Vaccinia Virus. LOIS B. EPSTEIN,* DAVID A. STEVENS,* AND THOMAS C. MERIGAN, San Francisco and Stanford, Calif.

Previous studies demonstrated that the nonspecific mitogen, phytohemagglutinin (PHA), induces interferon (IF) in combined lymphocyte-macrophage cultures from normal individuals. Purified protein derivative (PPD) also induces IF in similar cultures but only when derived from sensitized donors. In both instances IF production was associated with transformation of small lymphocytes to blastlike cells. In view of these results and the observation that live virus induces IF from leukocytes in vitro, the present studies were undertaken to determine whether a viral antigen (inactivated vaccinia virus) could induce transformation and IF production in combined cultures. Cultures were prepared from donors who had been vaccinated with live vaccinia virus from 2 to 30 yr previously. The cultures were harvested at 5–9 days after addition of heat-killed vaccinia virus (VH). Although there was significant lymphocyte transformation, no IF was detected in cultures from five of six donors. These five donors were reimmunized with live vaccinia virus and combined cultures prepared from 4 to 22 wk later. In

all, significant IF titers were detected, with the maximum (11–44 U) occurring at 4 wk. Despite this increase in IF after reimmunization, no significant rise in lymphocyte transformation was noted. As was the case with PHA and PPD, the presence of autochthonous macrophages greatly enhanced the production of IF in VH-treated cultures over that observed with cultures of lymphocytes alone. In addition, VH-induced IF had the same physical-chemical characteristics as did the PHA- and PPD-induced IF. (Research supported by NIH Grants CA 11067-03, AI 05629-09.)

89. The Effect of Fetal Thyroidectomy on Thyroid Kinetics in Fetal and Maternal Sheep. ALLEN ERENBERG,* KEIICHIRO OMORI,* WILLIAM OH,* AND DELBERT A. FISHER, Torrance, Calif.

Thyroid hormone kinetics and turnover have recently been reported in fetal sheep. The present study assesses the effect of fetal thyroidectomy on thyronine (T4) metabolism. Fetal sheep were thyroidectomized at 90–110 days' gestation. After placement of exteriorized catheters, tracer doses of T4-^{125}I and T4-^{131}I were injected into the fetus and mother, respectively, 4–30 days post-thyroidectomy. Serial blood samples were drawn for 72 hr during maternal perchlorate administration. Serum butanol extracts were prepared for dual isotope counting. From this data the maternal and fetal volumes of T4 distribution, half-times of T4 turnover, and mean fractional T4 clearance rates were estimated to be (12.6 and 0.97 liters, 1.22 and 0.99 days, and 0.50 and 0.77, respectively), values similar to those in nonthyroidectomized animals. Mean fetal serum T4 levels fell from a prethyroidectomy value of 12.2 μg/100 ml to 1.7 μg/100 ml on day 3 post-thyroidectomy; maternal levels did not change. Mean maternal and fetal T4 turnover values were 13.5 and 8.5 μg/kg per day (normal 5.5 and 40), respectively. Fetal serum thyroid-stimulating hormone (TSH) values were 300–1500 μU/ml 20–29 days post-thyroidectomy, confirming fetal hypothyroidism. Placental transfer of T4 was bidirectional with < 1 μg/day being transferred in the maternal to fetal direction. The high fetal to maternal TSH and maternal to fetal T4 gradients indicate that the fetal pituitary-thyroid system is autonomous and that placental T4 transport is inadequate for fetal needs. (Research supported by grant HD 04270-04 from NIH.)

90. An In Vitro Model of Gluten-Sensitive Enteropathy (GSE): Evidence that Gluten Is Not Directly Toxic to Gastrointestinal (GI) Epithelium. Z. MYRON FALCHUK,* CLEMENTINE SESSOMS,* AND WARREN STROBER,* Bethesda, Md. (introduced by J. E. Rall).

The toxicity of gluten in GSE may be direct or indirect. To study this question an in vitro model of GSE was sought. Jejunal biopsy specimens from patients with GSE and control individuals were maintained in organ culture for 48 hr. Alkaline phosphatase (p-tase) activities of specimens were obtained to estimate their functional state. P-tase activities in nine normal controls, and in nine disease controls with low base line p-tase levels, showed minimal increases during culture (mean: 1.6-fold). In contrast, specimens from seven GSE patients in relapse with initially low p-tase values, showed significantly greater increases in p-tase activity (mean: sevenfold) ($P < 0.001$) during culture with final values usually coming to normal. However, when specimens from patients in relapse were cultured in the presence of a peptic-tryptic digest of gluten (PT), no increase of p-tase activity was noted. This inhibition of increase of p-tase activity can be considered an in vitro model of GSE. We now turned to the question of the mechanism of gluten toxicity. Specimens from individual GSE patients obtained before and after in vivo gluten challenge were studied. In four patients before challenge, two- to threefold increases of nearly normal base line p-tase activities were unaffected by the presence of PT in the culture medium; in no case did activities fall. In contrast, after in vivo gluten challenge, cultures showed fivefold increases in activity during culture which could be abolished by the presence of PT. Thus, PT toxicity in vitro is expressed only after in vivo exposure to gluten, suggesting that gluten is not directly toxic to GI mucosa, but must first activate an endogenous effector mechanism.

91. Interbacterial Transfer of R Factor during Human Shigellosis. W. EDMUND FARRAR, JR.,* MARGENE EIDSON,* PATRICIA GUERRY,* STANLEY FALKOW,* LEWIS B. DRUSIN,* AND RICHARD B. ROBERTS,* Atlanta, Ga., Charleston, S. C., Washington, D. C., and New York (introduced by Joseph C. Ross).

Documentation of in vivo transfer of R factors in the human intestine, under conditions in which exogenous superinfection by resistant strains can be excluded, is rare. We have recently observed a probable instance of such in vivo transfer. A newborn infant acquired shigellosis during a nursery outbreak caused by a strain of *Shigella sonnei* resistant to streptomycin (Sm), tetracycline (Tc), and ampicillin (Am), designated strain R-1. After therapy with oral and intramuscular kanamycin (Km), he excreted organisms with the resistance pattern SmTcAmKm (strain R-2). Strain R-1 transferred all three resistance determinants during mixed cultivation with *Escherichia coli* K-12, regardless of which drug was used to select recipients, indicating that all three determinants were components of a single R factor. When strain R-2 was grown with *E. coli* K-12, most resistant recipients exhibited resistance patterns of SmTcAm or Km alone, indicating that R-2 possessed at least two factors. SmTcAmKm-resistant *E. coli* exhibited two distinct molecular species of extrachromosomal DNA, of 70 million and 43 million molecular weight. SmTcAm-resistant cells had only the 70 million molecular weight plasmid DNA, whereas cells resistant to Km only possessed only plasmid DNA of 43 molecular weight. These findings suggest that R-1 acquired a second R factor conferring resistance to kanamycin from another organism in the patient's gastrointestinal tract. To our knowledge this is the first report of acquisition of a second R factor by an already multiresistant organism during the course of a clinical infection. (Research supported by grants from CDC, National Science Foundation, and U. S. Army.)

92. Echocardiographic Detection of Dyskinetic and Akinetic Segments of the Left Ventricle. HARVEY FEIGEN-

BAUM, BETTY C. CORYA,* SONIA CHANG,* LEE L. KONECKE,* AND JOHN C. FISCHER,* Indianapolis, Ind.

Echocardiography provides a noninvasive means of visualizing internal cardiac structures. The purpose of this study was to see whether or not echocardiography could detect abnormally moving segments of the left ventricle. Echocardiograms were done on 25 normal individuals and on 20 patients with angiographically proven akinetic or dyskinetic left ventricular segments. The ultrasonic transducer was directed so as to record echoes from the true posterior left ventricular wall (PLV), the interventricular septum (IVS), the inferior left ventricular wall (ILV) near the posterior papillary muscle, and the anterior left ventricular wall (ALV). In the normal individuals, the PLV and ILV moved anteriorally about 1 cm during ventricular systole. The IVS and the ALV moved posteriorally about 0.5 cm during ventricular systole. In five patients with angiographically proven akinetic or dyskinetic septums, the IVS showed diminished, absent, or paradoxical motion while the PLV motion was increased. In two patients with angiographic dyskinetic anterior walls, the ALV demonstrated paradoxical motion. Three patients had diaphragmatic myocardial infarctions, and the ILV motion was reduced. In all three patients the IVS motion exceeded 0.5 cm. Five patients had akinetic or dyskinetic segments involving the apex or lateral walls. Although these areas were not visualized on the echocardiogram, indirect evidence was suggested by exaggerated motion of the IVS and/or the PLV. In five patients the echocardiograms were technically unsatisfactory. These results indicate that echocardiography can detect akinetic and dyskinetic segments of the left ventricle both directly, by recording diminished echo motion of the involved areas, and indirectly, by noting exaggerated motion in the remaining noninfarcted muscle. Unfortunately, satisfactory echograms could not be obtained on all patients. (Supported by NIH grants HE-09815-06, HE-6308, HTS 5363, HE-5749, and the Indiana Heart Association.)

93. Evidence for the Existence of Two Classes of Glucocorticoid Receptors in Rat Kidney. DAVID FELDMAN,* JOHN W. FUNDER,* AND ISIDORE S. EDELMAN, San Francisco, Calif.

Previous studies documented the properties of high affinity cytoplasmic aldosterone receptors (type 1) in the rat kidney. We recently demonstrated the presence of high affinity cytoplasmic glucocorticoid receptors (type 2) which bind dexamethasone (DM) somewhat better than corticosterone (B). We now present evidence for the existence of a third class of renal cytoplasmic receptors (type 3) which have a high affinity for B and very low affinities for both DM and aldosterone. The relative affinities of the type 3 receptors were: B > cortisol > deoxycorticosterone > progesterone > aldosterone > DM = spirolactone. By Scatchard analysis, the equilibrium dissociation constant of the type 3-B complex was 3×10^{-8} M (25°C). In density gradients this complex sedimented at 4S or in low Ca^{++} at 8S. In kidney slices, type 3 receptors are differentially distributed, the papilla having twice the concentration of the cortex. Specific nuclear uptake occurs in both zones of the kidney even when type 1 and 2 receptors are blocked, and is proportional to the type 3 receptor concentration. The nuclear complex thus generated sediments at 3S. The type 3 receptors are distinguishable from plasma transcortin by virtue of differences in rates of dissociation, kinetics of binding in vivo, ion dependence of sedimentation coefficient, and nuclear uptake. The evidence indicates, therefore, that the mammalian kidney contains one mineralocorticoid and two glucocorticoid receptors and we propose that each receptor regulates distinct functions. Current efforts are directed towards ascertaining the separate physiological roles of these receptors. (Supported by NIH Grants HL-06285 and HL-05725.)

94. Gluconeogenesis in Human Diabetes: Evidence of a Primary Abnormality in Hepatic rather than Peripheral Processes. PHILIP FELIG,* JOHN WAHREN,* EROL CERASI,* AND ROLF LUFT,* New Haven, Conn. and Stockholm, Sweden (introduced by L. R. Freedman).

Increased gluconeogenesis has long been recognized in severe ketotic diabetes. The mechanism of glucose overproduction and whether augmented gluconeogenesis occurs in milder diabetes has not been established. The present study was undertaken to characterize the gluconeogenic pattern in nonketotic diabetics, and to determine if an abnormality in intrahepatic processes or augmented peripheral release and availability of glucose precursors is the primary metabolic defect responsible for augmented gluconeogenesis. Splanchnic and leg exchange of individual amino acids, lactate, pyruvate, and glucose was determined by arterial, hepatic venous, and femoral venous catheterization in seven nonketotic diabetics (24 hr after insulin withdrawal) and in 29 healthy controls. Although splanchnic glucose output did not differ significantly between groups (diabetics, 3.0±0.2 mg/kg per min; controls, 2.6±0.5 mg/kg per min), splanchnic uptake of alanine, other glycogenic amino acids, lactate, and pyruvate was 50–150% greater in the diabetics. Total uptake of glycogenic substrates could account for 32% of hepatic glucose output in the diabetics but for only 16% in the controls. The greater uptake of glucose precursors in the diabetics was due to a two- to threefold increment in the splanchnic fractional extraction of these substrates. In marked contrast, the arterial levels of alanine and other glycogenic amino acids were reduced by 20% in the diabetics, while their output from the leg was equivalent to that in controls. Conclusions: In nonketotic diabetics total splanchnic glucose output is not elevated but the relative contribution from gluconeogenesis is increased twofold. The augmented gluconeogenesis is a consequence of increased hepatic extraction of glucose precursors resulting secondarily in depletion of circulating substrate. These results suggest that the liver rather than the periphery is the primary site of the gluconeogenic abnormality in diabetes.

95. Possible Mechanism of Compensated Hemolysis in Hereditary Spherocytosis. LOUIS A. FERNANDEZ* AND ALLAN J. ERSLEV,** Philadelphia, Pa.

Compensated hemolysis is frequently observed in patients with hereditary spherocytosis (HS) and characterized by an accelerated erythropoiesis despite a normal hemoglobin. Since tissue hypoxia is the usual driving force for erythro-

poiesis it has been suggested that the oxygen affinity of red cells is high in HS causing tissue hypoxia. The mean P_{50} of 15 normals (Hgb: 15.1, Retic: 1.6, MCHC: 34.1) was 27.8 mm Hg and the mean P_{50} of 12 HS patients (Hgb: 13.3, Retic: 9.9, MCHC: 37.9) was 28.4 mm Hg. Although the 2,3-diphosphoglycerate (2,3-DPG) of the red cells was lower in HS (2.65 mM/liter) than in normals (4.66 mM/liter), the effect of 2,3-DPG on the P_{50} was apparently cancelled out, possibly by the effect of a high MCHC. In any case, it appears that compensated hemolysis cannot be explained on a high oxygen affinity of red cells in circulating blood. However, it may be more relevant to consider the oxygen affinity of blood actually perfusing the oxygen sensor responsible for the regulation of red cell production. This oxygen sensor is believed to be located in the kidney and probably in the medulla which is relatively hypoxic. Since medullary blood is hyperosmolar, the P_{50} was measured in normal and HS red cells before and after exposure to hyperosmolar plasma (Na, 200 mEq/liter, Cl, 181 mEq/liter; and urea, 520 mOsmolar/liter). The P_{50} of red cells from eight normals showed a decrease of 1.3 mm Hg, while the P_{50} of red cells from six patients with HS showed a decrease of 4.7 mm Hg. Consequently, the oxygen affinity of red cells from patients with HS is higher than that of normals in a hyperosmolar environment and, if the oxygen sensor is located in the renal medulla, could explain the existence of compensated hemolysis in patients with HS.

96. The Effect of Prostaglandin (PGA_1) on Adrenal Function in Man. MARSHAL P. FICHMAN,* GLENN LITTENBERG,* JANNIE WOO,* AND RICHARD HORTON, Los Angeles, Calif.

Prostaglandins stimulate steroidogenesis in vitro. PGA_1, the only known prostaglandin not rapidly metabolized by the lung, was administered to 15 normal and 5 anephric patients. Aldosterone was measured by radioimmunoassay, cortisol by competitive binding, and renin (PRA) by bioassay. In 13 supine normal subjects given a subdepressor dose (< 0.6 μg/kg per min) by constant infusion for 1 hr, PGA_1 strikingly increased plasma aldosterone from 4.2±2.0 (SD) to 13.7±11.4 ng/100 ml ($P < 0.01$). However, plasma cortisol did not increase significantly ($P > 0.5$). Surprisingly, plasma renin (215±158) also did not increase (285±233 mμg/100 ml, $P > 0.4$). No change in serum Na and K was observed. 4-hr subdepressor infusions to normals further increased aldosterone, and were associated with fever and marked rises in cortisol without change in renin. No consistent changes in thyroid-stimulating hormone or growth hormone was observed. Dexamethasone (0.5 mg × 6 hr × 8) eliminated the fever and cortisol response, but did not prevent the rise in aldosterone. Depressor doses of PGA_1 (≤ 2.5 μg/kg per min) further increased aldosterone with variable increases in plasma renin in normals. 1-hr infusions of ≤ 0.6 μg/kg per min of PGA_1 in anephric patients increased plasma aldosterone in $\frac{4}{5}$ without changing cortisol, but in contrast to normals lowered blood pressure. These studies indicate that PGA_1 increased aldosterone independent of alterations in ACTH, renin, and serum electrolytes. PGA_1 may be an important regulator of aldosterone secretion in man. (Research supported by NIH.)

97. Altered Disease in Rats due to Mutants of Reovirus Type 3. BERNARD N. FIELDS* AND CEDRIC S. RAINE,* Bronx, N. Y. (introduced by M. D. Scharff).

Temperature-sensitive mutants of reovirus type 3 have been isolated and characterized genetically and biochemically. In contrast to the wild-type virus which produces an acute necrotizing encephalitis in rats infected by intracerebral inoculation, certain mutants induce a slowly progressive communicating hydrocephalus. Other mutants induce no overt disease. The biochemical defects in those mutants which induce hydrocephalus resides in the inability to assemble outer coat peptides. The assembled viral outer protein shell thus appears to be responsible for the lytic cell response and acute encephalitis due to wild reovirus type 3. Since pseudomyxovirus viral factories have been found in certain diseases such as subacute sclerosing panencephalitis and systemic lupus erythematosis, the use of well-characterized viral mutants could provide a model system for studying pathogenesis of certain slow virus infections and the possible virus etiology of autoimmune diseases. It is possible that mutations in genes coding for outer viral coat peptides are responsible for an altered virus-cell interaction in these diseases. (Supported by grants from the NIH, National Science Foundation, and ACS.)

98. Rapid Serum Bactericidal Assay by ^{51}Cr Release. JOSHUA FIERER* AND A. I. BRAUDE,** San Diego, Calif.

Complement-mediated destruction of Gram-negative bacteria is a major defense mechanism. We have devised a rapid, sensitive assay for serum bactericidal activity that directly measures the effect of serum on the bacterial cell wall. Bacteria grown in broth were resuspended in sodium chromate (Rachromate) for $\frac{1}{2}$ hr at 37°C. After washing in cold saline, labeled bacteria were added to serum in a ratio of 1:10. Portions were removed, filtered through Millipore filters (0.45 μ), and then counted in a gamma counter. Filterable radioactivity was expressed as per cent of total radioactivity of each sample after subtraction of activity released by saline control. The ^{51}Cr is associated with endotoxin as demonstrated by radioautography. Release of ^{51}Cr by normal serum began almost immediately and proceeded exponentially for 7 min until 70–80% of the label was filterable. This was accompanied by a 2 log fall in bacterial counts. Heated, hydrazine-treated, and EDTA-treated serum released only 5% of counts. Normal serum released only 28% of ^{51}Cr from a serum-resistant *Escherichia coli*. This assay is a direct measure of complement-mediated disruption of the cell wall, the process that leads to bacterial death and thus permits the demonstration of subtle defects in bactericidal activity. For example, diluting serum 1:4 caused a 40% fall in ^{51}Cr release but only 12% decrease in killing ability. This bactericidal assay is rapid and reproducible and avoids the errors inherent in colony counting. It is a direct measure of the action of serum on bacterial cell walls. (Supported by NIH Fellowship 1F03 A143980-01 and NIH Grant A.I. 10108-01.)

99. Human Growth Hormone: Evidence for Its Conversion or Secretion of a Second Factor. S. EDWIN FINEBERG* AND THOMAS J. MERIMEE, Boston, Mass.

Human growth hormone (HGH) is secreted throughout the life-span of normal individuals, but its physiologic role is unknown in the adult. To establish its function in the adult, acute metabolic effects were compared after intravenous infusions and arterial perfusions. The previously reported actions of HGH after intravenous administration were not seen when physiologic or pharmacologic concentrations were perfused across the tissues of the human forearm. (*a*) A 30 min intravenous infusion of HGH resulted in a mean plasma concentration of HGH of 79.7±6.4 ng/ml; low dose and high dose HGH perfusions achieved mean plasma concentrations of 29.7±2.6 ng/ml and 231.9±8.4 ng/ml. The intravenous infusion caused an early decrease of free fatty acid (FFA) concentration of 21.0±3.6% ($P < 0.05$) and at 2 hr, an increase to 146.0±7.3% of the mean control concentration. Neither intra-arterial dose had an early effect on FFA and only the high dose perfusion caused increased lipolysis at 2 hr. (*b*) Systemic glucose concentrations were lowered 9.5±1.9% ($P < 0.05$) by intravenous HGH; both low and high dose arterial perfusions failed completely to increase glucose uptake and actually caused a small decrease in glucose uptake across muscle. (*c*) K^+ uptake was stimulated acutely by low and high dose perfusions across peripheral muscle. Change in uptake per 100 ml of forearm tissue was, for low dose, 9.0±3.04 μmoles; and for high dose, 11.0±3.8 μmoles. The data demonstrate that the acute insulin-like effects of systemically administered HGH do not result from actions of HGH on peripheral tissues, regardless of the dose. The data are consistent with one of two interpretations: (*a*) HGH must be converted to a more active form, or (*b*) HGH stimulates the secretion of a second factor.

100. Interferon Induction and Development of Tissue Viral Resistance in Chickens. MARTIN S. FINKELSTEIN,* New York (introduced by A. Taranta).

Although interferon-inducing agents have been found effective in preventing experimental viral illnesses, their role is often ambiguous since they may also stimulate the immune and reticulo-endothelial systems, cause fever, inactivate viruses directly, interfere with viral replication by noninterferon means, and produce various toxic effects. These studies in chickens employed the following: two different interferon inducers (poly rI/rC and statolon), administered by different routes to stimulate interferon in the trachea or in the serum; infection in vitro of extirpated trachea and kidneys to measure tissue anti-viral resistance of a respiratory and visceral organ; and three different viruses with varying degrees of sensitivity to interferon to determine the specificity of resistance. Interferon titers were measured in the serum and in the tracheal scrapings, in order to relate tissue resistance with the amount of interferon present at the time of sacrifice and infection. It was found that: (*a*) interferon-like protection can be confined to specific organs, depending upon the interferon inducer used and its route of administration. (*b*) There is significant correlation between the degree of tissue protection with the amounts of interferon present in the serum or tracheal scrapings (particularly when large amounts of interferon are present), but the presence of small amounts of interferon, or the absence of detectable interferon frequently is not indicative of protection or a lack of tissue protection. And (*c*) the technique of testing the specificity and degree of viral resistance of extirpated organs provides an important index of the efficacy of interferon inducers administered in vivo in the protection against a specific infection. (Supported by USPHS Grant No. AI 09977-01 A1.)

101. Role of Pulmonary Surfactant in Pathogenesis of Emphysema in Smokers. THEODORE N. FINLEY, San Francisco, Calif.

The major hypotheses on the pathogenesis of emphysema, (*a*) alveolar overdistention due to obstruction and (*b*) weakness or tearing of the alveolar wall, make no mention of the acellular surface-active layer, pulmonary surfactant. This layer tends to prevent either alveolar collapse or overexpansion. From the considerations below a weakness and tearing of alveolar walls could be the direct result of airway obstruction and a breakdown in the surfactant lining of the alveoli due to smoking. The principal component of pulmonary surfactant is dipalmitoyl lecithin (DPL). A normal human lung at 7 liter lung volume, with alveolar surface area of 70 m^2, requires 210 mg of DPL for a packed monolayer (DPL area per molecule 35 A^2). Animal lavage studies yield about 0.5 mg of "alveolar" DPL per gram of lung. A 700 g normal human lung from a smoker or nonsmoker would be expected to contain 350 mg of alveolar DPL. From bronchopulmonary lavage data in eight nonsmokers, about 70% of alveolar DPL is free (noncellular) giving a value of 250 mg DPL. This would be more than enough to form a packed monolayer at all lung volumes. However, from lavage data in eight cigarette smokers. only 20% or 70 mg of alveolar DPL would be free. At all but the smallest lung volumes a DPL monolayer would not be tightly packed. During expansion of the alveolar surface a break in the DPL monolayer would occur in some alveoli. Factors that lead to overexpansion of such alveoli, such as airway obstruction, could cause a break in the protective surface film in smokers. In these alveoli direct contact to cell walls of noxious agents in cigarette smoke, proteolytic enzymes, bacteria, etc., would be possible. This could result in weakness and tearing of alveolar walls, i.e., emphysema.

102. Changes in Brain Amines after Portal Flow Diversion and Acute Hepatic Coma. JOSEF E. FISCHER,* J. HOWARD JAMES,* AND ROSS BALDESSARINI,* Boston, Mass. (introduced by W. Gerald Austen).

Previous work from this laboratory has suggested that the accumulation of false neurotransmitter amines may explain some of the pathophysiology of hepatic failure including some of the cardiovascular, renal, and central nervous system symptomatology. In the following experiments the origin of some of these amines is investigated and further changes in neurotransmitters are documented. Phenylethylamines, such as tyramine or alphamethyltyramine, were given by gastric tube to rats 2 months after portacaval shunt and their sham-operated controls. Animals were killed at 3 hr and brains and hearts were assayed for total and β-hydroxylated amines. The brains and hearts of the shunted animals contained 2–5 times the β-hydroxylated derivatives as that of the controls.

(Tyramine: sham 1.83±0.17, shunted 4.97±0.18 ng Octopamine/g heart.) (α-Methyltyramine sham 0.74±0.19, shunted 1.76±0.32 nCi α-methyloctopamine/brain, $P < 0.02$; for heart sham 7.8±1.4, shunted 38.8±4.7 nCi α-methyloctopamine $P < 0.001$.) These results strongly suggest that the increased accumulation of amines perhaps have their origin in the gut. Acute hepatic coma was prepared in rats by hepatic artery ligation 24 hr after end-to-side portacaval shunt. Animals were killed when moribund and brains and hearts were assayed for serotonin, norepinephrine, and dopamine. While dopamine was unchanged, norepinephrine was markedly decreased in the brains of the comatose rats: sham 318.1±13.9 (n = 21), coma 231.5±21.5 ng/g (n = 16) $P < 0.001$. Serotonin was markedly increased: sham 398±0.20, coma 517 ±0.30 ng/g (n = 10) $P < 0.002$. These findings further suggest that the recently reported efficacy of L-DOPA in the arousal of patients in hepatic coma may be due not only to its effects on restoring normal levels of the adrenergic transmitters, dopamine and norepinephrine, but also perhaps secondary to its recognized property of diminishing the accumulation of serotonin. To date L-DOPA has been administered to 11 patients in deep, unresponsive hepatic coma, with prompt awakening in 6, delayed response in 2, and no noticeable effect in 3. The characteristics of patients who showed a response as well as the cardiovascular changes secondary to L-DOPA administration will be discussed. (Supported by a Grant No. AM NS 15347-01 from NIH.)

103. Extracellular Products of Human Glomerular Cells in Tissue Culture. ALFRED J. FISH,* DAVID M. BROWN,* ROBERT L. VERNIER,** AND ALFRED F. MICHAEL, Minneapolis, Minn.

Although sites of biosynthesis are unknown, glomerular basement membrane (GBM) contains a collagen moiety with a high hydroxylysine/hydroxyproline ratio, a glucose-galactose moiety, and a heteropolysaccharide containing sialic acid; a polyanionic material is found on glomerular epithelial cells. Using autopsy kidneys from infants, sterile decapsulated glomeruli were isolated and explanted in tissue culture from which morphologically distinct "epithelial" cells surviving 13–15 passages were derived. By electron microscopy glomerular cells, when compared to fibroblasts, had larger rounded nuclei, multiple nucleoli, and more generous cytoplasm. The extracellular matrix between epithelial cells contains granular membranous material distinct from the fibrillar products adjacent to fibroblasts. Fluorescein-labeled antiserum to human GBM stained the extracellular material produced by both glomerular cells (GC) and fibroblasts (FB). Absorption of the anti-GBM serum with (*a*) purified GBM inhibited the staining of membranous material in GC, FB, and the GBM of normal human kidney (NHK) sections; (*b*) lyophilized washed FB inhibited the staining of FB membranes, partially inhibited that of GC, but did not inhibit NHK staining; and (*c*) lyophilized GC and FB inhibited staining of both FB and GC, but not NHK. These studies indicate that the membranous material from glomerular cells share antigenic determinants with GBM. Glomerular cell preparations contain 3-hydroxyproline and convert proline-^{3}H to hydroxyproline-^{3}H. No polyanionic material was detected in extracellular membranes with alcian blue or colloidal iron (pH 1.9), although positive staining was seen in extracellular globules. We hypothesize that human glomerular cells in tissue culture are capable of synthesizing some component(s) of GBM and the glomerular polyanion. (Supported by USPHS, Minnesota Heart Association, the Minnesota Medical Foundation, Twin Cities Diabetes Association, and the American Heart Association.)

104. Nucleotide Sequence Analysis of Human Hemoglobin Messenger RNA. BERNARD G. FORGET* AND CHARLES A. MAROTTA,* Boston, Mass., and New Haven, Conn. (introduced by Sherman M. Weissman).

Hemoglobin messenger RNA (mRNA) has previously been demonstrated in the 9S RNA region of total reticulocyte RNA fractionated by means of sucrose gradient centrifugation. We have isolated 9S RNA, free of all other RNA, by polyacrylamide-gel electrophoresis of total reticulocyte RNA, and the gel-purified 9S RNA was shown to stimulate amino acid incorporation by a cell-free protein synthesizing system. 9S RNA and other RNA components were digested with T1 ribonuclease and labeled by transfer of the terminal phosphate of γ-^{32}P-labeled ATP by means of polynucleotide kinase prepared from phage T4 infected *Escherichia coli* cells which were deficient in their ribonuclease content. Two-dimensional oligonucleotide maps suitable for further sequence analysis were obtained from less than 0.2 μg of RNA; thus the method could be applied to the analysis of small quantities of mRNA isolated from reticulocytes of patients with sickle-cell anemia and other hemoglobinopathies. Rabbit reticulocyte 9S RNA could be separated into two bands by polyacrylamide-gel electrophoresis. Each component was shown to given nearly identical oligonucleotide maps which were similar to the fingerprint obtained from the single 9S RNA of reticulocytes from patients with sickle-cell anemia. By contrast the patterns given by 28S and 18S RNA were very different. This approach was shown to be applicable to the analysis of the mRNA of thalassemic cells and provides the first direct chemical approach to fine structure analysis of the hemoglobin genes in man. (Research supported by grants from the NIH and ACS.)

105. Mechanism of Action of Arabinosyl Cytosine (ara-C): Assignment of Replicative Function to a Human DNA Polymerase. R. M. FOX,* G. V. RAMA REDDY,* AND M. GOULIAN, La Jolla, Calif.

Recently, multiple DNA polymerases have been found in both animal and microbial cells; however, their precise functions remain largely unknown. Studies employing arabinosyl cytosine triphosphate (ara-CTP) to distinguish biological roles of microbial enzymes in repair or replication of DNA, led to a similar investigation with human enzymes. Two DNA polymerases were purified from a permanent diploid line of cultured human lymphocytes. Similar to other results with animal cells, the two enzymes were distinctive in cellular location, behavior on DEAE-cellulose, optima for pH and [Mg^{++}], and response to SH inhibitors. One of the polymerases of nuclear origin was unaffected by ara-CTP, in contrast to the marked inhibition of a second poly-

merase of predominantly cytoplasmic location. Ara-C specifically inhibits DNA replication of intact cells, and its principal form in the cell is known to be the triphosphate. Therefore, the inhibition of the "cytoplasmic" DNA polymerase by ara-CTP permits the tentative assignment of replicative function to that enzyme, despite its parodoxical location. Additional support for this interpretation comes from studies with human peripheral blood lymphocytes, in which phytohemagglutinin-stimulated DNA synthesis, like normal replication, is inhibited by ara-C. Correlated with stimulation by phytohemagglutinin is an increase in the level of "cytoplasmic" enzyme but not "nuclear" enzyme. These results combined with studies on several microbial enzymes identify a class of ara-CTP-inhibitable DNA polymerases, which appear to have an essential role in replication. This also clarifies older observations, made before recognition of the multiplicity of DNA polymerases, that ara-CTP inhibits mammalian but not microbial DNA polymerases. (Supported by USPHS [5-R01-CA11705], ACS [P-567], and NHMRC [Australia].)

106. Islet Phospholipogenesis and Glucose-Stimulated Insulin Secretion. NORBERT FREINKEL AND NASSER COHANIM,* Chicago, Ill.

Glucose-stimulated insulin secretion may be subdivided into: effects of glucose or glucose metabolites upon islet "receptor" → increased Ca^{++} flux → microtubular realignments and emiocytosis. To assess associated membrane events, we have correlated insulin secretion by isolated rat islets with concurrent incorporation of orthophosphate-^{32}P into the choline (PC), ethanolamine (PE), and inositol (PI) phosphoglycerides. Incubation with 50 mg/100 ml glucose did not stimulate insulin release although ^{32}P incorporation increased as incubation was prolonged (PC = PE > PI). Increasing glucose to 100 mg/100 ml stimulated insulin release minimally coincident with qualitatively different increases in the relative rates of phospholipogenesis (PE > PC = PI). At 200–300 mg/100 ml glucose, labeling of PC and PE plateaued; PI increased in parallel with increasing insulin secretion at 300–500 mg/100 ml glucose. To document that effects upon PI and PE were linked to "stimulated" insulin secretion, responses to 50 vs. 300 mg/100 ml glucose were correlated in the following situations. Islets from 48-hr fasted rats exhibited less stimulated insulin secretion than fed animals coincident with reduced PE and PI labeling but unchanged PC. Islets from 48-hr fasted, 24-hr refed rats displayed greater stimulated insulin secretion coincident with greater increases in PE and PI than PC. Islets "pulse-labeled" with ^{32}P exhibited greater turnover of PI than PC during "chase" with ^{31}P and 300 mg/100 ml glucose. Contrariwise, incubation without Ca^{++}, or with tubulo-active agents (colchicine) blocked stimulated insulin secretion but did not affect PI or PE selectively. Thus, the selective effects upon PE and PI may occur before the Ca^{++} and microtubular aspects of the secretory sequence. We conclude that "triggering" insulin secretion in islets with glucose elicits the same changes in selected acidic phosphoglycerides that characterize secretory stimulation in other structures and may connote some primary perturbation of a membrane receptor. (Supported by USPHS AM-10699 and AM-05071.)

107. Effect of Differentiated Myeloid Cells on Hematopoietic Stem Cell (HSC) Proliferation. W. FRIED, W. H. KNOSPE, AND F. E. TROBAUGH, JR., Chicago, Ill.

Previous studies showed that HSC transplanted into mice given cytoxan 4 days before X-irradiation regenerate faster than those in mice which were only irradiated. The factors, responsible for this enhancement of HSC replication, were studied. Five mg of cytoxan was injected into CF_1 mice and controls received saline. 1–10 days afterward they received 1000 R X-irradiation and 10^6 marrow cells. HSC in their femoral marrow were assayed at various times afterward by the spleen colony method of Till and McCulloch. Injection of cytoxan 4 days before X-irradiation significantly enhanced regeneration of transplanted HSC, whereas injection 7 days before resulted in a significant delay of HSC regeneration. The number of HSC and nucleated marrow cells were determined, and differential cell counts were done 1 hr after X-irradiation on femoral marrow cells of mice which received saline or cytoxan 4 or 7 days before X-irradiation. The nucleated cell count, number of HSC, and number of differentiated cells of mice given cytoxan 4 days before radiation were less than in controls which received saline only. In those treated 7 days preradiation the number of HSC remained less than control, the nucleated cell count was no different from control, but differentiated cell counts revealed 85% of the marrow cells to be differentiated myeloid cells (bands and segs) as compared to < 50% in the control group. These results suggest the conclusion that the proliferation of hematopoietic stem cells is modulated by cell: cell interactions with mature myeloid elements in the milieu. (Research supported by grants from NIH, ACS, and Leukemia Research Foundation.)

108. Hemodynamic Identity of Progressing Essential (Man) and Spontaneous (Rat) Hypertensive Heart Disease. EDWARD D. FROHLICH* AND MARC A. PFEFFER,* Oklahoma City, Okla. (introduced by Eugene D. Jacobson).

Left ventricular functional characteristics of progressing hypertensive heart disease have not been shown clinically or experimentally, although studies in essential hypertensive man demonstrated group hemodynamic differences even before cardiac failure. Because of ethical considerations in pursuing these studies in uncomplicated essential hypertension with developing left ventricular hypertrophy (LVH), 20 young (Y; aver. 24 wk) and 10 old (O; aver. 77 wk) spontaneously hypertensive rates (SHR) were compared with the same number of age- and sex-matched normal Wistar controls (NCR). Measurements were made under ether anesthesia using electromagnetic flow probes (ascending aorta) and superior vena cava (SVC) and aortic catheters. Aortic maximum flow acceleration (Accel) and peak velocity

Rats	MAP (mm Hg)	Heart rate (beats/min)	CO (ml/min)	LVER (ml/sec)	Accel (ml/sec²)	Peak-Q (ml/sec)	LV: body wt ($\times 10^{-3}$)
NCRY	85	342	95	3.36	250	5.28	1.97
SHRY	115*	397*	90	2.98*	251	5.22	2.81*
NCRO	85	270	71	2.90	245	4.75	1.98
SHRO	114*	356*	65	2.27*	174*	3.75*	3.55*

* Indicates at least $P < 0.01$ significance; CO = cardiac output; LVER = left ventricular ejection rate.)

(Peak-Q) were used as indices of left ventricular contractility. There was no evidence of pulmonary or hepatic congestion, and SVC pressures were normal. Thus, the characteristics of the young and old SHR were very similar to essential hypertensive man with left atrial hypertrophy (early LVH) and gross LVH, respectively. The data also demonstrate that left ventricular function is significantly and progressively impaired in LVH resulting from systemic hypertension, even before development of cardiac decompensation.

109. Serum Precipitins in the Diagnosis of Disseminated Candidiasis. J. DAVID GAINES* AND JACK S. REMINGTON,** Palo Alto, Calif.

Although the problem of disseminated candidiasis (DC) is well recognized, less than 50% of cases are diagnosed premortem. A rapid, reliable means of diagnosis of infection with *Candida* is needed to allow for early instigation of therapy. A prospective study was performed to define the usefulness of the *Candida* precipitin test for diagnosis of DC in a university and community hospital population. Precipitins were found in 26 (27%) of 95 patients with suspected DC. In 21 (80%) of the 26 the diagnosis of DC was subsequently established. The most common underlying condition in these cases was multiple surgical procedures. Five patients with positive precipitins were lost to follow-up. Two patients with DC (one with terminal leukemia and one with multiple abdominal surgery) had no precipitins. Two leukemics with candidemia just before death had no precipitins and no evidence of DC at postmortem. Cross-reactions due to other fungal infections were not observed; seven of the precipitin negative patients were found to have aspergillosis and one, coccidioidomycosis. There were no positive reactions among a general outpatient population used as controls. Precipitin lines in conventional immunodiffusion methods often did not appear for 48–72 hr. Development of a method which employs a combination of electrophoresis and immunodiffusion provided results in all cases within 2 hr, was simple to perform, and agreed perfectly with the Ouchterlony method. This method is easily adaptable for use in clinical laboratories.

110. First Step in Digitalis Action: Direct Study of Ouabain Binding to the Plasma Membrane. JERRY GARDNER,* THOMAS CONLON,* AND CATHERINE FRANTZ,* Bethesda, Md. (introduced by Leonard Laster**).

The initial step in the mechanism of action of cardiac glycosides is thought to involve an interaction with the plasma membrane. To characterize this interaction we have investigated the binding of ouabain-^{3}H to the plasma membrane of intact human erythrocytes. We have found that ouabain binding is reversible, does not depend on cellular energy metabolism, exhibits a high degree of chemical specificity, and can be detected at ouabain concentrations as low as 1×10^{-10} mole/liter. Ouabain binds to a single class of glycoside binding sites via a saturable reaction. There are 1200 glycoside binding sites per cell and the ouabain concentration at which half-maximal binding occurs (K_B) is 1.5×10^{-7} mole/liter. Both monovalent and divalent cations act at sites which are functionally distinct from the glycoside binding site and their effect is to alter the affinity of the glycoside site for ouabain. Furthermore, the site at which monovalent cations exert their effects on ouabain binding is functionally distinct from that site at which divalent cations exert their effects. Na, Li, and Cs increase ouabain binding ($K_B = 5.0 \times 10^{-10}$, 9.7×10^{-9}, and 2.8×10^{-8} mole/liter, respectively), K and Rb decrease ouabain binding ($K_B = 2.6 \times 10^{-7}$ and 1.8×10^{-7} mole/liter, respectively), and Ca, Ba, and Mg increase ouabain binding ($K_B = 8.3 \times 10^{-8}$, 4.5×10^{-8}, and 1.7×10^{-8} mole/liter, respectively). In addition to altering the affinity of the glycoside site, divalent cations also regulate the affinity of the monovalent site for monovalent cations. These observations indicate that the initial action of cardiac glycoside is association with a membrane "receptor complex" which has three functionally distinct sites: a monovalent cation site, a divalent cation site, and a glycoside binding site. The particular cation-determined functional configuration of the membrane complex, in turn, determines the affinity of the glycoside binding site for ouabain.

111. Nephron Structure and Function in Precystic Kidneys of Rats Fed Diphenylamine. KENNETH D. GARDNER, JR.,* AND SIDNEY SOLOMON,* Honolulu, Hawaii (introduced by Roy H. Maffly).

To document pathophysical factors responsible for cyst development in affected mammalian kidneys, standard microdissection and micropuncture techniques were used to examine anatomical and functional properties of precystic nephrons in rats fed 1% diphenylamine for 5–9 months. Latex castings disclosed focal narrowing and dilatation of proximal and distal nephrons. Pressures in vivo in 30 visibly dilated proximal tubules in 10 precystic kidneys averaged ($\pm$SE) 26.2$\pm$1.6 cm water. Pressures in 24 relatively undilated tubules in the same kidneys averaged 19.6$\pm$0.8 cm water. The difference between means was significant ($P < 0.001$). When individual proximal tubules were injected with aqueous lissamine green and tritiated inulin, two excretory patterns were observed. One was characterized by prompt excretion of green highly radioactive urine from the injected left kidney. It followed injection of 6/19 tubules in seven precystic kidneys and 12/12 free-flowing tubules in seven control rats. The second was characterized by the absence of dye-tinged urine and the excretion of less highly radioactive urine by both kidneys. It followed injection of 13/19 precystic nephrons and was produced intentionally in 6/6 normal nephrons by preliminary partial occlusion with oil, thus implicating obstruction as a cause for the two patterns of dye-isotope excretion that were observed in precystic kidneys. Dye-isotope excretion correlated with intratubular pressure ($r = -0.698$; $P < 0.001$) but not with urinary flow within the range observed (1.64 – 15.98 μl/min). These results delineate two populations of nephrons in the precystic kidneys of diphenylamine-fed rats and support a concept that obstruction and increased intratubular pressure participate in cyst formation in the susceptible mammalian kidney. (Supported by NIH Grant HE 13137.)

112. Induction of Nasal Interferon by Rhinoviruses and a Topical Drug. BIENVENIDO G. GATMAITAN,* EDITH D. STANLEY,* AND GEORGE GEE JACKSON,** Chicago, Ill.

Rhinovirus (RV) 21, passage 3, induced nasal interferon (IF) when administered intranasally to volunteers. Virus shedding from the nose preceded interferon production, which was measurable on day 2, reached peak titer on days 4–6, and sharply declined thereafter. In antibody-free subjects the pattern of virus shedding was not altered by the appearance or titer of IF. In three subjects with preexisting serum antibodies, virus excretion was inversely related to peak IF titers. Persons in whom nasal IF was measurable had an ameliorated illness compared to those with infection who failed to produce IF. The severity of the symptoms, however, was not related to the titer of IF produced. A passage 14, "attenuated" strain of RV 29 induced similar IF responses. A chemical inducer (*N,N*-dioctadecyl-*N,N'-bis* (2 hydroxyethyl) propanediamine), administered by intranasal spray, induced nasal IF, similar to virus administration. Application of the drug 24 hr before RV challenge did not prevent infection. The inducer and virus were additive in elevating the IF titer in nasal secretion. Thus, IF which appears in nasal secretion after RV infection, or a chemical inducer may diminish symptoms, but the IF titers reached did not prevent RV infection or change the patterns of virus shedding.

113. Hormone-Receptor Interactions in Circulating Cells: Studies in Normal and Pathologic States in Man. JAMES R. GAVIN,* JUANITA A. ARCHER,* MAXINE A. LESNIAK,* PHILLIP GORDEN,* AND JESSE ROTH, Bethesda, Md.

Since polypeptide hormones exert their effects on cell receptors, the accessibility of the circulating cell makes this tissue desirable for studies in man. We find that circulating and cultured human lymphocytes have receptors for insulin that have the same affinity and biologic specificity for insulin as the receptors studied in liver and fat cells of rats. To determine whether the number of receptors and/or the affinity of the receptor for insulin is the same or different in normal individuals, acromegaly, obesity, and hypopituitarism, the maximal binding of insulin-^{125}I (B_{max}) and the half-maximal displacement of insulin-^{125}I ($\frac{1}{2}$ B_{max}) by unlabeled insulin have been determined. In the 8 studies in normals and 15 patient studies the mean range B_{max} equals 2.45–3.53% and the mean range $\frac{1}{2}$ B_{max} equals 6.5–9.4 ng/ml. The apparent affinity constant for the normals and patients equals 10^9 M^{-1} (limit of sensitivity = 1–2 ng/ml); no statistical differences could be demonstrated between the normals and the patient groups under these experimental conditions. In the cultured cell, however, an additional order of insulin receptor sites with an apparent affinity constant of 10^{10} M^{-1} has been detected, increasing the sensitivity to 0.1 ng/ml. With this method the purified insulin component of circulating insulin has the same biologic specificity as pancreatic porcine insulin, whereas the proinsulin-like component has a similar biologic specificity to porcine proinsulin. Specific growth hormone (HGH) receptors are also present in human cultured lymphocytes. HGH-^{125}I is bound to the cultured lymphocytes and displaced only by biologically active HGH. The ability of an HGH preparation to displace HGH-^{125}I from the lymphocyte receptor is directly proportional to the biological activity of the preparation. The sensitivity of this method is between 5 and 10 ng/ml of HGH. When plasma from an acromegalic patient is filtered on G-100 Sephadex (superfine), the major peak of immunoreactive HGH displaces HGH-^{125}I from the cultured lymphocytes in a manner indistinguishable from a purified pituitary HGH. These data indicate that it is now possible to study directly in man hormone receptors and the interaction of the hormone receptor with its circulating polypeptide hormone.

114. Complement Components C8 and C9 in Sera and Urine of Patients with Chronic Membranoproliferative Nephritis (CMPGN). H. GEIGER,* N. DAY,* AND R. A. GOOD,** Minneapolis, Minn. (introduced by Robert Ulstrom).

In chronic membranoproliferative nephritis (CMPGN) activation of the complement system, particularly through the alternative pathway, has been shown. Studies in CMPGN regarding the complement system focus mainly on C3 and its conversion. No information is available about C8 and C9 in sera of patients with CMPGN. To date, determinations of C8 and C9 in the urines of patients with renal diseases have not been reported. In this study hemolytic C8 and C9 were measured in sera and urines of 12 patients with CMPGN. In serum C8 was found to be significantly decreased in 8 patients. Only 3 patients showed significantly low C9 titers, whereas in 2 patients C9 was found to be elevated. The C8 and C9 titers did not always correlate with hemolytic C3. Hemolytic C9 could be measured in urine of all CMPGN patients; in 11 of 12 patients C8 was detectable in urine. Hemolytic activity of C8 and C9 in urine could be inhibited by EDTA, suramin, and heat inactivation (56°C, 30 min). When Clearance C8/Clearance C9 index was compared with Clearance IgG/Clearance Transferrin index low selectivity for the IgG/transferrin index (> 0.12) was found in 11 of 12 patients; whereas in 10 of 12 patients the C8/C9 index revealed low selectivity (> 0.05) when compared to cases of idiopathic nephrotic syndrome. Low selectivity using complement index was also found, in cases of chronic glomerulonephritis (5) and nephrotic syndrome (3) of unknown etiology, congenital nephrosis (2), lupus erythematosus (2), and kidney transplantations (3). In summary, hemolytic C8 and C9 can be measured regularly in unconcentrated urine of patients with proteinuria. Determination of C8/C9 index provides a reliable tool to detect low selectivity in cases with proteinuria. (Aided by NIH.)

115. Functional Mapping of the Hypothalamus: RNA Synthesis in Response to Hypertonic Saline. JACK M. GEORGE,* Columbus, Ohio (introduced by James V. Warren**).

As a new noninvasive approach to localization of hypothalamic function, we have performed radioautography on 9-μm serial sections of hypothalamus using rapid incorporation of cytidine-^{3}H into RNA as an index of polypeptide and protein synthesis. Two groups of seven mice each were given water (control) or 3% saline for 2 days and then each mouse was injected with 0.15 μCi cytidine-^{3}H intraperitoneally. The mice were killed 90 min later and radioautographs prepared. Treatment of histologic sections with ribonuclease before coating with photographic emulsion pre-

vented the appearance of > 95% of silver grains. Control mice had 0.6 silver grains/μm^2 (mean) ±0.2 (SE) in the supraoptic nucleus compared to 2.4 grains/μm^2 ±0.2 ($P < 0.001$) in the saline-treated mice. This result agreed with a previous pilot experiment involving four mice. Hypertonic saline is known to stimulate synthesis of vasopressin in the supraoptic nucleus. There was also a significant difference in cytidine-^{3}H incorporation in the ventromedial area adjacent to the third ventricle: control 4.0 grains/μm^2 ±0.3; saline 6.6 grains/μm^2 ±0.8 ($P < 0.01$). This area may be involved in reception and transmission of the hypertonic stimulus. There were no significant differences between control and saline groups in nine other hypothalamic areas counted. We conclude that dehydration is associated with increased incorporation of cytidine-^{3}H into RNA in precise areas of the hypothalamus. Functional mapping of hypothalamic response to stimuli is possible. (Supported in part by Bremer Foundation.)

116. Role of Complement in *Escherichia coli* Bacteremia in the Squirrel Monkey. DAVID N. GILBERT,* JACK A. BARNETT,* AND JAY P. SANFORD, Dallas, Tex.

Endotoxins are capable of activating the terminal components of the complement sequence in vitro and in vivo. Serum complement levels fall after intravenous administration of endotoxin. Complement activation might be either primary or coincidental in the pathophysiology of Gram-negative bacillary bacteremia. Availability of a nontoxic protein fraction of cobra venom which inactivates C3 made feasible in vivo studies on the influence of complement depletion on experimental *Escherichia coli* bacteremia. Squirrel monkeys (27 controls, 31 cobra factor [CoF]–treated animals) were depleted of complement (means CH_{50} levels: control 226 U, CoF-treated 31 U; means C3 levels: controls 187 U, CoF-treated 36 U) and lethal inocula of viable or heat-killed ^{32}P-labeled *E. coli* (4×10^9 organisms/kg) were administered intravenously. Striking neutropenia occurred rapidly in controls (the average leukocyte count decreased from 19,000/mm^3 to 3500/mm^3 with 1 min) while the rate of occurrence of neutropenia was 20–30 times slower in CoF-treated animals. This blunted response was paralleled by a decreased sequestration of neutrophils in the pulmonary circulation during the initial minutes postchallenge. In complement-depleted monkeys, the initial rate of clearance of either viable or ^{32}P-labeled *E. coli* was slower and resultant levels of bacteremia and endotoxemia (measured by actinomycin D-mouse assay) were significantly higher than in controls. The late defect in bacterial clearance was corrected by administration of fresh monkey serum. These observations are consistent with the following mechanism: phagocytosis of intravascular bacteria and detoxification of endotoxin is more efficiently performed by marginated neutrophils and margination is complement mediated. Thus, complement-mediated neutrophilic leukocyte function is an important host defense mechanism in Gram-negative bacillary bacteremia. (Research supported by grants from NIAID, NIH, USA R and D Comm., DOD.)

117. Decrease in the Hemolytic Anemia of Sickle Cell Disease after Administration of Sodium Cyanate. PETER N. GILLETTE,* CHARLES M. PETERSON,* JAMES M. MANNING,* AND ANTHONY CERAMI,* New York (introduced by Attallah Kappas).

Studies in these laboratories have recently shown that cyanate can inhibit the sickling phenomenon in vitro by specifically carbamylating amino-terminal residues of hemoglobin S (*Proc. Nat. Acad. Sci. U.S.A.* 1971. **68:** 1180). This observation has led to the initiation of clinical studies designed to evaluate the possible use of cyanate in the treatment of this disease. In a group of patients with sickle cell disease cyanate treatment in vitro of a small aliquot of erythrocytes was associated with an increase towards normal in the apparent 50% chromium-51 survival time when these cells were returned to the patient (*Proc. Nat. Acad. Sci. U.S.A.* 1971. **68:** 2791). If cyanate decreased sickling, then chronic administration would be expected to diminish the hemolytic anemia. Eight of ten patients with sickle cell disease receiving cyanate orally over 2–4 months showed a mean increase in hemoglobin from 8.5 to 10.7 g/100 ml and increased hematocrit from 26 to 31%. The maximum increase in blood indices was 40% of control values in one patient. Mean total serum bilirubin declined from 2.1 to 1.4 mg/100ml. Carbamylation (as moles of cyanate per mole of hemoglobin tetramer) achieved from a given oral dose was variable. The two individuals who did not respond to oral cyanate did show appropriate responses when treated intravenously. Toxic side effects observed were dose related, reversible, and well tolerated. These results demonstrate the hematologic efficacy of cyanate as a possible treatment for sickle cell disease and establish its short-term safety in humans. (Research supported by grants from NIH, National Science Foundation, the NF-MOD.)

118. Effect of Immunoregulatory Alpha-Globulin on Antigen and Mitogen Binding by Lymphocytes. A. H. GLASGOW,* S. R. COOPERBAND, K. SCHMID,* AND J. A. MANNICK, Boston, Mass.

We, and others, have demonstrated an immunosuppressive factor in plasma which may be isolated with the α-globulins. We have hypothesized that this factor serves a regulatory function in vivo and have called this factor immunoregulatory α-globulin (IRA). We have previously found that IRA inhibits graft rejection and primary and secondary antibody responses. In vitro studies have demonstrated that IRA inhibits blast transformation and proliferation induced by both antigens and mitogens, without cytotoxicity. The inhibition is easily removed by washing the lymphocytes, and IRA is ineffective if added after exposure to activating agent. These studies suggest that IRA acts upon the early events involved in antigen and mitogen activation. We have therefore examined the earliest event in activation—antigen and mitogen binding. Antigen binding by human blood or murine spleen lymphocytes was studied by: (*a*) rosette formation with sheep erythrocytes, alone, and as carrier for diptheria or tetanus toxoid or tuberculin, and (*b*) uptake of free BSA-^{125}I. Mitogen binding was studied with ^{125}I-labeled PHA and Concanavalin A. IRA at 1–3 mg/ml inhibited the binding of all antigens: 80% inhibition of rosette formation, and 60% inhibition of free antigen uptake. IRA did not interfere with antigen binding by preformed antibody (measured as agglu-

tinin titer). There was little inhibition of PHA binding and a minimal inhibition of Conconavalin A uptake, although lymphocytes cultured with IRA did not show the normal increased uptake of Conconavalin A which occurred with transformation. These studies suggest that IRA interferes with the earliest events of antigen uptake at the surface of lymphocytes, but not by altering the antigen or immunoglobulin. Since it does not alter mitogen uptake it must exert its antiproliferative action metabolically, and at a membrane site near the antigen-binding site, but distant from the mitogen-binding areas. (Research supported by grants from NIH and U. S. Dept. of the Army.)

119. Importance of Cell-Cell Interaction in the Proliferation of Human Hematopoietic Cells in Liquid Culture. DAVID W. GOLDE* AND MARTIN J. CLINE, San Francisco, Calif.

To examine the role of cellular interaction in the proliferation and differentiation of human hematopoietic tissue, normal and neoplastic human marrow cells were cultured in liquid suspension utilizing an in vitro diffusion chamber. The apparatus consists of a small glass bulb in which the cell suspension is separated from a large volume of medium by dialysis membrane. This system permits intimate cell contact while allowing for adequate nutrient supply and metabolic waste removal. At intervals, total and differential counts were performed and radioautographs prepared after incubation with thymidine-^{3}H. Under appropriate conditions, granulopoiesis and mononuclear cell proliferation persisted for several weeks in cultures of normal human bone marrow. Proliferation depended on cellular proximity provided by a high cell density within the chamber. This situation obviated the need for exogeneous stimulating factors required in semisolid systems. Growth of mononuclear cells on the dialysis membrane was a concomitant of all normal marrow cultures and may be necessary for the growth and differentiation of other cell lines. Maturation of early red cell precursors to erythrocytes was noted in cultures up to 1 wk old, but no proliferation of erythroid elements was observed. Leukemic cells from untreated patients had a high proliferative capacity and labeling index in cultures 1–2 wk old. In one culture initiated with leukemic myeloblasts only mature polys were observed at 25 days. It is concluded that proliferation and differentiation of human hematopoietic cells can be accomplished in liquid culture, can proceed in the absence of exogenous stimulating factors, and are dependent on cell-cell interaction. (Research supported by ACS Grant No. C I-60.)

120. The Biochemical Basis of Androgen Resistance in the Testicular Feminization Syndrome in the Mouse. JOSEPH L. GOLDSTEIN* AND JEAN D. WILSON, Seattle, Wash. and Dallas, Tex.

Previous work has demonstrated that male pseudohermaphroditism in the X-linked testicular feminization syndrome (Tfm) in the mouse, and probably in man, results from resistance to testosterone action during androgen-mediated sexual differentiation of male embryos; this androgen resistance is believed to result from diminished androgen binding to nuclear receptor sites in target tissues. To characterize the mechanisms by which this defect might arise, the binding of dihydrotestosterone-1,2-^{3}H has been examined in extracts of the submandibular gland, a tissue in which androgen-mediated synthesis of several proteins is deficient in Tfm mice, utilizing equilibrium dialysis, gel filtration on Sephadex G-100, and sucrose density sedimentation analysis. To ensure that secondary testosterone deficiency was not responsible for the effects observed, control, castrated, and testosterone-treated groups were studied. Both by gel filtration and by density gradient, specific dihydrotestosterone binding in the cytosol was localized in a discrete protein peak of approximate 3S size and mol wt less than 60,000, easily separable from the larger androgen-binding protein of mouse serum. In castrated animals no difference in the concentration of binding protein was demonstrated between male, female, and Tfm animals. In testosterone-treated male and female animals the cytosol-binding protein was markedly decreased, a finding compatible with the concept that the cytosol-binding protein-androgen complex is consumed by conversion to less soluble nuclear binding proteins. In contrast, the cytosol-binding protein of Tfm animals increased after testosterone treatment so that binding in Tfm cytosol was 5–10 times greater than that of cytosol from testosterone-treated male animals. These findings suggest that deficient nuclear binding of androgen in the Tfm mouse results from inability to transfer cytosol-binding protein to nuclear receptor sites. This abnormality could result from mutation in cytosol protein not involving androgen binding, from abnormality in nuclear receptor complex, or from a defective component in the transfer process.

121. Receptors in Plasma: New Parameter of Target Organ Disease. THEODORE GOODFRIEND* AND FREJ FYHRQUIST,* Madison, Wis. (introduced by George Rowe).

Plasma from some severely ill patients contained macromolecules with many of the known characteristics of receptors for angiotensin. These characteristics include high affinity, specificity, and saturability of hormone binding, and inhibition by chemicals such as diethylstilbesterol and SKF 525-A. The molecular weight of the binding macromolecules was approximately 800,000, and they migrated in the β_1-globulin region on electrophoresis. Assay involved simple gel filtration of plasma mixed with labeled hormone. Maximum binding capacity of positive plasmas was 0.01 pmole/ml. A few plasmas also contained macromolecules of different molecular weight which bound labeled insulin or glucagon. Of 2000 plasmas tested, 106 were positive for the angiotensin binders. One of the conditions associated with positive plasma was surgical relief of arterial occlusion (four patients). This was mimicked experimentally by occlusion and release of limb circulation in anesthetized dogs. After the release, dog plasmas transiently contained binders for angiotensin which resembled those from patients. Other diseases associated with plasma angiotensin binders included glomerulonephritis (6), septicemia (3), and chemotherapy for a variety of tumors (23). Relative specificity of binders for congeners of angiotensin was characteristic of the pathology. For example, postocclusion human and dog plasmas bound octapeptide 20 times better than heptapeptide, while glomerulonephritis plasmas bound octapeptide 5 times better than heptapeptide. There was no pattern of blood pressure or

chemistry, including enzymes, that differentiated patients with binders from normals or others with similar diagnoses. The appearance of receptors in plasma may be a parameter of target cell damage, with the binding characteristics indicating the location of the diseased organs. Their presence in blood does not appear to interfere with hormone action, but their absence from targets may.

122. Evidence for Participation of the Central Nervous System (CNS) in the Transition from the Fed to the Fasted State. CHARLES J. GOODNER, DONNA KOERKER,* PERTTI TOIVOLA,* CHARLES C. GALE,* AND JOHN ENSINCK, Seattle, Wash.

The role of the endocrine pancreas in the adaptation to fasting has been emphasized but the central nervous system (CNS) may also participate via the autonomic and neuroendocrine systems. We have examined this adaptation in conscious baboons after conditiong to two isocaloric feeding schedules: food ad lib. between 8 a.m. and 8 p.m., or food once per day at 4 p.m. Blood was sampled and urine collected at 4-hr intervals from 8 a.m. to 12 p.m. during 2 days' feeding, 3 days' fasting, and 2 days' refeeding. The metabolic changes of fasting were heralded by a 20% fall in glucose occurring at the time of the first missed meal. This occurred 8 hr earlier in the ad lib.-conditioned than in the once per day-conditioned animals (at 8 a.m. vs. 4 p.m., respectively). Insulin fell progressively reaching basal concentrations after 36 hr of fasting; thereafter glucose and insulin remained low and relatively constant until refeeding. FFA, glycerol, and glucagon rose but, in contrast to glucose and insulin, displayed a circadian pattern in parallel with urinary NE and E. Basal growth hormone did not change with fasting. In other studies, ganglionic or β-adrenergic blockade, after 36 hr of fasting, produced a 50% decline in FFA, glycerol, and insulin. The effect of conditioning to different feeding schedules, the cyclic pattern of lipolysis and glucagon secretion, and the results of blocking studies indicate an important role for the CNS in the adaptation to fasting. We have previously demonstrated that CNS glucoreceptors can affect lipolysis. The sequence of changes in early fasting suggests that a fall in glucose may be the signal for both the CNS and pancreatic control systems to initiate the adaptation to fasting. (Research supported by grants from NIH.)

123. Segment Power as an Index of Left Ventricular Function in Man. L. GOULD,* W. KENNEDY,* G. HAMILTON,* M. FRIMER,* AND H. T. DODGE, Seattle, Wash.

LV wall segment power (Ps) was calculated at every 0.01 sec during the cardiac cycle as the product of wall stress and normalized velocity of wall contraction in radial, equatorial, and meridianal directions, in contrast to merely the circumferential direction as in the past. Primary data were obtained from biplane angiocardiograms (12/sec) and catheter-manometer systems having uniform amplitude responses to 20–25 cycles/sec. 73 patients with various degrees of myocardial dysfunction (nonvalvular) were studied, including 9 patients with normal hearts and coronary arteriograms as controls. Ps values ranged from 715±198 g-cm/cm^3-sec in normals (ejection fraction, 60±6%) to 234±79 g-cm/cm^3 in primary cardiomyopathy (ejection fraction, 19±6%). Values for Ps correlated with other indices of ventricular performance with the following r values: ejection fraction +0.85, maximum external pump power/EDV +0.91, systolic work/LV mass +0.94, EDV/m^2 −0.73, ED equatorial stress −0.44, ED pressure −0.40, maximum normalized velocity of circumferential shortening +0.90, maximum rate of ejection +0.54, maximum dp/dt +0.56, maximum dp/dt/P +0.44. In patients with localized contraction abnormalities ejection fraction was significantly reduced, whereas Ps was only modestly diminished; in patients with diffuse generalized LV dysfunction Ps was reduced to the same extent as ejection fraction. The results suggest that Ps may also permit quantitative assessment of LV function in the presence of localized contraction abnormalities. Ps includes more variables than any other index of ventricular performance and may be interpreted in terms of muscle mechanics (force-velocity relationships) without assuming any particular model for the contractile characteristics of muscle. Thus, it may have some advantages over other measurements used to characterize LV performance. (Supported by NIH Grant 118303.)

124. Defective Regulation of Protein Kinase Activity in Three Liver-Derived Tissue Culture Lines. DARYL K. GRANNER, Iowa City, Iowa (introduced by Stanley G. Korenman).

The only proven mechanism of adenyl cyclase (AC)-mediated effects is through activation of protein kinase (K). c-AMP binds to a protein (R) which inhibits the activity of K; cAMP binding dissociates R from K thereby activating the latter. This model has been tested in three hepatoma cell lines (HTC, RLC, and H4-II-E) because they all contain subnormal amounts of AC, and in at least one (HTC cells) tyrosine aminotransferase is not induced by added cAMP, whereas it is in liver. All three cell lines have markedly decreased amounts of cytosol R as compared to liver (L, 2.33 ±0.15 pmoles cAMP bound per mg protein; H4-II-E, 0.58 ±0.02; RLC, 0.46±0.03; and HTC, 0.32±0.02). As predicted from the model: (*a*) cytosol K is higher in all these cell lines than liver (L, 9.51±0.82 pmoles ^{32}P transferred per mg protein; HTC, 47.0±2.3; RLC, 95.2±15); (*b*) cAMP causes a smaller increase in K activity when added to crude or partially purified extracts of HTC (+217%) or RLC cells (+183%) than liver (+793%); (*c*) other cyclic nucleotides (cIMP, cGMP, cCMP, dbcAMP) stimulate K activity in proportion to their ability to inhibit cAMP binding to R; (*d*) K activity of HTC cells can be reduced to the liver value by addition of liver R, and in this reconstituted system cAMP is effective. We conclude that these multiple defects in this important pathway afford a unique opportunity to study the regulation of related metabolic processes. (Supported by USPHS Grant No. 12191.)

125. Prophylactic Polymyxin B for the Prevention of Colonization by Nosocomial Gram-Negative Bacilli of the Upper Respiratory Tract of High-Risk Patients. SHELDON GREENFIELD,* DANIEL TERES,* LEONARD S. BUSHNELL,* JOHN HEDLEY-WHYTE,* AND DAVID S. FEINGOLD, Boston, Mass.

A prospective study was designed to try to prevent hospital-acquired pneumonia with Gram-negative bacilli in high-risk patients. Since the etiologic organism in pneumonia most

often enters the lung by aspiration from the upper respiratory flora, the first goal of the study was to prevent colonization of the upper respiratory passageways. We report here the success of prophylactic polymyxin B given by aerosal, one method employed in the study, in preventing colonization of the respiratory tract with Gram-negative bacilli. 43 patients on admission to the Respiratory-Surgical Intensive Care Unit (RSICU) were randomized into control and the polymyxin-treated groups. Treatment consisted of 2.5 mg/kg per day of polymyxin B by aerosol in six divided doses into the posterior pharynx and also trachea if an endotracheal tube or tracheostomy was present. The two groups were similar with respect to age, concomitant administration of antibiotics, and number with tracheostomies. The patients in the control group spent an average of 7.1 days in the RSICU with a total of 62 days on mechanical ventilation; comparable figures for the polymyxin group were 9.0 days and 120 days. Of the 19 control patients, 13 became colonized with Gram-negative bacilli, whereas of the 24 patients treated with polymyxin aerosol, 4 became colonized ($P < 0.01$). No adverse reactions to the antibiotic were recognized. We conclude that polymyxin B given prophylactically by aerosol can reduce the incidence of colonization of the upper respiratory tract by nosocomial Gram-negative bacilli in high-risk patients.

126. Mechanisms of Endotoxin Tolerance: Effect of Exchange Transfusion. SHELDON E. GREISMAN, Baltimore, Md.

Following a single intravenous injection of a lethal dose of bacterial endotoxin, most of the toxin is rapidly removed by the reticuloendothelial system (RES); appreciable amounts, however, continue to circulate for hours. It has been proposed that enhanced clearance of this residual circulating toxin by the RES accounts for endotoxin tolerance. The present studies were designed to critically test this thesis by determining whether early removal of the residual circulating endotoxin by exchange transfusion would significantly reduce mortality. Femoral artery cannulation was performed in healthy, unanesthetized New Zealand albino rabbits (2.0–2.5 kg). A control group of randomly selected animals then received 2.5 mg *E. coli* endotoxin intravenously followed by 4000 U heparin 20 min later (equivalent to amounts received by test animals during exchange transfusion). The test group received 2.5 mg *E. coli* endotoxin intravenously, and 20 min later exchange transfusion performed as follows: 10-ml aliquots blood were rapidly withdrawn via the femoral artery cannula and 10-ml fresh heparinized aliquots of blood (filtered to remove microemboli) were rapidly returned via the same cannula. Volume of exchange transfusion equaled 14% body weight and accomplished exchange of approximately 80% of the original blood volume. Control exchange transfusions performed *before* endotoxin injections did not enhance mortality. Mortality in nonexchanged animals was 83% (19 deaths/23 total); mortality in exchanged animals was 70% (16 deaths/23 total). It is concluded that mortality after sudden massive endotoxemia cannot be reduced significantly by removal 20 min later of a major portion of the residual circulating toxin. The findings are consistent with the concept that tolerance to endotoxin is dependent not upon enhanced clearance of toxin from the blood by the RES, but upon diminished susceptibility of the cellular elements of the RES to the injurious effects of the toxin.

127. Defective Bile Salt Synthesis as Cause of Cholesterol Gallstones. SCOTT M. GRUNDY, ALLAN L. METZGER, AND RONALD ADLER, Phoenix, Ariz.

Cholesterol solubilization in bile depends largely on bile salts. Conversion of cholesterol into bile salts is thus crucial for removal of cholesterol from the body. This conversion is under feedback regulation by bile salts in the enterohepatic circulation. In the present study, we have found a defect in regulation of bile salt formation that is probably the major cause of cholesterol gallstones in the American Indian population. Hepatic secretion rates for bile salts were determined in 16 Indian and 7 Caucasian women with and without gallstones, respectively. Our method employed duodenal intubation with 3-lumen tubes; markers and liquid formula were infused continuously for 18–24 hr. Secretion rates were determined by marker dilution principles. Average rates of bile salt secretion in Indian women with stones were markedly reduced, as compared to normal Caucasian women (470 $\pm$35 vs. 1504$\pm$271 mg/70 kg per hr; $P < 0.001$). These reduced secretions could have been due to abnormalities in either synthesis or reabsorption of bile salts. These two parameters were compared in six Indian women with stones and six normal Caucasian women. Fecal bile salt excretions were determined for 2–6 wk. Average excretions for the two groups were not significantly different. Since bile salts in the enterohepatic circulation were relatively depleted in Indians, even normal fecal excretions indicated reduced fractional reabsorption. However, previous studies have shown that Caucasians can rapidly replenish bile salt pools in the presence of sizeable intestinal losses; therefore, we conclude that homeostatic regulation of bile salt synthesis is defective in Indian women. The resulting decrease in hepatic secretion of bile salts exceeds limits required for solubilizing cholesterol in bile; hence, cholesterol gallstone formation is almost inevitable.

128. Mechanism of Action of an *Escherichia coli* Enterotoxin. R. L. GUERRANT,* N. F. PIERCE,* U. GANGULY,* W. B. GREENOUGH III,* AND C. K. WALLACE,* Baltimore, Md. (introduced by C. C. J. Carpenter).

Enterotoxigenic *Escherichia coli* can cause acute watery diarrhea. We compared the effects of *E. coli* enterotoxin (ECT) and cholera toxin (CT) upon jejunal function. ECT was the lyophilized, dialyzed, cell-free supernatant of liquid culture of a jejunal isolate from a man with severe diarrhea. Net water fluxes were determined in ligated segments of canine jejunum using a dilution marker (PSP). Isotonic ECT (500 μg/cm) induced maximum net secretion (8.2$\pm$1.4 μl/cm $\cdot$ min, mean $\pm$SE) within 10 min. 15 and 75 min after ECT removal net absorption had returned, being 4.0$\pm$2.2 and 4.4$\pm$1.1 μl/cm $\cdot$ min, respectively. Isotonic CT induced maximum net secretion (14.6$\pm$2.1 μl/cm $\cdot$ min) only after 150 min. Jejunal mucosal adenyl cyclase activity increased 193$\pm$49% after 10 min ECT exposure ($P < 0.01$), 59$\pm$31% ($P > 0.10$) 75 min after ECT removal, and 246$\pm$60% ($P < 0.01$) 150 min after CT exposure. ECT perfusion

500 μg/min) did not increase jejunal clearance of ^{51}Cr-labeled serum albumin, or diminish enhanced sodium absorption when the perfusion solution contained 60 mmoles/liter glucose. ECT perfusion during sustained maximum secretory response to CT did not increase the net secretory rate. Choleragenoid, a natural toxoid of CT, was employed to compare mucosal binding of ECT and CT. Prior exposure of rabbit jejunum to choleragenoid (0.2 μg/cm) blocked response to CT whereas 7 μg/cm choleragenoid did not alter ECT response. We conclude that ECT induces prompt fluid loss and mucosal adenyl cyclase activation, which are not sustained after ECT removal. Failure of choleragenoid to inhibit ECT indicates different mucosal binding for CT and ECT. This may contribute to the striking difference in time course of their effects. (Supported by NIH Grant AI-07625 and Career Development Award to N. F. Pierce.)

129. Very Low Density Lipoprotein Metabolism in Abetalipoproteinemia. CHRISTIAN GULBRANDSEN,* VIRGINIA EVANS,* ALEXANDER NICHOLS,* AND ROBERT S. LEES,* Cambridge, Mass. and Berkeley, Calif. (introduced by Richard J. Wurtman).

We have shown that human plasma very low density lipoproteins (VLDL) are converted to low density lipoproteins (LDL) by the squirrel monkey. To determine whether such conversion occurs in man, and to localize further the metabolic lesion in abetalipoproteinemia, we infused isolated human VLDL into each of two boys with that disease. VLDL, obtained from a patient with type IV hyperlipoproteinemia after 3 days of fat-free feeding, was injected intravenously at a dose equivalent to 11 mg protein/kg body weight in one subject and 3 mg/kg in the other. Serial plasmas from the recipients were analyzed for VLDL and LDL by analytical ultracentrifugation. In addition, VLDL protein was analyzed by the Lowry technique and LDL protein by radial immunoassay after preparative ultracentrifugation at density 1.006. Both patients showed sequential degradation of VLDL with gradual downward shift of the major S_f peak with time and produced lipoproteins which were immunologically, electrophoretically, and ultracentrifugally LDL. In the subject who received the larger amount, the infused VLDL had a half-life of 6 hr; peak LDL protein concentration of 2.62 mg/dl occurred at 12 hr. In the other patient the infused protein had a half-life of 1.5 hr; peak LDL protein concentration of 2.42 mg/dl occurred at 4 hr. VLDL incubation in the recipients' plasma in vitro at 37°C for 72 hr produced negligible amounts of LDL protein. The data show (*a*) that patients with abetalipoproteinemia can catabolize VLDL, (*b*) that sequential VLDL degradation occurs in them, and (*c*) that LDL is a product of VLDL catabolism in humans. (Supported by NIH, American Heart Association, and National Dairy Council.)

130. Failure of Cyclic GMP to Stimulate Short-Circuit Current. P. F. GULYASSY AND G. A. PORTER,* San Francisco, Calif., and Portland, Ore.

An increase in short-circuit current (SCC) across toad bladders was observed in response to cyclic GMP by Bourgoignie et al. (*Science [Washington]*. 1969. **165:** 1360). We recently observed marked variations in the purity of four different commercial preparations of cyclic GMP. Cyclic GMP from Boehringer-Mannheim Corp. (BMC) and Nutritional Biochemicals (NB) were found to be pure by column, paper, and thin-layer chromatography and had less than 1% noncyclic nucleotide as shown by release of inorganic phosphate with *Crotalus atrox* venom. The snake venom released 10–11% of the phosphate in "cyclic GMP" obtained from Calbiochem (CB) and P-L Biochemicals, Inc. (PL) and anion exchange chromtography showed six to seven impurities (by O.D. 260). We compared the effects of pure and impure cyclic GMP on short-circuit current. In six paired cross-over studies mean per cent change in SCC (compared to control period) was $+6.9\pm8.4$ SE (NS) for pure cyclic GMP (BMC) and $+23.5\pm6.8$ ($P < 0.025$) for impure cyclic GMP (CB). On subsequent cross-over re-addition of the serosal media, responses were $+42.3\pm10.9$ ($P < 0.025$) to cyclic GMP (BMC) and $+80.9\pm10.0$ ($P < 0.001$) to cyclic GMP (CB). In another set of eight paired studies potassium cyclic GMP (CB) gave -2.3 ± 2.2 (NS) when compared to paired KCl controls on first addition and $+17.9\pm6.6$ ($P < 0.05$) on re-addition. Pure sodium cyclic GMP (NB) gave -0.9 ± 1.2 (NS) compared to paired NaCl controls on initial addition in eight studies. Thus, the rise in SCC seen by Bourgoignie et al. with "cyclic GMP" (CB) may have been due to an impurity or a derivative formed during incubation. (Supported by grants from NIH.)

131. Primary Hyperparathyroidism: Immunochemical Characterization of Parathyroid Hormone in the Circulation. J. F. HABENER,* G. V. SEGRE,* D. POWELL,* P. DEE,* AND J. T. POTTS, JR., Boston, Mass.

We recently reported that parathyroid hormone (PTH) is secreted from the parathyroids in vivo as a polypeptide of mol wt 9500 and, after secretion, it is cleaved and a hormonal fragment of mol wt 7500 is detected in the circulation. Using radioimmunoassays that specifically detect the carboxyl-terminal (sequence 53–84) and amino-terminal (sequence 14–19) regions of the PTH molecule, we have measured immunoreactive PTH in plasma, and in fractions from gel filtration of plasma obtained from the general circulation, parathyroid effluent blood, and extracts of adenomas in patients with primary hyperparathyroidism. Results show that C-reactive PTH in the general circulation measures 6–20 times higher than N-reactive PTH, whereas in the effluent blood and in extracts of adenomas, C- and N-reactive PTH measure the same. Gel filtration reveals that 85–95% of the immunoreactive PTH in the general circulation consists of a late-eluting hormonal fragment of mol wt 7500 that reacts in the C-assay and not in the N-assay. The 5–15% of immunoreactive PTH in the plasma, that reacts in the N-assay, elutes coincident with intact, native PTH. Since a continuous peptide sequence consisting of at least the first 21 amino acids from the N-terminus is required for biological activity, we conclude that much of circulating immunoreactive PTH is biologically inactive. Our recent observations show that PTH is biosynthesized as a prohormone of mol wt 11,500, is cleaved, and is stored in and secreted from the gland as a molecule of mol wt 9500.

Thus, it appears that the metabolism of PTH is complex and involves at least two specific cleavages; the first occurs within the gland, in preparation for secretion of active hormone, and the second occurs after secretion and represents inactivation of the hormone.

132. Growth Hormone-Releasing Factor in Acromegalic Plasma. Reduction during Successful Medical Management. THAD C. HAGEN,* A. M. LAWRENCE,* AND LIDIA KIRSTEINS,* Chicago, Ill. (introduced by Henry T. Ricketts**).

Lack of absolute functional autonomy of growth hormone secretion in patients with active acromegaly has argued for continuing hypothalamic modulation of pituitary growth hormone release in this disease. This laboratory has demonstrated a negative feedback of growth hormone upon its further secretion in normal man; this exists also in acromegaly but threshold for feedback is set higher. These observations suggest acromegaly may be caused by chronic hyperstimulation of somatotrophs by hypothalamic growth hormone-releasing factor. We have reported that plasma from patients with active acromegaly enhances in vitro monkey pituitary growth hormone release 4- to 5-fold over that obtained with normal plasma. This effect is heat stable and dialyzable. In patients with active acromegaly, medical therapies designed to suppress hypothalamic secretory activity have resulted in clinical benefit and lowering of high basal growth hormone levels. Pretreatment plasmas greatly enhanced monkey pituitary growth hormone release; activity was substantially reduced in plasmas obtained during successful treatment of acromegaly. Cessation of therapy resulted in a marked rebound phenomenon in one patient. Finally, in one patient rendered panhypopituitary after ^{90}Sr hypophysectomy, plasma growth hormone-releasing activity greatly exceeded that of pretreatment samples, a situation reminiscent of the postadrenalectomy syndrome in Cushing's disease. We conclude that growth hormone-releasing activity of acromegalic plasma is substantially higher than that of normal plasma; since this activity is heat stable and dialyzable in both normal and acromegalic plasmas, differences in growth hormone-releasing potential appears quantitative rather than qualitative. Since successful medical therapy of acromegaly, aimed at suppressing the hypothalamus, is associated with reduction of this factor in acromegalic plasma, it is concluded that this activity, present in the peripheral circulation, is most likely of hypothalamic origin.

133. Increased Stem Cell Response to Erythropoietin Induced by Androgens. WILLIAM N. HAIT,* DOV GORSHEIN,* JOANNE H. JEPSON,* EMMANUEL C. BESA,* AND FRANK H. GARDNER, Philadelphia, Pa.

The ability of androgens to increase plasma erythropoietin (ESF) levels, as demonstrated in the rodent, is an inadequate explanation of the observed erythropoietic response in man. Human anemias, attributed to a shortage of erythroid precursors, display extraordinarily high ESF titers. ESF titers often decrease during androgen therapy as recognizable erythroid elements in the marrow increase. The following experiments demonstrate the ability of 19-nortestosterone decanoate (19-ND) to enhance erythropoiesis by increasing the pool of erythroid-committed precursors responsive to ESF. 19-ND was unable to induce measurable ESF levels in mice maintained in 60% ovygen environment (HOX). Mice made polycythemic (PCT) by prolonged hypoxia ($HCT \geq 55$) were subsequently maintained in HOX. PCT-HOX animals receiving, respectively, (*a*) 1.25 mg 19-ND followed by 0.5 U ESF 24 hr later, (*b*) appropriate vehicles, (*c*) ESF, and (*d*) 19-ND were compared to identical PCT groups maintained in HOX. ^{59}Fe incorporation into RBC of PCT-HOX animals receiving vehicle (1.2±0.4%) was significantly lower than their PCT counterparts ($P < 0.10$) indicating further suppression of endogenous ESF by HOX. PCT-HOX animals also exhibited lower ($P < 0.05$) ^{59}Fe incorporation after 19-ND injection. The administration of a single dose of 19-ND only 24 hr before ESF injection enhanced erythropoiesis in both systems when compared to ESF alone ($P < 0.01$). These observations imply that: (*a*) 19-ND cannot induce ESF production in HOX, and that (*b*) it rapidly triggered G_0 or G_1 pluripotential stem cells into an erythroid-specific G_1 cell cycle responsive to ESF, and that (*c*) further differentiation of such erythroid-committed cells requires ESF. (This research supported by a grant from the John A. Hartford Foundation.)

134. Control of Breathing in Uremia: Enhanced Ventilatory Response to CO_2 after Hemodialysis. ROBERT HAMILTON,* PAUL EPSTEIN,* LEE HENDERSON,* NORMAN EDELMAN,* AND ALFRED FISHMAN,** Philadelphia, Pa.

The hyperventilation that persists in uremic patients after hemodialysis remains unexplained. To test whether this is due to increased sensitivity of the respiratory chemoreceptors, we studied the ventilatory response to CO_2 ($\Delta\dot{V}/\Delta P_{CO_2}$) in eight patients with uremia. Despite a mean increase in plasma HCO_3^- of 4.86 mmoles/liter, the $\Delta\dot{V}/\Delta P_{CO_2}$ increased from a predialysis value (mean±SE) of 3.16±0.71 liter/min per mm Hg to 5.59±1.25 immediately after dialysis ($P < 0.005$) and decreased to 3.45±0.94 24 hr later. To determine whether these changes were due to (*a*) dialysis of a toxin depressing ventilation, (*b*) extracorporeal circulation per se, (*c*) acid-base changes in the CSF, or (*d*) an effect of the dialysis disequilibrium syndrome, we produced chronic renal failure (without metabolic acidosis) in three goats by renal infarction. In 51 studies of $\Delta\dot{V}/\Delta P_{CO_2}$ in these unanesthetized goats, uremic animals showed a significant increase in $\Delta\dot{V}/\Delta P_{CO_2}$ immediately after hemodialysis ($P < 0.05$), as in uremic patients; nonuremic goats did not show this response. The $\Delta\dot{V}/\Delta P_{CO_2}$ of the goats was slightly greater after production of uremia ($P < 0.05$), thus refuting the thesis of toxic depression. Changes in $\Delta\dot{V}/\Delta P_{CO_2}$ were unrelated to changes in acid-base status of plasma or CSF. However, adding urea to the dialysate, to match the concentration in the plasma, prevented the postdialysis increase in $\Delta\dot{V}/\Delta P_{CO_2}$ in uremic goats. The data suggest that postdialysis hyperventilation is due to increased respiratory sensitivity to CO_2. This increase in respiratory sensitivity is not related to dialysis of a depressant toxin, extracorporeal circulation per se or changes in CSF acid-base status. It is a consequence of the dialysis disequilibrium syndrome. (Research supported by NIH Grants HE-08805, HE-00340, 5T01 AM-05634, and 5M01 FR-00040.)

135. Plasma Activator of Plasminogen: Cause of a Familial Bleeding Diathesis. JAMES W. HAMPTON,* FRED B. OLDHAM,* DAVID BANNERJEE,* EKREM KALMAZ,* AND ROBERT DELANEY,* Oklahoma City, Okla. (introduced by Colin M. MacLeod**).

12 members of both sexes in four generations of a family were demonstrated to have enhanced plasma activator of plasminogen which could be inhibited in vitro or in vivo by epsilon amino caproic acid. Plasma activator activity was measured by a semiquantitative caseinolytic assay modified from the method of Johnson and Tse. In all 12 members the plasma activator was elevated (15–45 streptokinase-like units with no activity in normals even after prolonged exercise). Plasma activator was confirmed by a fibrin plate method which utilized test plasma, a partially purified human plasminogen, and a fibrin substrate which was free of plasminogen or activator activity. 9 of the 12 patients had a history of excessive bleeding and showed serum fibrin degradation products, low fibrinogen, short euglobulin lysis times, and reduced antiplasmin activity, which could be interpreted to indicate in vivo activation of the fibrinolytic system. Low factor VIII activity in one affected member had prompted the incorrect diagnosis of classic hemophilia. In four affected members plasma activator increased 2- to 7-fold with exercise. The activator is present in the supernatant of Cohn fraction I-O and in the 0.4–0.5% ammonium sulfate precipitate. Chromatography on Sephadex G-100 indicates a pattern similar to tissue activator. Urokinase antiserum gave no precipitin reaction on double immunodiffusion. The nature of the plasma activator has previously been subject only to conjecture and its presence has not been described in association with familial in vivo fibrinolysis. (Research supported by NIH Grants HE 12316, 2 TO1 AM 05107, and 5-K3-HL-14,227.)

136. In Vitro Synthesis of Porphyrins by Skin. L. HARBER, D. BICKERS,* A. RIFKIND,* AND A. KAPPAS, New York.

Although the pathway of porphyrin biosynthesis has been extensively studied in erythropoietic and hepatic tissue, no similar studies have been reported in skin. This investigation was designed to demonstrate the in vitro synthesis of porphyrins in skin using γ-aminolevulinic acid (ALA) as a substrate. Induction of the mitochondrial enzyme, γ-aminolevulinic acid synthetase (ALA-S), was also attempted using allylisopropylacetamide (AIA) and the 5β-steroid, etiocholanolone (Etio). Multiple biopsies were obtained from five foreskins removed during routine circumcision of 3- to 5-day-old infants. Each biopsy specimen, 13–15 mg wet wt, was incubated in tissue culture. ALA ranging from 10^{-6}–10^{-2} M, was added to each incubating specimen. Epidermal porphyrin production was qualitatively noted by fluorescent microscopy in 4–6 hr and quantitated by spectrofluorimetry. A dose-related production of porphyrin ranging from 3.71 to 171.4 $\mu\mu$moles was detected in the biopsy specimens incubated with ALA. Porphyrin production after ALA incubation was also noted using the above technique in the skins of chick embryos, rats, guinea pigs, and adult humans. Intense activity was also seen in plucked human hair follicles. Control incubation of ALA without skin, and skin incubated without ALA, did not lead to porphyrin production. Similarly, no porphyrin production was observed in skin incubated with ALA precursors, nor was porphyrinogenesis induced by AIA or Etio. These studies demonstrate the presence of enzymes necessary to convert ALA to porphyrins in skin. Minimal or no ALA-S activity was noted in any of the skin specimens studied. (Research supported by USPHS grants and an NIH Training Grant.)

137. Plasminogen Kinetics in Man. LAURENCE A. HARKER, GOTTFRIED SCHMER,* AND SHERRILL J. SLICHTER,* Seattle, Wash.

Fibrinolytic activity in vivo was evaluated by measurements of plasminogen turnover in 16 normal subjects and in 37 patients. Plasminogen for ^{125}I labeling was prepared from single-donor normal plasma by one-step EACA-agarose affinity chromatography that involved plasminogen replacement chromatography. The protein was biochemically homogeneous with caseinolytic activity of 30 R-C units per mg and about 85,000 mol wt. Plasminogen turnover was determined from concentration divided by $t_{\frac{1}{2}}$ disappearance of its ^{125}I label. Plasminogen levels were measured by caseinolytic, affinity chromatographic, and immunologic techniques. In vivo platelet and fibrinogen turnovers were measured simultaneously. Plasminogen-^{125}I disappeared logarithmically in vivo with a half-time of 30.9±2.1 hr. The mean plasminogen turnover was 0.11±0.01 mg/ml per day. In four normal individuals undergoing strenuous exercise intermittently over 24 hr, the $t_{\frac{1}{2}}$ was shortened to 17.2±1.2 hr, and turnover increased twofold (0.21 mg/ml per day). In five patients infused with urokinase the $t_{\frac{1}{2}}$ was reduced to 3.3±1.2 hr. Ongoing thromboembolism in nine patients was associated with increased plasminogen destruction ($t_{\frac{1}{2}}$ 16.3±2.3 hr) and a turnover of 0.34±0.08 mg/ml per day. Parallel changes were observed in platelet and fibrinogen kinetics. Other patients with ongoing "intravascular clot coagulation" (malignancy in 11, malaria in 2, bacteremia in 3) rapidly consumed plasminogen (mean $t_{\frac{1}{2}}$ 7.7±1.8 hr, turnover 0.65 mg/ml per day) at rates comparable with the increase in platelet and fibrinogen consumption. In these patients heparin therapy reduced both fibrinogen and plasminogen consumption to normal values, while EACA corrected plasminogen consumption to normal without diminishing the destruction of fibrinogen. On the basis of these data it is concluded that fibrinolysis is a highly reactive system which responds to a wide variety of physiological and pathological stimuli, and that fibrinolytic activation appears to be quantitatively coupled with the rate of fibrin formation. (Research supported by Grants HE-11775 and HE-06242 from the NIH.)

138. A Unique Mechanism for Collagenolysis Associated with Invasive Neoplasm in Rabbits. EDWARD D. HARRIS, JR.,* CHARLES S. FAULKNER II,* AND SUMNER WOOD, JR.,* Hanover, N. H., and Rahway, N. J. (introduced by Thomas P. Almy**).

Malignant tumors invade and destroy normal connective tissues, presumably by enzymatic action. We have partially characterized collagenolytic activity associated with the ascitic form of rabbit V2 carcinoma in New Zealand white

rabbits. 30×10^6 carcinoma cells were injected into thigh muscle of rabbits. Tumors grew rapidly; in 3–4 wk animals were sacrificed, tumors excised, and explants incubated at 37°C in modified Eagle's medium without serum. Rabbit tumor collagenolytic activity (RTCA), assayed using native collagen substrate, was concentrated from pooled culture medium. The RTCA was partially purified by chromatography on Bio-Gel A 0.5 and found to be active at pH 7–8, inhibited by chelating agents, and without caseinolytic activity. Against collagen in solution at 10°–27°C the RTCA decreased specific viscosity (η_{sp}) to 13–20%, while synovial collagenase yielded products retaining 40–45% of initial η_{sp}. Reaction products electrophoresed on acrylamide revealed bands not seen as products of other collagenase action. Examination of SLS aggregates of the reaction products by electron microscopy have indicated that the collagen molecule is cleaved by RTCA at a site 57% from the C-terminus, and that the preferential site of cleavage by other mammalian enzymes—25% from the C-terminus—was spared by RTCA. The discovery of a tumor-associated enzyme with a qualitatively different mechanism of action from functionally similar enzymes in nonmalignant tissues is unusual and may have significance in further study of mechanisms of tumor invasion. (Research supported by grants from NIH, Arthritis Foundation, Easter Seal Foundation, and by the Merck Institute for Therapeutic Research.)

139. Mechanism of Action of Type II Antiarrhythmic Drugs. LURA A. HARRISON,* JOHN WITTIG,* AND ANDREW WALLACE, Durham, N. C.

The purpose of these experiments was to test the feasibility that the unique properties of the Purkinje-muscle (PM) junction might serve as a useful model for testing the actions of antiarrhythmic agents. Previous studies have indicated that action potential (AP) duration is greatest in distal Purkinje fibers (3–4 mm from the PM junction). Premature beats initiated proximal or distal to the area of maximal AP duration may propagate with decrement or block. These studies were performed on the right bundle branch and distal Purkinje fibers removed from the dog heart, utilizing standard microelectrode techniques. Action potential duration was 215±5 msec in proximal right bundle, 315±10 msec in distal Purkinje fibers, and 170±7 msec in muscle. Premature beats with a coupling interval comparable to AP duration at the area of maximal duration conducted with decrement. Earlier beats were confined to the fibers proximal to the area of maximal AP duration. Lidocaine (2.34 μg/ml) shortened AP duration by 100±7 msec at the area of maximal AP duration, but had little effect on proximal right bundle or muscle. Decremental conduction and block were abolished by lidocaine. Norepinephrine (NE) 1×10^{-5} M prolonged AP duration, increased decremental conduction, and prolonged the functional refractory period of distal fibers. Propranolol 0.25 — 1.0 mg/liter shortened AP duration at the area of maximal AP duration, and abolished decremental conduction and block of premature beats. These observations demonstrate that the unique properties of the PM junction may contribute to the genesis of arrhythmias and that this is the site of maximal effect of type II antiarrhythmic agents. The action of these agents on the PM junction is dose dependent and at therapeutic concentrations they exert effects which very probably contribute to their antiarrhythmic effectiveness. (Research supported by US PHS.)

140. Glucose Homeostasis in the Newborn Rat: Role of Gluconeogenic Substrates and Ketones. MOREY HAYMOND,* IRENE KARL,* ANTHONY PAGLIARA,* AND DAVID KIPNIS, St. Louis, Mo.

Fasting in the newborn rat results in hypoglycemia which may be due to a functional defect(s) in the gluconeogenic enzymic system or to deficient substrate availability. Term rats were litter mated into fasted (maintained at 30°C, constant humidity) and fed (6 animals per mother) groups. Blood was obtained by decapitation at timed intervals for the microfluorometric determination of glucose, β-hydroxybutyrate, acetoacetate, lactate, pyruvate, glutamate, alanine, and glutamine. Fasting glucose increased from 77±3 mg/100 ml (mean ±SEM) to 100±3 mg/100 ml by 6 hr and then fell to 26±1 mg/100 ml by 18 hr. Acetoacetate increased slightly (73±8 to 179±14 μM) while β-hydroxybutyrate remained unchanged (111±7 μM). Lactate (7.2±0.5 mM) and pyruvate (0.198±0.022 mM) elevated at birth, fell throughout the fast (18 hr, 1.2±0.06 and 0.047±0.01 mM, respectively). Alanine also decreased from 867±42 to 275±9 μM. Glutamine initially decreased (1141±53 to 824±39 μM) and then increased to 1036±49 μM. Intraperitoneal administration of alanine (500 mg/kg) in fasted animals, rapidly increased blood glucose (Δ 25 mg/100 ml). Fed animals maintained glucose > 80 mg/100 ml at all times. Lactate, pyruvate, and glutamate were similar to fasted animals except lactate at 18 hr was higher (2.4±0.1 vs. 1.23±0.06 mM) and glutamate higher (356±11 vs. 275±9.13 μM). Alanine decreased at 2 hr (867±42 to 598±34 μM) and then remained constant. Glutamine levels remained unchanged. β-Hydroxybutyrate (111±7 to 872±5 μM) and acetoacetate (73±8 to 1010±38 μM) demonstrated significant changes throughout. These studies demonstrate that glucose homeostasis in the fasted newborn rat is not limited by functional hepatic gluconeogenic enzyme deficiencies, but rather appear to be due to deficiencies of endogenous gluconeogenic and fatty acid substrates. In fed animals, ketone body formation derived from mother's colostrum (22% fat) appears to be an important source of energy which may spare glucose. (Research supported by USPHS Grant AM 1921 from the NIH.)

141. Hyperlipidemia in Coronary Heart Disease: Lipoprotein Characteristics of a Classification Based on Genetic Analysis. W. R. HAZZARD,* J. L. GOLDSTEIN,* H. G. SCHROTT,* A. G. MOTULSKY,** AND E. L. BIERMAN, Seattle, Wash.

One current classification of the hyperlipidemias (phenotypes I–V) is based on the level of plasma triglyceride (TG), content of cholesterol (C) in low density lipoproteins (LDL), and electrophoretic mobility of isolated lipoprotein (LP) fractions. An alternative classification has emerged from a genetic analysis based on the distribution and segregation of plasma C and TG in more than 2000 relatives of 130 consecutive hyperlipidemic survivors of myocardial infarction. Three genetically distinct disorders were

identified by this approach: hypertriglyceridemia (HTG), hypercholesterolemia (HC), and combined HTG + HC (Goldstein, Hazzard, et al., *Clin. Res.*, April 1972). In an attempt to relate these genetic disorders to LP phenotypes, isolated LP fractions from index cases of 44 families were characterized LDL-C was lowest in HTG (154±50 mg/100 ml [mean ±SD], n = 16), intermediate in combined HTG + HC (198±47, n = 20), and highest in HC (266±52, n = 8). Since close correlations were noted in all genetic groups between total C and LDL-C ($r = 0.93$) and total TG and VLDL-TG ($r = 0.93$), whole plasma C and TG accurately reflected LP levels. However, because of the overlap in individual LDL-C values of index cases with the different genetic disorders, LP phenotyping (using suggested 95% normal limits for LDL-C) agreed with the genetic diagnosis in only 50% of cases—those at the extremes of HTG (pure type IV) and HC (pure type IIa). Index cases with combined HTG + HC showed three different LP phenotypes; IV (40%), IIb (40%), and IIa (20%). Thus these studies demonstrate that the specific genetic disorder in the individual patient with hyperlipidemia and coronary heart disease cannot be reliably diagnosed from determination of his LP phenotype. They also suggest that the simple measurement of plasma TG and C in relatives is likely to yield more meaningful information regarding the genetic nature of his lipid disorder than detailed analysis of his lipoproteins. (This work was supported by grants from NIH.)

142. Histidine-Dependent Zinc Loss, Hypogeusia, Anorexia, and Hyposmia. R. I. HENKIN, H. R. KEISER,* AND D. BRONZERT,* Bethesda, Md.

Zinc metabolism, gustation, and olfaction were studied in five patients with scleroderma and in three normal volunteers on and off histidine, given for 2-day periods, in doses of 8, 16, 32, 48, and 64 g (patients) and 8, 16, and 32 g (volunteers). In patients, serum zinc (mean±1 SEM, μg/100 ml) decreased directly with histidine administration (base line 82±8; 32 g, 71±6; 64 g, 50±3) whereas urinary zinc excretion (mean±1 SEM in μg/24 hr) increased (base line 402±25; 32 g, 2552±513; 64 g, 8490±408). In volunteers, serum and urinary zinc exhibited similar changes. Administration of ≧32 g histidine produced elevations of 5- to 80-fold in thresholds for four taste qualities (NaCl, sucrose, HCl, and urea) and each patient reported anorexia and decreased taste acuity (hypogeusia); thresholds for three vapors (pyridine, nitrobenzene, and thiophene) increased 100-fold above normal, indicating decreased olfactory acuity (hyposmia). Similar, although less severe, changes occurred in volunteers. With withdrawal of histidine a gradual return toward normal occurred in serum and urinary zinc, hypogeusia, anorexia, and hyposmia. Zn^{++}, 100 mg given orally during administration of 64 g histidine, returned hypogeusia and hyposmia to normal within 8 hr. Zinc in serum is complexed primarily with macromolecular species (α_2-macroglobulin and albumin) but also with micromolecular species (histidine and cysteine). In vitro, increased histidine concentrations strip Zn^{++} from Zn^{++}-albumin complexes forming Zn^{++}-histidine complexes. The present studies suggest that this ligand shift can occur in vivo and is associated with hyperzincuria, subsequent hypozincemia, and depletion of zinc from receptor sites in gustatory and olfactory systems.

143. The Metabolism of the Steroid and Glycine Moiety of Glycine-Conjugated Bile Acids in Man. GERSHON W. HEPNER,* ALAN F. HOFMANN, AND PAUL J. THOMAS,* Rochester, Minn.

Bile acids are deconjugated and dehydroxylated during enterohepatic cycling in man. To measure the magnitude of these bacterial biotransformations. cholyl-^{3}H glycine-1-^{14}C, chenodeoxycholyl-^{3}H glycine-1-^{14}C, and deoxycholyl-^{3}H glycine-1-^{14}C were synthesized and administered to 12 healthy volunteers. Bile samples were collected daily for 7 days and the specific activity decay curve of each moiety was determined. Breath excretion of ^{14}C was assessed by interval $^{14}CO_2$ specific activity determinations. The daily fractional turnover rate of the glycine moiety was about 3 times greater than that of the steroid moiety in the three conjugated bile acids. Assuming six enterohepatic cycles daily, about 20% of the glycine-conjugated pool was deconjugated per cycle; the greater part of the steroid moiety thus liberated was reabsorbed. ^{14}C excretion correlated highly ($r = 0.95$) with daily fractional turnover of the glycine moiety for all three bile acids; hence the rate of deconjugation can be easily assessed quantitatively by interval $^{14}CO_2$ specific activity determination. The daily fractional turnover of the steroid moiety of the two dihydroxy bile acids was nearly identical and less than that of the cholyl moiety, indicating that dihydroxy bile acids are absorbed more efficiently (99% per cycle) than the trihydroxy acid (95% per cycle). Input of deoxycholic acid from the intestine was about 0.3 mmoles/day or one-quarter of all the cholic acid lost from the enterohepatic circulation. These data describe (*a*) a simple method for determining the metabolism of the two moieties of a conjugated steroid participating in enterohepatic cycling, (*b*) a simple method for quantifying bile acid deconjugation in healthy man, and (*c*) new insights into the intestinal conservation of the bile acid nucleus. (Supported by NIH Grant AM 6908.)

144. The Assimilation or Production of Gaseous Nitrogen by Tissues. J. H. HERRON,* H. A. SALTZMAN, B. A. HILLS,* AND J. A. KYLSTRA,* Durham, N. C.

The classic assumption that gaseous nitrogen (N_2) is metabolically inert has been challenged by recent observations of uptake or elimination in the steady state by men breathing air. Since accurate measurement of a small difference between large volumes of inhaled and exhaled N_2 is difficult, gas exchange was evaluated in six resting men equilibrated with a normoxic gas (P_{O_2} of 150 mm Hg) containing only 0.2% N_2, thereby reducing the error of measurement by about two orders of magnitude. The respired concentrations of O_2, CO_2, and N_2 were measured on a gas chromatograph. Respired volumes were measured in a Tissot gasometer. The measured volumetric differences between exhaled and inhaled N_2 were small, averaging 1.2±2.53 (SD) ml/min STPD when fasting and 1.7±2.99 to 2.9±2.17 ml/min STPD 1–5 hr after ingesting protein. These results indicate that, under the conditions tested, any chemical assimilation or production of gaseous nitrogen by

tissues is insignificant relative to the standard error of measurement (1 ml/min STPD). (Research supported by grants from NIH (HL07896) and ONR.)

145. The Kinetics of Hepatic Ferritin. CHAIM HERSHKO,* JAMES D. COOK,* AND CLEMENT A. FINCH, Seattle, Wash.

The kinetics of iron storage and release by two liver cell populations was measured in the rat after intravenous injection of purified ferritin labeled with ^{59}Fe. 97–99% of soluble ferritin was taken up by the hepatocytes, while the complex, ferritin-antiferritin, was localized exclusively in the Kupffer (RE) cells. Release of radioiron from the Kupffer cells was complete in 5 days. Turnover in the hepatocytes was much slower (2–3%/day), corresponding to the daily exchange of iron between plasma and liver as measured by plasma iron turnover. Chemical determinations combined with cell differentiating studies showed that over 95% of hepatic ferritin iron was localized within the parenchymal cells of the liver. A fivefold increase in the release of radioiron from hepatocytes occurred when erythropoiesis was stimulated by phlebotomy, and the liver ferritin concentration fell from 97 to 28 μg/g tissue in 5 days. Radioiron loss from the parenchymal pool was also induced by the chelating agent desferrioxamine, whereas RE cell radioiron was inaccessible for chelation. Desferrioxamine was shown to interact selectively with parenchymal ferritin ^{59}Fe and not with other radioiron complexes processed by the hepatocyte. Thus, hepatic ferritin is located almost completely in the hepatocyte; this ferritin may be mobilized by chelate action or in response to physiologic needs. (Research supported by Grant HE-06242 from the NIH.)

146. Elevated Cadmium in Emphysematous Lungs. RUSSELL N. HIRST, JR.,* H. MITCHELL PERRY, JR.,** MARCOS G. CRUZ,* AND JOHN A. PIERCE,** St. Louis, Mo.

Several lines of evidence suggest a relationship between cadmium and emphysema. Each cigarette contains about 1 μg of cadmium. Occupational exposure to cadmium has been implicated in some human emphysema. Inhalation of cadmium aerosols by dogs and intratracheal instillation of cadmium into hamsters has induced emphysema. The livers of patients dying with emphysema contain significantly more cadmium than the livers of other patients. The present study reports levels of cadmium in emphysematous lungs. Tissues for metal analysis were obtained at autopsy and stored frozen. One lung was prepared by the Gough technique and graded for emphysema after the 1970 method of Thurlbeck and associates using a scale of 0–100. 12 subjects with emphysema scores greater than 60 were compared to 12 subjects with scores less than 20. Cadmium, zinc, lead, sodium, potassium, and calcium concentrations were determined by atomic absorption spectroscopy in 1-g aliquots of kidney, liver, and lung after ashing at 450°C for 48 hr. Mean values for cadmium were 82±60 μg/g tissue ash in the emphysematous lungs, and 20±13 μg/g in the control lungs ($P < 0.005$). Mean cadmium levels also were greater in the other tissues from emphysematous as compared to control patients (liver 191 vs. 63 μg, $P < 0.025$; kidney 2846 vs. 1745 μg, $P < 0.10$). Zinc, lead, sodium, potassium, and calcium did not differ significantly in the lungs, livers, or kidneys between the emphysematous and control patients. The finding of an elevated cadmium level in emphysematous lungs merits further study. The source of the cadmium, the rate of clearance, and the chemical state all are important. The role of cadmium in the pathogenesis of emphysema in man remains uncertain.

147. Role of the Frank-Starling Mechanism in Strenuous Exercise. LAWRENCE D. HORWITZ,* JAMES M. ATKINS,* STEPHEN J. LESHIN,* AND STANLEY A. DUNBAR,* Dallas, Tex. (introduced by Jere H. Mitchell).

In six dogs running on a level treadmill, measurements were made of left ventricular pressure with a solid-state transducer, aortic flow with an electromagnetic probe, and left ventricular internal transverse diameter with sonocardiometer crystals. The animals ran for 3-min periods at 3–4 mph (mild exercise), 6–8 mph (moderate exercise), and 10–13 mph (severe exercise). Heart rate increased from a standing control of 104±7 (mean±SEM) beats/min to 183 ±14 in mild, 222±13 in moderate, and 259±15 in severe exercise. Stroke volume increased from 33±4 cc to 35±4 cc in mild, and 37±3 cc in severe exercise. Mean left ventricular end-diastolic diameter was 30.6 mm initially, 30.8 mm in mild, and 31.9 mm in severe exercise; corresponding end-systolic diameters were 23.4, 22.9, and 23.1 mm. Mean left ventricular end-diastolic pressure rose 4 mm Hg in mild and 12 mm Hg in severe exercise, while peak systolic pressure rose 26 and 75 mm Hg, respectively. Therefore, despite extremely high heart rates a small increase in stroke volume occurs with exercise ($P < 0.05$ at both mild and severe levels). In mild exertion the increase in stroke volume is due primarily to increased cardiac muscle fiber shortening resulting in a lower end-systolic diameter, whereas in severe exertion there is further augmentation in stroke volume due to an enlarged end-diastolic diameter—evidence that the Frank-Starling mechanism is operative. (Research supported by Grant HE 14355 from the NIH.)

148. Colfibrate-Induced Antidiuresis. JOAN HOWANITZ,* MARCIA VAN GEMERT,* MYRON MILLER,* AND ARNOLD M. MOSES, Syracuse, N. Y.

Five patients with diabetes insipidus, who were able to release some antidiuretic hormone (ADH) on dehydration, developed an antidiuresis during treatment with clofibrate 500 mg q.i.d. while on random fluid intake (urine osmolality 141 vs. 340 mOsm/kg). The antidiuresis was similar to that which results from ADH with no change in sodium, potassium, solute, or creatinine excretion. Clofibrate diminished the ability of these patients to excrete a maximally dilute urine after water loading (minimum U_{osm} 52 vs. 123 mOsm/kg). When oral water loads were given to 14 normal subjects treated with clofibrate, there was also impaired ability to excrete the water and to dilute the urine maximally (minimum U_{osm} 68 vs. 87 mOsm/kg). In these normal subjects immunoassayable urinary ADH excretion during water loading increased from a mean of < 1 μU/min before treatment to 32.3 μU/min during clofibrate treatment. Similarly, in four patients with diabetes insipidus on ad lib. fluid intake, urinary ADH excretion increased from 1.3 μU/min to 2.9 μU/min during clofibrate treatment. The antidiuretic action of the

drug in patients with diabetes insipidus was partially overcome by water loading or ethanol administration, both of which are capable of inhibiting ADH release. To further study the mechanism of action, 40 mg of clofibrate was injected into rats with total ADH deficiency (Brattleboro strain) and no antidiuresis occurred. When clofibrate was injected into these rats along with exogenous ADH, there was no augmentation of the action of the ADH. These combined clinical and animal data support the conclusion that clofibrate has no intrinsic antidiuretic action and exerts its antidiuretic effect by causing release of endogenous ADH from the neurohypophysis. (Supported by VA Research Funds.)

149. Influence of Renal Transplantation on Aminonucleoside Nephrotic Syndrome. JOHN R. HOYER,* JEAN RATTE,* AND ALFRED F. MICHAEL, Minneapolis, Minn.

Although pathogenesis of the idiopathic nephrotic syndrome is unknown, our recent demonstration of recurrent disease after renal transplantation suggests importance of humoral mechanisms. Exchange of renal grafts between rats with aminonucleoside of puromycin (PA) nephrosis and normal rats was performed to analyze humoral and renal factors. All results reported are urinary protein (mean±1 SD mg/24 hours) of groups of three or more grafts on specified posttransplant day. Renal transplantation of kidneys from PA-injected donors (150 mg/kg intravenously) to bilaterally nephrectomized normal rats revealed the following. Kidneys from rats with massive proteinuria (495±50 mg) transfer the disease: 427±66 mg on day 3 (normal transplanted controls 31±14 mg). Kidneys transplanted from groups of PA rats 1, 4, 24, and 48 hr after injection (during the 4–5 day induction period before proteinuria) developed massive proteinuria at the expected time; mean proteinuria in each group was greater than 500 mg on day 7. One kidney transplanted 15 min after injection excreted 677 mg on day 7. A normal kidney's influence on proteinuria was studied in two ways. (*a*) 4 hr after injection of donors with PA (100 mg/kg), kidneys were transplanted to normal unilaterally nephrectomized (UnNe) rats. Proteinuria was not increased in the first 9 days (33±24 mg). After removal of the normal kidney, proteinuria developed (351±92 mg 3 days later). (*b*) Transplantation of a normal kidney to UnNe rats given PA 4 hr previously resulted in decreased proteinuria (57±16 mg on day 7) compared to UnNe Pa rats (657±101 mg). These studies demonstrate: (*a*) the nephrotic syndrome can be transferred by transplantation of the nephrotic kidney; (*b*) crucial biochemical events leading to massive proteinuria after a latent period of several days occur in the kidney within minutes after PA injection; and (*c*) presence of a normal kidney reduces protein excretion of a "nephrotic" kidney.

150. Culture of Human Endothelial Cells Derived from Umbilical Cord Veins. ERIC A. JAFFE,* RALPH L. NACHMAN, AND CARL G. BECKER,* New York.

Recent immunofluorescence studies have shown that human platelets and human endothelial cells contain immunologically similar smooth muscle actomyosins which are absent in mature connective tissue. Clinical and experimental observations suggest the possibility that platelets interact with endothelial cells in maintaining normal blood vessel integrity. To study these relationships, we have attempted to grow human endothelial cells in culture. Human umbilical veins were incubated with buffered collagenase solutions. The enzymatically removed intimal cells were cultured in medium 199. Comparison of stained histologic sections of umbilical veins obtained before and after enzyme treatment revealed a selective loss of the endothelial cell monolayer lining the vessel's interior. In tissue culture, on plain and collagen-covered cover slips, these cells grew as a monolayer of closely opposed polygonal cells, 50×70 μ with prominent intercellular interdigitations. Comparison of these cells with standard fibroblast cell lines grown in culture revealed gross morphologic differences. Thrombosthenin, the smooth muscle actomyosin, was extracted from human platelets and partially purified. Fluoresceinated antithrombosthenin (F-AT) brilliantly stained human endothelial cells and vascular and uterine smooth muscle in tissue sections. The cultured endothelial cells obtained from the umbilical veins stained brilliantly with F-AT, while standard fibroblasts cultures stained only weakly. Mixed cell agglutination reaction studies suggested the presence of ABO blood group antigens on the umbilical vein endothelial cells and their absence in mature fibroblasts and smooth muscle cells in blood vessel media. These results suggest that the cells cultured from umbilical veins are endothelial cells. Culture of these cells offers an opportunity to clarify the interaction of platelets and vascular endothelium using an in vitro model of the blood vessel wall.

151. The Effect of Pericardial Tamponade on Coronary Hemodynamics in the Awake Dog. M. M. JARMAKANI,* PHILIP A. MCHALE,* AND JOSEPH C. GREENFIELD, JR., Durham, N. C.

In order to evaluate the effects of pericardial tamponade on coronary hemodynamics, phasic coronary blood flow was measured in chronic dog preparations during changes in epicardial pressure induced by infusion of saline into the pericardial space. Statham electromagnetic flow probes were implanted on the ascending aorta (Ao) and left circumflex coronary artery (LCCA) in eight adult mongrel dogs weighing 25–35 kg. Pressures were measured in the aorta (AP), pericardium (PCP), left ventricle (LV), through suitable catheters. All studies were done 7–15 days after the surgical procedure. Tamponade was induced with saline infusions of 25-cc increments. Maximal pericardial pressure ranged from 25–55 mm Hg. Pericardial pressures were maximal during diastole at low PCP, but were maximal during systole with a high PCP. Total coronary blood flow (CBF) decreased with PCP > 30 mm Hg. Systolic CBF decreased gradually with increasing pericardial pressure and correlated with coronary intramural pressure (AP-PCP). At maximum PCP systolic coronary flow was retrograde in five of eight experiments. Hyperemic coronary flow was abolished at pericardial pressures > 25 mm Hg. These data suggest that during pericardial tamponade coronary artery intramural pressure is a major determinant of coronary flow. Intramyocardial pressure also may play a significant role in regulating coronary blood flow. (This research supported by NIH grants HE09711 and HE10179.)

152. Seizures, H_2O_2 Formation, Lipid Peroxides in Brain during Exposure to Oxygen under High Pressure (OPH). S. A. JERRETT,* D. JEFFERSON,* AND C. E. MENGEL, Columbia, Mo.

Our previous studies showed that OHP caused H_2O_2 to form in RBC's causing lysis. The present study was undertaken to determine: (*a*) if H_2O_2 occurs in brain during OHP and (*b*) any relation to lipid peroxidation and seizures. Chow-fed (CF), tocopherol-deficient (TD), and tocopherol-supplemented (TS) mice were exposed to 100% O_2 at 45 psia for 60 min. Before OHP mice were given aminotriazole (AT) 2100 mg/kg intraperitoneally. At room air, brain H_2O_2 of TD mice was higher (34 U) than that of CF mice (27 U) ($P < 0.01$). OHP CF mice showed 41 vs. 71 U for OHP TD mice ($P < 0.005$). Tocopherol supplementation prevented H_2O_2 formation. Lipid peroxides paralleled H_2O_2 formation. Seizures occurred in 100% of TD, 50% of CF, and 0% of TS mice. To examine relationships between lipid peroxidation, H_2O_2 formed, and seizures. TD rats were given AT and exposed to 100% O_2 at 60 psia for 5, 15, and 35 min. The first significant increase in lipid peroxides was at 5 min 0.62 μmole (OHP rats) vs. 0.55 μmole (rats at room air) ($P < 0.025$). H_2O_2 in OHP rats (79 U) was first greater than rats at room air (52 U) at 15 min ($P < 0.01$). At these times there was no clinical evidence of seizures. These data show that a close relationship exists between H_2O_2 formation and lipid peroxidation in brain, both of which precede clinically evident seizure activity, during OHP. (Research supported by grants from the Department of Naval Research and National Aeronautics and Space Administration, and National Institutes of Health.)

153. The Role of Acetaldehyde in the Myocardial Depressant Effects of Ethanol. M. J. JESRANI,* V. SETHI,* M. I. KAHN,* H. A. OLDEWURTEL,* AND T. J. REGAN,** Newark, N. J.

Ethanol has been shown to acutely depress left ventricular (LV) function but the contribution of its first metabolite, acetaldehyde (ACH), is unknown. Using doses to effect blood levels associated with moderate ethanol ingestion, 15 intact anesthetized dogs were infused with ACH in saline 50 μg/kg per min for 2 hr. Peak blood concentrations were less than 1 μg/ml by gas chromatography. LV dp/dt maximum decreased from 2015 to 1775 mm Hg/sec ($P < 0.02$). Stroke volume declined despite a significant rise of end-diastolic pressure and volume, measured by indicator dilution. Aortic pressure and heart rate were unchanged. To test the role of its first metabolite, ethanol was administered intravenously (1.5 g/kg) over 2 hr to groups A ($n = 8$) and B ($n = 5$). The latter were pretreated with pyrazole 6 mmoles/kg orally to inhibit the production of ACH. Group A had a rise of LVEDP from 5.0±0.4 to 9.8±0.6 mm Hg and stroke output declined from 14.7±1.4 to 7.6±1.1 ml/min, which persisted for 3 hr without significant change in heart rate or arterial pressure. With similar control hemodynamic values, in group B animals the infusion of ethanol produced no significant change in left ventricular end-diastolic pressure, stroke output, or dp/dt maximum throughout the observation period when ethanol oxidation was inhibited. Thus, ACH at blood levels found after moderate ethanol intake has a depressant action on the left ventricle and appears to have a major role in the myocardial response to ethanol.

154. Fetal Gene Expression in Human Tumor Cells. OLIVER W. JONES,* ANDREW TAYLOR,* AND MARY A. STAFFORD,* La Jolla, Calif. (introduced by William L. Nyhan).

It has recently been postulated that viral information, including that portion of the viral genome responsible for transforming normal cells to tumor cells, is vertically transmitted in vertebrates (R. J. Huebner and G. J. Todaro. *Proc. Nat. Acad. Sci. U.S.A.* 1969. **64**: 1087). The postulate also states that genes for certain tumor viruses, which later in life may act as determinants of cancer, may be important also as gene determinants during embryogenesis. The viral information manifest through fetal gene expression should be detectable during a certain period of fetal development. Derepression of the fetal gene activity should occur in tumor cells and in cells undergoing transformation with oncogenic viruses. We have discovered a thymidine kinase variant in normal human fetal tissue that may represent one form of virogene expression. Thymidine kinase has been partially purified from human fetal and postnatal liver, spleen, and fibroblasts. Properties of fetal enzyme different from those in postnatal tissue include molecular size, electrophoretic characteristics, pH optima, heat stability, utilization of phosphate donors, inhibition by dCTP, and intracellular compartmentalization. Fetal form of thymidine kinase dominates during a brief period of fetal development and is not detectable in postnatal cells. The enzyme is detectable in human cancer tissue but not in benigh tumors. Fibroblast cell lines cultured from human donors were transformed with SV_{40} DNA virus. The form of thymidine kinase produced in transformed cells changes from postnatal to fetal. Thus, a human fetal gene for thymidine kinase may fulfill criteria of virus expression, vertically transmitted as part of the natural genetic apparatus in human cells. (Supported by grants from the American Cancer Society and National Institutes of Health.)

155. Survival of *Toxoplasma gondii* within Phagocytic Vacuoles: Evidence for Absence of Lysosomal Fusion. THOMAS C. JONES* AND JAMES G. HIRSCH,** New York.

Observations have been made on the interaction between the obligate intracellular protozoan, *Toxoplasma gondii,* and several types of mammalian cells in tissue culture. Parasites freshly isolated from the peritoneal cavity of infected mice were viable (> 95% excluded trypan blue) and were highly infectious in vivo for mice and in vitro for Hela cells and for mouse macrophages and fibroblasts. The mechanism of entry of toxoplasmas into the various cell types was phagocytosis, as revealed by electron microscopic observations during the early minutes after toxoplasma-cell contact. All or nearly all parasites that entered Hela cells divided. In macrophages, however, two populations were seen: approximately 50% of the organisms remained morphologically normal and multiplied after a lag period of several hours, whereas the other half of the toxoplasmas became morphologically abnormal within 1/2 to 3 hr and had undergone autolysis by 6 hr. The toxoplasmas which appeared to be degenerating were seen in typical phagocytic vacuoles. These vacuoles were converted to phagolysosomes by fusion with primary

lysosomes (Golgi vesicles) and with secondary lysosomes (dense granules), as demonstrated by electron microscopic studies using cells prelabeled with thorotrast or using cytochemical acid phosphatase reactions. In sharp contrast, well-preserved or dividing toxoplasmas in all three cell types were within vacuoles with an unusual vacuolar membrane surrounded by a layer of rough endoplasmic reticulum or by mitochondria. In macrophages these phagocytic vacuoles remained negative for acid phosphatase and for thorotrast markers of lysosomal fusion. These studies thus suggest that survival of toxoplasmas within cells may be related to the absence of fusion of lysosomes with parasite-containing vacuoles. (Research supported by a grant from NIH.)

156. Renal Enterobacterial Infection as the Cause of Equine Infectious Anemia. ROBERT K. JORGENS* AND KATHLEEN E. ROBERTS,** Owego, N. Y.

More evidence has accrued to assign renal infection as the causative factor of several degenerative diseases of man (*J. Clin. Invest.* 1970, 1971. Abstracts.) Treatment universally reversed the clinical situation. Comparative studies on equines revealed that one of their degenerative diseases, equine infectious anemia, was causally related to renal enterobacterial infection. These studies revealed that this disease is neither infectious nor solely equine. Horses were successfully treated and the renal lesion as well as the anemia reversed by a vaccine identical with that used in humans.

157. Immunologic Studies of Human Bronchoalveolar Cells and Secretions. P. F. JURGENSEN,* G. N. OLSEN,* J. E. JOHNSON,* E. W. SWENSON,* E. M. AYOUB, AND R. H. WALDMAN,* Gainesville, Fla. (introduced by L. E. Cluff**).

The cellular and humoral immune responses to immunization with influenza virus vaccine either subcutaneously or by aerosol were studied. Bronchoalveolar (BA) lavage, nasal washing, and venipuncture were carried out on 21 volunteers before and after immunization. Cell-mediated immunity was assessed by studying the inhibition of macrophage migration (IMM) using the BA lymphocytes, and determining the β-*N*-acetylglucosaminidase (BNAG) level of the alveolar macrophages as a measure of lysosomal acid hydrolase activity. Immunoglobulin and antibody levels were measured on the supernatants. The results indicate that the IgG: IgA ratios are different in the various body fluids studied, being about 5:1 in serum, 1:2 in nasal secretions, and 3:1 in BA washings. Influenza-neutralizing antibody titers in BA fluid and in nasal washings were highest after aerosol immunization, with the mean titer rising 5-fold in the BA fluid of volunteers immunized by aerosol, and 3-fold in those immunized subcutaneously. The serum antibody titers, however, rose 4-fold and 25-fold respectively. After aerosol immunization, BA lyphocytes gave 30% inhibition in the IMM test, while circulating lymphocytes gave 5% inhibition. After subcutaneous immunization, a reverse pattern was seen, with BA lymphocytes giving 16% inhibition, while circulating lymphocytes gave 28% inhibition. The level of BNAG in alveolar macrophages rose significantly after aerosol, but not after subcutaneous immunization. These data confirm and extend the results of several studies in recent years which indicate that the lower respiratory tract is a relatively independent immunologic organ. (This work was supported by NIH Grant AI10295-01 and by the Irwin Strasburger Foundation.)

158. Prolonged Steady-State Protein and Secretory Immunoglobulin A Synthesis and Secretion by Intestinal Mucosa. MARTIN F. KAGNOFF* AND ROBERT M. DONALDSON, JR., Boston, Mass.

Investigations into intestinal synthesis and secretion of immunoglobulins are currently hampered since the limited viability of available in vitro preparations allow steady-state conditions for only 1–2 hr. We have examined *de novo* synthesis and secretion of protein and secretory immunoglobulin A (sIgA) by rabbit jejunal mucosal biopsies cultured in an organ culture system. Incorporation of leucine-^{14}C into tissue protein by these biopsies was linear with time, and the rate of protein synthesis was the same at the beginning and end of 24 hr of incubation. After a 3–6 hr lag, biopsies steadily secreted radiolabeled protein into the incubation medium. In "pulse-chase" experiments, the kinetics of protein turnover were linear and radiolabeled protein was steadily secreted during the 6 hr "chase." Cyclohexamide inhibited protein synthesis by more than 99%. Radiolabeled sIgA secretion by single biopsies was assayed after 3–24 hr incubation using anti-rabbit sIgA covalently linked to a solid phase immunoadsorbant. Increases in specific binding to the bromoacetylcellulose-antibody immunoadsorbant were linear with time between 6 and 24 hr. After 24 hr, components of SIgA composed 13–22% of the total radiolabeled protein secreted by the intestinal mucosa. To document that sIgA-^{14}C was present in the incubation medium as an intact molecule containing secretory component (SC), sIgA-^{14}C was purified from pooled concentrated medium by sequential column chromatography on Sephadex G-200, DEAE cellulose, and Sepharose 4B. This purified material uniformly gave a single radioactive precipitin arc by immunodiffusion and immunoelectrophoresis with specific antibodies against sIgA, SC, and α-chain. These results demonstrate that, when incubated in an organ culture system, intestinal mucosal biopsies synthesize and secrete protein and sIgA under steady-state conditions for prolonged periods. (Research supported by NIH Grants AM 05025 and AM 11867.)

159. A Possible Mechanism of Connective Tissue Defect in Homocystinuria. ANDREW H. KANG,* Boston, Mass. (introduced by Donald B. Martin).

Homocystinuria is an inherited disorder of methionine metabolism characterized biochemically by homocysteinemia, homocystinemia, and homocystinuria. Clinically, the affected patients manifest wide-spread deformities and malfunctions of connective tissue including joint laxity, kyphoscoliosis, genu valgum, severe osteoporosis, ectopia lentis, and vascular abnormalities. Although the basic defect has been shown to be the deficient activity of the enzyme, cystathionine synthetase, the mechanism for connective tissue alterations has not been elucidated. In view of the clinical similarities to the many of the features observed in osteolathyrism, the possible influence of the metabolites on the properties of collagen was investigated. Purified rat and chick skin tropocollagen, rich in cross-link precursor alde-

hydes, solubilized in 0.05 M Tris–0.16 M NaCl, was incubated at 37°C in the presence of homocysteine, homocystine, or methionine to form insoluble native-type fibrils, and after the fibril was completed, each sample was examined for reversibility of insolubility upon cooling to 4°C. The results obtained clearly showed that the collagen solution containing homocysteine fails to form insoluble fibrils. Furthermore, much less of the reducible amino acid residues, δ-hydroxylysinonorleucine, the aldol condensation product, and the post-histidine compound, which have previously been shown to be involved in collagen cross-linking, are formed in the preparations containing homocysteine as compared with the control and the samples containing methionine or homocystine. It is concluded that homocysteine interferes with the formation of the reducible intermolecular cross-links which help stabilize the collagen supramolecular complex. These in vitro results were tentatively confirmed by analyses of the skin biopsy samples from two patients with vitamin B_6 unresponsive homocystinuria, which showed similarly decreased content of the cross-link-related compounds. (Supported by grants from NIH and National Science Foundation.)

160. Evidence of a Genetic Defect in Pyruvate Oxidation in Three Patients with Friedreich's Ataxia. R. A. P. KARK,* J. P. BLASS,* AND W. K. ENGEL,* Los Angeles, Calif. and Bethesda, Md. (introduced by C. M. Pearson.)

17 patients with various spinocerebellar degenerations were screened for abnormalities of oxidative metabolism. Muscle slices from 9 patients oxidized 2-^{14}C-pyruvate to $^{14}CO_2$ at rates 6 standard errors below the mean for 17 normal and disease-control patients (1.23±0.13 μmoles hr^{-1} g^{-1} protein; mean±SEM) as did muscle from 6 of 16 patients with motor neuropathies. There was no relation between pyruvate oxidation and the mild-to-moderate neuropathic changes evident in muscle by histochemical study. Three of the nine patients (from two families) whose clinical diagnoses were Friedreich's ataxia were investigated further. Blood pyruvate levels were elevated in the basal state. Skin fibroblasts from these three were studied after some 25 serial passages in tissue culture. The test fibroblasts oxidized pyruvate to $^{14}CO_2$ more slowly than did cells from nine normal and disease-controls when oxidation was stimulated by 8 μM dinitrophenol. Results were similar with 1-^{14}C-pyruvate (9.7–14.9 vs. 19.3–31.3 μmoles hr^{-1} g^{-1} protein for controls) and with 2-^{14}C-pyruvate (2.52–4.14 vs. 4.86–10.62 for controls). The rates of oxidation of 1-^{14}C-palmitate and of 1-^{14}C-glutamate were within the lower range of the controls. The lower rates of oxidation after multiple serial passage in culture probably reflect genetically determined deficiencies in the fibroblasts since material present in the original sample would have been diluted by a factor of 10^6 or more. Some patients with "Friedreich's ataxia" appear to have a biochemical defect which is genetically determined, which is as yet unidentified, but which is reflected in reduced oxidation of pyruvate. (Supported by NICHD Grant HD-05061.)

161. Effect of Hypothyroidism on Sodium Reabsorption (T_{Na}) and Renal Na-K-ATPase. ADRIAN I. KATZ* AND MARSHALL D. LINDHEIMER,* Chicago, Ill. (introduced by Leslie J. DeGroot).

Decreased Na-K-ATPase in crude homogenates of several organs from thyroidectomized (Tx) rats has been reported and correlated with reduced oxygen consumption and increased sodium content of corresponding tissue slices. The present study was designed to evaluate further the effect of hypothyroidism on Na-K-ATPase in the kidney, and to determine whether its decrease is the direct effect of thyroid hormone deficiency or represents only an adaptive response to decreased reabsorptive sodium load. Age- and weight-matched Tx (Free Thyroxine Index = 1.4±0.1 SEM) and control (FTI = 7.3±0.6) rats were studied simultaneously. T_{Na} was lower in Tx rats than in controls (110.4±9.2 vs. 152.3±9.0 μEq/min per 100 g, $P < 0.005$) and was accompanied by quantitatively similar differences in renal microsomal Na-K-ATPase (63.6±4.0 vs. 89.2±7.9 μmoles P_i/mg protein per hr, $P < 0.01$). This decrement was greater in outer medulla than cortex and appeared specific, since enzymes not involved in sodium transport (Mg^{++}-ATPase, glucose-6-phosphatase, 5′-nucleotidase) remained unchanged. K_mNa (22 mEq/liter) was similar in Tx and controls. Triiodothyronine (10^{-12} to 10^{-5} mole/liter) had no effect in vitro on microsomal Na-K-ATPase of either Tx or control animals. In order to increase filtered and reabsorbed sodium per kidney, Tx rats were uninephrectomized. 3 wk later T_{Na} in the remaining kidney increased markedly, exceeding that calculated per kidney of sham-operated controls. Despite persistence of the hypothyroid state, Na-K-ATPase activity also increased significantly when compared to sham-operated Tx animals ($P < 0.005$), and was similar to that of intact controls. The decrease in renal Na-K-ATPase in thyroid deficiency appears to be mediated primarily through decreased tubular sodium transport, since it can be reversed by increasing T_{Na} despite continuing hypothyroidism. (Research supported by a grant from NIH.)

162. Metabolism of Proinsulin, Insulin, and C-Peptide. ADRIAN I. KATZ* AND ARTHUR H. RUBENSTEIN,* Chicago, Ill. (introduced by Richard L. Landau**).

Proinsulin comprises a significant proportion of circulating basal immunoreactive insulin, and its concentration varies independently of insulin after beta cell stimulation. However, the relative contribution of secretion and metabolism in determining proinsulin levels is uncertain. Similarly, the metabolic fate of C-peptide is unknown. Urinary clearance, renal extraction, and metabolic clearance rates (MCR) of bovine proinsulin, insulin, and C-peptide were measured in separate experiments in rats. Each peptide was infused at different rates to obtain a wide range of steady-state plasma levels. Concentrations in arterial and renal venous blood and in urine were measured by radioimmunoassay. Renal hemodynamics were evaluated by measuring GFR (inulin clearance) and renal plasma flow (PAH clearance and extraction). Urinary clearance of both insulin (7.2±0.6 μl/min) and proinsulin (3.4±0.6 μl/min) was low, neither exceeding 0.2% of simultaneously measured GFR. However, the kidney extracted significant amounts of both peptides (insulin, 40.2±2.0%; proinsulin, 34.9±1.5%). Insulin MCR was much higher than that of proinsulin (16.4±0.4 ml/min

vs. 6.7±0.3 ml/min; $P < 0.001$); both were independent of plasma concentration. Renal extraction contributed 53.9± 6.2% of proinsulin MCR, but only 25.2±3.6 of insulin MCR ($P < 0.005$). C-peptide was handled similarly by the kidney (12.0±2.4 μl/min urinary clearance; 41.3±3.5% extraction); its MCR was slow, approaching that of proinsulin. We conclude the following. (*a*) Renal handling of proinsulin, insulin, and C-peptide is similar, being characterized by high extraction rates and very low urinary clearances. (*b*) Proinsulin and C-peptide MCR are comparable, approximating 40% that of insulin. This difference is probably due to substantial extraction of insulin, but not of the other two peptides, by the liver. (*c*) The kidney is a major site for degradation of all three peptides, contributing 25% of insulin MCR and more than 50% of the MCR of proinsulin and C-peptide.

163. Episodic Secretion of Aldosterone in Supine Man. FRED H. KATZ,* PEGGY ROMFH,* AND JUDITH A. SMITH,* Denver, Colo. (introduced by Karl E. Sussman).

Frequent blood sampling has shown that cortisol is secreted in bursts occurring during only a portion of the day in response to bursts of ACTH. Since aldosterone is also known to vary in "steady-state" subjects and to respond to ACTH, we have measured plasma aldosterone, renin activity, and cortisol by sensitive radioimmunoassay and competitive protein-binding methods every 30 min in healthy supine individuals over 24 hr. The subjects were maintained on 130 mEq sodium–70 mEq potassium diets for 4 days and acclimated to the metabolic ward. Blood was drawn from an indwelling needle. In the first two subjects studied plasma aldosterone varied from 0 to 296 pg/ml. These men had six and eight secretory bursts of cortisol and four and six of aldosterone, respectively. The aldosterone peaks in the prewaking hours were synchronous with those of cortisol. The lesser aldosterone peaks that occurred later in the day appeared to lag somewhat behind those of cortisol. Active cortisol secretion occurred 34 and 46% of the time and aldosterone secretion 21 and 31% of the time. Aldosterone secretion rates in these supine subjects, calculated by the method of Weitzman et al., were 41 and 101 μg/24 hr. Plasma renin activity also varied within the normal supine range. Secretion of renin seemed concordant with the other two hormones during the major secretory episodes but large renin peaks later in the day were not followed by corresponding aldosterone peaks. These results demonstrate episodic basal secretion of aldosterone for the first time and suggest that the ACTH-cortisol system has an important role in this periodicity. (Supported by grants from The Population Council, NIH, and Veterans Administration.)

164. A Soluble Receptor for IF-B_{12} from Guinea Pig and Human Ileum. MAX KATZ* AND BERNARD A. COOPER, Montreal, Canada.

Absorption of vitamin B_{12} in many species requires intrinsic factor (IF) which binds B_{12} and attaches specifically to a receptor in the intestine. We have solubilized a factor from guinea pig and human ileal mucosa which specifically binds IF-B_{12} complex. Mucosa from guinea pig ileum was sonified, treated with Triton X-100, and 100,000 *g*'s supernatant was dialyzed and lyophilized. Portions of this extract were incubated with $^{57}CoB_{12}$ and gastric juice from subjects and from a patient described previously, whose gastric juice contained a nonfunctional IF unable to bind to guinea pig mucosal homogenate (GPMH). Filtration on Sephadex G-200 revealed some radioactivity excluded from the gel in the case of the normal, but not the abnormal, gastric juice. Fractionation on Sepharose 4-B of normal IF-$^{57}CoB_{12}$ and extract revealed several peaks of radioactivity with molecular sizes ranging from 400,000 to 1,600,000. These procedures were applied to terminal ileal mucosa obtained from a single human subject. A material was present in the lyophilized extract of the 100,000 *g* supernatant which bound IF-$^{57}CoB_{12}$ and was excluded from Sephadex G-200. Dissociation of $^{57}CoB_{12}$ from human IF in the presence of excess $^{59}CoB_{12}$ was faster ($t_{\frac{1}{2}}$ 150 min) at 37°C than when the $^{57}CoB_{12}$-IF was attached to GPMH. No dissociation of the latter was observed over 360 min. The macromolecular factor described above, which bound only the normal IF-B_{12} complex, probably is soluble receptor for IF-B_{12}. When IF-B_{12} complex was bound to receptor on GPMH, the rate of dissociation of $^{57}CoB_{12}$ was much less than when not so bound. (Research supported by grant from MRC of Canada.)

165. Site of Association of Protein, Lipid, and Carbohydrate Components of Low Density Lipoprotein in Intestinal Epithelial Cells. JACQUES I. KESSLER, Montreal, Canada.

The cellular equivalent of microsomes is the site of synthesis of low density lipoprotein (LDL) by intestinal epithelium (Kessler. 1970. *J. Biol. Chem.* **245**: 5281). The site of association of the protein, lipid, and carbohydrate components of LDL is unknown. To elucidate this, corn oil containing palmitate-^{14}C was fed to rats and a reasonably pure suspension of lipid-loaded intestinal epithelial cells was obtained by ultracentrifugal flotation. The cells were incubated with ^{3}H-labeled amino acids and glucosamine-^{14}C, with and without puromycin. Medium LDL of solubilized rough (RSM) and smooth surface membranes (SSM) was isolated by immunoprecipitation and protein, lipid, and hexosamine radioactivities assayed. Aliquots of RSM and SSM were also incubated in fortified media containing ^{3}H-labeled amino acids and glucosamine-^{14}C. Lipid radioactivity occurred in medium LDL at 10 min and remained reasonably constant for 120 min. Protein and hexosamine radioactivity in medium LDL occurred at 40 min, increased linearly up to 80 min, and remained constant thereafter. LDL protein radioactivity of RSM was higher than that of lipid and hexosamine and at 30 min all radioactivities approached those of medium LDL. LDL radioactivities of SSM paralleled those of medium LDL. In contrast to RSM, protein radioactivity of SSM approached that of medium LDL after 80 min. Puromycin markedly decreased the radioactivities of RSM, SSM, and medium LDL. Inhibition of lipid incorporation was not manifest until 40 min. Protein radioactivity of incubated RSM was higher than that of lipid and hexosamine. The opposite was obtained with SSM. Radioactivities of medium LDL were greater when RSM and SSM was incubated together. These results indicate that the aproprotein of LDL is synthesized

by the RSM and subsequently associates with lipid and carbohydrate in the SSM before release.

166. Collagenase-Sensitive Adrenocorticotropic Hormone (ACTH) Receptor in Isolated Adrenal Cells. ABBAS E. KITABCHI* AND DONALD B. WILSON,* Memphis, Tenn. (introduced by Gene H. Stollerman**).

We have previously shown that isolated adrenal cells prepared by a trypsin digestion method (IACT) produce corticosterone in response to cyclic AMP (c-AMP) and microunit quantities of ACTH. Adrenal cells prepared by collagenase digestion method (IACC), however, exhibit a loss of ACTH response without alteration of c-AMP-induced steroidogenesis. In order to study the mechanism of action of ACTH in these two cell preparations, adenyl cyclase (AC) was measured in IACT and IACC preparations in response to ACTH and NaF by measuring conversion of ATP-α^{32}P to cyclic 3'5'-AMP-^{32}P. Since neither preparation contained an appreciable amount of cyclic nucleotide phosphodiesterase, AC was measured in these cells in the absence of methyl xanthines. ACTH and NaF stimulated AC in IACT preparations in a dose-related fashion. With 20 μU of ACTH and 20 mM NaF, AC activity increased from 44 to 152 and 136 pmoles/10 min per adrenal, respectively. AC activity in IACC, however, was below 10 pmoles in the control and experimental conditions. Lack of effect of ACTH in IACC was shown not to be due to residual collagenase in the preparation. We conclude that (*a*) loss of ACTH response in IACC has been demonstrated with concomitant diminution of AC activity; (*b*) trypsin-digested cells exhibited ACTH concentration-dependent AC activity; and thus, (*c*) collagenase treatment impairs ACTH binding and/or ACTH-activated AC in isolated adrenal cells. (Supported by grants from Veterans Administration and NIH.)

167. Metabolism of Thyroid Hormones by Phagocytizing Human Leukocytes. S. J. KLEBANOFF AND W. L. GREEN,* Seattle, Wash.

Myeloperoxidase (MPO), H_2O_2, and an oxidizable cofactor such as iodide, bromide, chloride, or thiocyanate ions form an antimicrobial system which may be operative in intact leukocytes. When iodide is the halide employed in the isolated system, iodination of the microorganisms occurs. Iodide also is converted to organic form by intact phagocytizing leukocytes. Thyroxine (T_4) and triiodothyronine (T_3) can replace iodide as the cofactor in the isolated MPO-mediated antimicrobial system. We have, therefore, studied the metabolism of ^{125}I-labeled T_4 and T_3 in vitro by intact human leukocytes and by purified MPO. Resting leukocytes metabolize T_4 and T_3 very slowly. Addition of phagocytizable particles, such as preopsonized zymozan or *Lactobacillus acidophilus,* stimulates hormone metabolism about 15-fold. The chief products are iodide and material which remains at the origin on paper chromatography. The latter consists, in part at least, of iodinated protein. MPO and H_2O_2 also rapidly degrade T_4 and T_3, forming iodide and origin material. Degradation by intact phagocytizing leukocytes or by MPO and H_2O_2 is inhibited by propylthiouracil, azide, cyanide, and ascorbic acid. Methimazole also inhibited the formation of iodinated origin material; however, the rate of deiodination of T_4 or T_3 is increased by this agent. Leukocytes of two patients with chronic granulomatous disease degraded T_4 and T_3 much more slowly than normal leukocytes, whereas intermediate rates were obtained with leukocytes from a patient with hereditary MPO deficiency. We conclude that the metabolism of T_4 and T_3 by intact phagocytizing leukocytes may be the major source of iodide for the cell and may be responsible for the accelerated thyroid hormone turnover observed during bacterial infections. (Research supported by USPHS Grants AI 07763 and AM-1000.)

168. Countercurrent Multiplication System—New Model. JUHA P. KOKKO* AND FLOYD C. RECTOR, Dallas, Tex.

The present study reports a new model for countercurrent multiplication in which both the descending limb of Henle (DLH) and thin ALH operate as purely passive equilibrating segments. This model is based in part on transport characteristics obtained by perfusing isolated segments of rabbit DLH in vitro. Water and solute transport was examined by imposing an osmotic gradient across the DLH by addition to the bath either (*a*) 112 mmoles/liter urea, or (*b*) combination of 204 mOsm/liter NaCl and 119 mOsm/liter urea. The rise in osmolal and volume marker ratios of collected to perfusion fluids were not statistically different. In both cases simultaneous urea influx was calculated from the appearance rate of the urea-^{14}C added to the bath. The collected urea concentration increased by 5.2±2.5 mmoles/liter in (*a*) and 7.8±3.0 mmoles/liter in (*b*). Under both conditions, intraluminal [NaCl] rose significantly above the ambient surroundings. For the passive equilibration model to be operative the following additional membrane characteristics should be present: thin ALH, more permeable to NaCl than urea; thick ALH, active NaCl transport; outer medullary and cortical collecting duct (CD), urea impermeable; inner medullary and papillary CD, urea permeable. The salient feature of the proposed model is that the energy generated by active outward NaCl transport by thick ALH (expressed as high urea concentration in outer medullary CD by virtue of water abstraction) is transmitted to the papilla (by way of urea diffusing down its concentration gradient). In turn, papillary interstitial urea abstracts water out of DLH generating high intraluminal NaCl concentrations which allows the entire system to operate by passive diffusion of NaCl out of the thin ALH. By this model, the observed medullary concentration gradients are developed without invalidating the mass balance equations. (Research supported by grants from NIH and Texas Heart Association.)

169. Evidence That the Inhibition of DNA Replication in Tumor Cells Is Not a Specific Function of Cyclic AMP. J. KOWAL, Cleveland, Ohio.

Cyclic AMP has been reported to inhibit replication in cultures of neoplastic cells and induce reversion towards a "normal" morphology. Up to 90% inhibition of thymidine-^{3}H (TdR-^{3}H) incorporation into the DNA of a clonal line of ACTH-responsive mouse adrenal tumor cells by cyclic AMP and ACTH has been described (Masui and Garren. 1971.

Proc. Nat. Acad. Sci. **68**: 3206). The effect of ACTH was attributed to its stimulation of intracellular cyclic AMP levels. We have investigated this in clonal lines and primary tumor cultures. For most experiments, cultures in their growth phase were incubated with ACTH or other compounds from 4 to 6 hr and pulsed with TdR-^{3}H during the final 1–2 hr. Maximal steroidogenesis (~ 10-fold) can be achieved with 2 mU/ml of ACTH. A decrease in TdR-^{3}H incorporation into DNA was not perceived until 20 times this level was reached. At 400 mU/ml, incorporation of TdR-^{3}H was decreased only to 75% of control cells. This effect was reversed by replenishing the cells with fresh medium when adding the TdR-^{3}H. 10 mM cyclic AMP maximally stimulates steroidogenesis; TdR-^{3}H incorporation is inhibited 30–40%. Adenosine stimulates steroidogenesis in the clonal line, but has no effect on primary cultures. However, adenosine inhibited TdR-^{3}H incorporation > 95% in both. Although 10 mM cyclic CMP has the same steroidogenic activity as 10 mM cyclic AMP, TdR-^{3}H incorporation was inhibited < 10% by cyclic CMP. Cytidine inhibited TdR-^{3}H incorporation > 60%. However, cyclic CMP is not metabolized by phosphodiesterase. The data suggest that this inhibition is not a property of cyclic AMP per se and may be due to adenosine or a common metabolite of adenosine and cyclic AMP as well as other nucleosides. The inhibition can be dissociated from the stimulation of steroidogenesis and intracellular cyclic AMP production. Possible reasons for the remarkably high concentrations of ACTH required for this effect are still under investigation.

170. Decreased Lysyl-Protocollagen Hydroxylase Activity in Fibroblasts from a Family with a Newly Recognized Disorder: Hydroxylysine-Deficient Collagen. STEPHEN M. KRANE, SHELDON R. PINNELL,* AND RICHARD W. ERBE,* Boston, Mass.

We have recently described two sisters, ages 9 and 12, with scoliosis, recurrent joint dislocations, and hyperextensible skin and joints. Parents and older sister appeared normal. Analysis of dermal collagen (normal by electron microsopy) revealed marked decrease in hydroxylysine (about 5% normal) with normal content of hydroxyproline and other amino acids. Hydroxylysine-deficient dermal collagen showed increased solubility in denaturing solvents. Hydroxylysine was also reduced in fascia and bone, and urinary ratios of hydroxylysine/hydroxyproline were low. We reasoned that the defect might be due to deficient lysyl-protocollagen hydroxylase. In the present study skin fibroblasts cultured from family members and controls were harvested 48 hr after reaching confluence. Cell lysates were assayed for lysyl-protocollagen hydroxylase using chick tibial ^{14}C- or ^{3}H-labeled lysine-protocollagen as substrate by measuring ^{14}C- or ^{3}H-labeled hydroxylysine formed. In addition, some lysates were also assayed for prolyl-protocollagen hydroxylase using ^{14}C-labeled proline-protocollagen by measuring hydroxyproline-^{14}C formed. Whereas in the affected sister, lysyl-protocollagen hydroxylase in fibroblast lysates averaged 11 and 12% of controls respectively, their levels of prolyl-protocollagen hydroxylase were not reduced, consistent with the specificity of the defect in collagen hydroxylation. Preliminary data reveal no alteration in apparent affinity for either the lysine-protocollagen or α-ketoglutarate substrates in the mutant cell. Fibroblast lysyl-protocollagen hydroxylase was within the range of controls in the unaffected sister and about half control in the mother. This novel disorder is the first inborn error of human collagen metabolism in which both the deficient product (hydroxylysine) and enzyme (lysyl-protocollagen hydroxylase) are identified. (Supported by NIH and Gebbie Foundation grants.)

171. Selective Measurement of Lipoprotein Lipase and Hepatic Triglyceride Lipase in Postheparin Plasma. RONALD KRAUSS,* ROBERT LEVY, HERBERT WINDMUELLER,* LAURA MILLER,* AND DONALD FREDRICKSON,** Bethesda, Md.

We have now obtained additional evidence that triglyceride lipase activity released into plasma by heparin contains not only lipoprotein lipase (LPL) but a different lipase of hepatic origin (HTGL). Using rat and human tissues in an assay with triolein-^{14}C (7.5 mg/ml), we found that preincubation with protamine sulfate inhibited more than 90% of adipose tissue LPL, but less than 10% of HTGL. With rat plasma as enzyme source, protamine inhibited 43% of the heparin-released triglyceride lipase activity. In completely hepatectomized rats plasma lipolytic activity was inhibited 91% by protamine. With varying degrees of subtotal hepatectomy, protamine-inhibitable activity was constant, while residual activity was directly proportional to the amount of liver remaining. Thus in this assay postheparin lipolytic activity that is protamine inhibitable provides an accurate measure of LPL and residual activity represents HTGL. This selective assay was then used to reevaluate clinical states previously associated with abnormalities in *total* postheparin lipolytic activity. In 26 normals LPL activity was 2.7±1.0 U (μM FFA/ml per hr), and HTGL was 11.4±3.1. In 10 patients with type I hyperlipoproteinemia, LPL was 0.30±0.15 and HTGL 8.9±3.3, whereas in 12 type V patients with comparable triglyceride levels, both activities were normal. In three normals low-fat diets caused an average 66% reduction of LPL with a 16% increase of HTGL. Oral contraceptives given to two normals and progesterone to one type V patient increased LPL an average of 67%; HTGL increased only 7%. In three hypothyroid patients, LPL was normal (1.8–4.7), but HTGL was low (1.6–5.2). These data point to the necessity of distinguishing LPL and HTGL in the clinical assessment of plasma postheparin lipolytic activity.

172. The Endocrinologically Normal Enlarged Sella. DOROTHY T. KRIEGER, New York.

Previous reports have indicated abnormalities of growth hormone responsiveness in most patients with radiological evidence of increased sellar size, even where tests of gonadal, thyroid, and adrenal function have been normal. In reviewing subjects with demonstrable sellar enlargement (limited to almost exclusively intrasellar involvement) studied by our laboratory, eight subjects were encountered with *normal* growth hormone responsiveness to insulin hypoglycemia. It was then realized that these subjects were unique in that they were all female, moderately to massively obese, all but

one had had at least one pregnancy, and their ages ranged from 22 to 61 yr. None had clinical evidence of endocrinopathy and all manifested age-appropriate urinary gonadotropin titers, normal basal levels of urinary 17-hydroxycorticosteroid, 17-ketosteroid, and plasma protein-bound iodine, and normal cortisol responses to hypoglycemia. Visual fields were normal in all instances save for two patients who demonstrated suggestive defects when tested with a small red object. None had evidence of increased intracranial pressure. Electroencephalograms and brain scans were normal in all. The sellar enlargement in all but one instance was unassociated with evidence of clinoid erosion. Cerebral angiography or pneumoencephalography were not warranted in all instances in view of the absence of symptoms necessitating such studies for diagnostic or therapeutic reasons. Where such studies were performed there was no evidence of suprasellar extension. The presumptive etiology of the sellar enlargement in these cases, based on radiological criteria, was that of chromophobe adenoma. In the absence of pneumoencephalographic studies in all instances, the possibility of an "empty sella" syndrome cannot be excluded. The present findings indicate that evidence of marked sellar enlargement is compatible with a normal endocrine status according to currently employed parameters of evaluating pituitary function.

173. An Enzymatic Explanation for Increased Hepatic Triglyceride Formation in Rats Fed High Sugar Diets. ROBERT G. LAMB* AND HAROLD J. FALLON, Chapel Hill, N. C.

Serum triglyceride (TG) levels are increased by high carbohydrate intake in man and animals. Previous studies performed in vitro and in vivo have shown an increased hepatic TG formation in rats fed high glucose and high fructose diets. The enzymatic basis for the increased TG formation was studied using liver microsomal preparations from rats fed 75% fructose or glucose for 3 days. Synthesis of diglyceride (DG) and TG from ^{14}C-labeled glycerol-3-P, palmitate, ATP, and CoA was increased 2- to 5-fold by these diets. The ratio of neutral lipid to phosphatidic acid (PA) formed was increased 2- to 3-fold. The increased DG and TG formation also was observed in studies with whole homogenate. Measurement of individual enzymatic reactions in the pathway suggest PA phosphatase is the rate-limiting step and is increased 2- to 3-fold by high sugar diets. Rat liver supernate (100,000 *g*) stimulates DG and TG formation by microsomes. The stimulating factor is precipitated by 40% $(NH_4)_2SO_4$ and has PA phosphatase activity. High sugar intake increases the stimulation by this factor 3- to 4-fold. The increase in PA phosphatase activity and TG formation was observed after 24 hr and was maximum after 3 days for 75% glucose and 11 days for 75% fructose. The results suggest that high sugar intake results in accelerated hepatic TG formation as a consequence of increased PA phosphatase activity. These changes may account for the rise in serum TG observed with high carbohydrate intake in animals. (This research supported by NIH Grant No. AM-09000.)

174. Reduction of Postperfusion Cytomegalovirus Infections after the Use of Leukocyte-Depleted Blood. DAVID J. LANG,* PAUL A. EBERT,* BRADLEY M. RODGERS,* H. PRESTON BOGGESS,* AND ROBERT S. RIXSE,* Durham, N. C. (introduced by Samuel L. Katz.)

After extracorporeal perfusion 50–60% of patients develop virologic and/or serologic evidence of cytomegalovirus (CMV) infection. It has been proposed by one of us (D. L. J.) that after perfusion, donor and/or recipient leukocytes may be stimulated to divide, as in a mixed lymphocyte culture, activating latent CMV associated with leukocytes. To test this hypothesis, alternate patients were perfused with leukocyte-depleted or whole blood (controls). Preoperative leukocyte counts performed on treated pump bloods averaged 42% of the controls. Virologic and serologic studies were performed preoperatively and 2, 10, 30, and 90 days after surgery. There were 10 patients in each group. Four of six controls who were CMV antibody-negative preoperatively, seroconverted after perfusion. Virus was recovered from the blood of three and the urine of two of these patients. One of four controls who were seropositive preoperatively developed a significant titer rise. In contrast, only one of eight patients perfused with leukocyte-poor blood who were seronegative before surgery developed a viremia and became antibody positive; another patient manifested a viruria but remained antibody-negative. In the latter case the preoperative pump blood manifested a high leukocyte count; the only instance in which the leukocyte concentration in the experimental perfusate was in the range of the controls. Two patients with preexisting CMV antibody exhibited no titer change and yielded no virus. These findings support the proposed hypothesis and suggest a means for reducing if not eliminating transfusion-associated CMV infections. (Research supported by NIH RCDA SK04-HD-19472-02.)

175. Antibodies Arising during Hepatitis That React with Antigens in Normal Stools. ROGER F. LANGE, N. RAPHAEL SHULMANN,** CARLA S. KNEPP, AND CECEIL N. COLEMAN, Bethesda, Md.

Six of 30 patients with acute viral hepatitis (HAA positive or negative) and 6 of 25 patients with high titer anti-HAA after multiple transfusions developed 7S serum antibodies against normal stool antigens detected by immunodiffusion and counter immunoelectrophoresis. Of 66 patients with cirrhosis and other forms of chronic liver disease, only two had anti-stool antibodies, and of 151 normal individuals, none had anti-stool antibodies. Stool antigen was prepared by extracting homogenized stool with saline and filtering through a 450 nm pore size. Stools from 43 of 57 normal individuals and 22 of 30 patients with hepatitis or other liver disease contained the stool antigen. Different stool extracts produced precipitin lines of identity or partial identity. The antigen was not detected in serum, urine, bile, extracts of human pancreas, stomach, intestine, or liver or in extracts of bacterial cultures of antigen positive stool. The antigen has chemical characteristics of protein, is destroyed by trypsin, and the molecular weight by Sephadex G-200 filtration varies from 60,000 to greater than 300,000. No virus-like particles were seen in antigen-antibody precipitates by electron microscopy and there were no antigenic determinants in common with HAA or its subtypes AY or AD. All precipi-

tins were soluble in saline, hence could not be analyzed histochemically. These findings indicate that antigenic substances normally present in the bowel can gain access to antibody-forming tissue during liver disease, particularly acute viral hepatitis. This new antigen-antibody system, in addition to HAA, must be considered in the pathogenesis of hyperglobulinemia, "auto-immune" phenomena, immune-complex disease, and immunologic cross-reactions associated with liver disease.

176. Coronary and Intensive Care Training for Nurses: A Two Year Study of Four Projects of a Regional Medical Program. STEPHEN B. LANGFELD* AND RONALD E. MILLER,* Haverford, Pa. (introduced by John K. Clark**).

The Greater Delaware Valley Regional Medical Program, recognizing the critical role of trained nursing personnel in achieving whatever benefits result from coronary care units, have implemented six programs for training nurses in coronary and intensive care. This paper presents and analyzes data covering four programs which have been in operation for 2 yr. Of the 361 trainees surveyed by questionnaires, data have been obtained from 295. 68% are currently employed in special care units and 82% of these are working full time. 66% of the nurses currently working are responsible for five or less patients. Training has permitted an increase in the organizational level of responsibility for 55% of the nurses who were previously working in special care units and for 78% of those previously employed in some other capacity. There has been an increase in the number of nurses performing each of a list of specified procedures with a marked shift toward authority for doing so devolving upon the nurse herself. Even though 77% considered their training adequate, 45% of these have sought additional training. Participants' grades at the end of the course showed significant improvement and appeared to be reliable predictors of subsequent performance. (Study supported by grant from HSMHA of USPHS to Greater Delaware Valley RMP.)

177. Observations on Instantaneous Flow across the Incompetent Mitral Valve In Vivo. SHLOMO LANIADO,* HYMAN MILLER,* EDWARD YELLIN,* AND ROBERT W. M. FRATER,* New York (introduced by Henry D. Lauson**).

Simultaneous mitral valve flow (measured by a toroidal electromagnetic flow probe at the mitral ring), left atrial, left ventricular and aortic pressures, electrocardiogram, and aortic flow were recorded in 15 dogs during normal sinus rhythm, irregular junctional rhythm, ectopic beats, and ventricular pacing. Cutting chordae tendinae created valvular insufficiency. The major fraction of regurgitant flow occurred in early ventricular systole: a rapid fall in velocity of flow was noted after aortic valve opening, presumably because a second outlet was available. With irregular ventricular rates, the ratio of regurgitant volume to total stroke volume for any beat was determined by the preceding cycle length and the dynamics of flow of the previous beat. The latter factor was more significant in short cycles. The distribution of ventricular ejection in one beat determined instantaneous aortic diastolic pressure and thus the impedance to forward flow the subsequent beat faced. Changes in absolute regurgitant volumes from beat to beat during arrhythmias were minimal, explaining the clinical observation that the murmur of mitral regurgitation is uniform in intensity in premature beats and during arrhythmias. "Double regurgitation" (diastolic as well as systolic) was noted when relaxation of left ventricular muscle was rapid and abbreviated, limiting the duration of the period of maximal increase in compliance of the ventricle. Inflow across the mitral valve in mid-diastole created a buildup of pressure in the left ventricle resulting ultimately in a brief mid-diastolic period of positive ventriculo-atrial pressure gradient accompanied by modest regurgitant flow. In certain cases of mitral regurgitation, it is speculated that the third heart sound may arise from an extra closure of the mitral valve in diastole.

178. The Cardiac Beta-Adrenergic Receptor: Solubilization, Characterization, and Purification by Affinity Chromatography. ROBERT LEFKOWITZ,* EDGAR HABER, GEOFFREY SHARP,* AND DONALD O'HARA,* Boston, Mass.

Inotropic and chronotropic effects of catecholamines (CA) are mediated by beta-adrenergic receptors (BAR) with consequent stimulation of adenyl cyclase. Receptors and enzyme are found in membrane-bound particulate fractions of tissue. This has retarded detailed study of the individual components of this system. The 78,000 *g* particulate fraction of ventricular myocardium contains both the BAR (i.e., CA are specifically bound in the order of their potency as beta-adrenergic agonists) and an adenyl cylase which is stimulated by these CA with the same rank order of potency. To solubilize the components, particles were treated with deoxycholate or Lubrol P.X. The BAR and cyclase were found in the supernatant after 1 hr centrifigation at 100,000 *g*. Affinity and specificity of catecholamine binding in the soluble supernatant was defined by the capacity of drugs to displace bound norepinephrine-^{3}H. The order of potency of beta-adrenergic drugs was isoproterenol > epinephrine ≧ norepinephrine > dopamine > Dopa. Propranolol inhibited binding, whereas metabolites and alpha-active drugs were < 0.1% as active. K_D for norepinephrine was 4×10^{-6} mmole/liter. PCMB abolished binding, suggesting participation of free SH groups. When filtered on Sepharose 4B, norepinephrine-binding activity eluted at volumes corresponding to molecular weights of 40,000 and 160,000. An affinity chromatography column (ACC) was constructed by covalently linking norepinephrine to agarose via an extended hydrocarbon side-chain. When aliquots of supernatant were passed over the ACC, 90–100% of the BAR was adsorbed. Protein concentration (Lowry) of the effluent was not measurably different from supernatant. Receptors could then be quantitatively eluted with epinephrine at pH 3.8, thus achieving a striking degree of purification in a single step. These purified receptors retain the specificity of the original particulate preparations. (Supported by NIH No. HL14150.)

179. Ultrastructural and Functional Correlates in Cardiac Cells Grown in Tissue Culture. MARIANNE J. LEGATO,* New York (introduced by Nicholas P. Christy**).

This electron microscopic study of 2- to 6-day-old rat ventricular cells grown in tissue culture contributed to our understanding of the development and function of intercellular connections, the mechanism of mitochondrial prolifera-

tion, and the manner in which myofilaments are generated and assembled into sarcomeric units. It is apparent that neither nexal linkages between cells or a transverse tubular system are present even in the oldest (6 days) cells of the preparation, although they resemble the adult working ventricular myofiber in almost all other respects. Since the cells beat in synchrony at this stage at rates as high as 180, it may be assumed that the T system is not necessary for excitation-contraction coupling, and that the nexus is not the only low-resistance junction through which intercellular transmission of impulses occurs. Z substance is abundant in the earliest cells and precedes the development of the first myofilaments. It is the Z substance which serves as the source for or template upon which myofilament assembly and sarcomeric construction occur.

180. Passive Hemagglutinating Antibodies (PHA) in Cerebospinal Fluids (CSF) of Patients with *Herpesvirus hominis* (HVH) Encephalitis. A. MARTIN LERNER, CARL B. LAUTER,* DAVID C. NOLAN,* AND MARY JANE SHIPPEY,* Detroit, Mich.

In order to assess incidence and spectrum of disease of *Herpesvirus hominis* (HVH) infections of the central nervous system (CNS), an accurate means of diagnosis is needed. Such a test is also needed to initiate and evaluate antiviral therapy. We have modified a type-specific microindirect passive hemagglutinating antibody (PHA) procedure for HVH and studied responses in rabbit and human sera and in human cerebrospinal fluids (CSF). PHA are 2.6–14 times to 4–22 times more sensitive than neutralizing antibodies to HVH, types 1 or 2, respectively. PHA to HVH were assayed in spinal fluids obtained early during illnesses of seven patients with encephalitis caused by type 1 virus (mean CSF-PHA = 65) and in two infants with congenital disseminated type 2 virus infection (mean CSF-PHA = 768). Specific CSF-PHA were found in eight of the nine patients with HVH encephalitis. The antibodies were IgM in congenital cases, but IgG in the others. PHA in CSF of patients with HVH encephalitis were similar in patients in whom the diagnosis was made by isolation of viruses at brain biopsy, and in others in whom craniotomies were not done. PHA in CSF do not correlate with degree of meningeal injury as indicated by pleocytoses or protein. Kinetics of rises in PHA in CSF and sera suggest that the anti-herpesvirus antibodies in the CNS are locally produced. In all but one patient in whom specimens were available, type-specific PHA were 1/16 or greater by the 7th day of illness. PHA to HVH were virtually absent in CSF from 35 control patients with various diseases requiring lumbar puncture (mean CSF-PHA, *H. hominis* type 1 or 2, < 10). PHA to HVH in CSF of patients with encephalitis are a rapid, specific, inclusive, and noninvasive means of diagnosis.

181. Exercise Testing in Patients with Occluded Aorto-Coronary Saphenous Vein Bypass Grafts. STEPHEN J. LESHIN,* LAWRENCE D. HORWITZ,* ROGER R. ECKER,* GUNNAR BLOMQVIST,* W. L. SUGG,* AND CHARLES B. MULLINS,* Dallas, Tex. (introduced by Norman M. Kaplan).

Multistaged exercise tests are used to objectively quantify functional improvement after coronary bypass graft surgery. However, the correlation between functional improvement and graft patency is unknown. This relationship was examined in seven patients with nonfunctioning grafts at postoperative cardiac catheterization. Four patients had all grafts either occluded or nonfunctional. Three patients had one open, functioning graft and one graft which was either occluded or nonfunctional. Nonfunctional grafts could be visualized angiographically but no dye appeared to move into distal coronary vessels. Angiography and graded bicycle exercise were performed preoperatively and repeated an average of 8.5 months after surgery (range 4.5–13.0 months). The clinical histories and results of exercise tests correlated well. In four patients angina was partially or completely relieved and exercise performance improved (average preop maximal work load 260 kpm vs. 490 kpm postop). In one patient angina disappeared, but congestive heart failure caused severe clinical limitation and the maximal exercise work load was unchanged. Two patients continued to have angina; in one of the exercise test was unchanged and in one the maximal work load decreased from 300 to 150 kpm. Of the four patients who showed improvement by exercise testing, three had no functioning grafts and the other had one of two grafts open. Myocardial infarction during surgery or postoperatively was documented in two of the four clinically improved patients. Thus, the results of exercise testing cannot be used as the sole index of graft patency. Angiographic confirmation of graft patency is necessary before clinical improvement can be attributed to improved myocardial perfusion. (Research supported by NIH Grant HE 06296.)

182. Restoration of Catecholamine Responsiveness of Solubilized Myocardial Adenylate Cyclase by Phosphatidylinositol. GERALD S. LEVEY,* Miami, Fla. (introduced by William J. Harrington).

Catecholamines serve critical roles in cardiac function. They augment heart rate and contractility and activate myocardial adenylate cyclase by a beta adrenergic receptor mechanism. This receptor is thought to be closely related to or identical with the membrane-bound adenylate cyclase. We have described the preparation of a solubilized myocardial adenylate cyclase which is unresponsive to catecholamine stimulation. Since membrane lipids play an important role in maintaining the functional integrity of the hormone-receptor complex, we examined the effect of several phospholipids on restoring the responsiveness of the solubilized adenylate cyclase to three catecholamines, norepinephrine, epinephrine, and isoproterenol. The addition of phosphatidylinositol restored catecholamine activation of the solubilized adenylate cyclase, whereas phosphatidylserine and phosphatidylethanolamine did not. Half-maximal activation was achieved with norepinephrine 8×10^{-8} mole/liter, epinephrine 1×10^{-8} mole/liter, and isoproterenol 1×10^{-9} mole/liter, concentrations approximately 100-fold lower than those required in particulate preparations and similar to the sensitivity observed in intact physiologic preparations. Maximal concentrations of the catecholamines produced an increase in cyclic 3′,5′-AMP accumulation of approximately 150%, similar to that obtained in particulate preparations. Catecholamine activation of the solubilized adenylate cyclase in the presence

of phosphatidylinositol was abolished by the beta-adrenergic blocking agent DL-propranolol. When norepinephrine was present at 2×10^{-6} mole/liter, its effect was abolished by 1×10^{-6} M DL-propranolol, a concentration of blocker that was without effect in the presence of 2×10^{-5} M norepinephrine. The minimally effective concentration of phosphatidylinositol for restoring norepinephrine responsiveness (0.05 μg/50 μg enzyme protein) is comparable to that calculated to be present in heart muscle. These results provide further evidence for the importance of membrane lipids in hormone-sensitive adenylate cyclase systems and define a role for phosphatidylinositol as a possible molecular component of the cardiac beta-adrenergic receptor. (Research supported by NIH Grant 1 RO1 HE 13715-01.)

183. A Nonimmunoglobulin Protein—the Major Component of Some Human Amyloid Fibrils. MARK LEVIN,* EDWARD C. FRANKLIN, BLAS FRANGIONE, AND MORDECHAI PRAS,* New York.

Recent studies have demonstrated the identity of the major subunit of many amyloid fibrils to the variable region of light chains. Starting with purified amyloid fibrils from five patients with secondary amyloidosis or FMF, we have isolated a homogeneous protein which appears to be similar in all, and whose partial amino acid sequence is unlike any known immunoglobulin. In order to separate carbohydrate from the protein component of amyloid, fibrils from subjects with secondary amyloidosis and FMF were treated with 0.05 M HCl. More than 50% of the material was extracted as a homogeneous protein with a mol wt of ±6000, which bound Congo red and could be made to assume a fibrillar appearance. Partial amino sequence studies of two of these proteins were identical. The amino acid sequence of the first 50 residues derived from three cyanogen bromide fragments of one of these was unrelated to any known immunoglobulin. After complete reduction and alkylation of two amyloid fibrils, this component was recovered in the second peak on Sephadex G-100 filtration while the first peak consisted primarily of material which resembled light chains on peptide maps. Although similar material could not be extracted in significant amounts from tissues of two patients with multiple myeloma and macroglobulinemia, the possible existence of small amounts of a similar component could not be excluded with certainty. The finding of a homogeneous component not related to immunoglobulins as the major component of some forms of amyloid raises questions about the role of immunoglobulins in the pathogenesis of amyloid and the possibility of chemically defining different types of amyloid. (Research supported by grants from NIH and A.F.)

184. Inhibition of Insulin Secretion by Diphenylhydantoin (DPH) In Vitro and In Vivio: Selective Effects in Diabetes and Obesity. SEYMOUR R. LEVIN,* JAMES W. REED,* KING-NIEN CHING,* JOHN W. DAVIS,* ROBERT BLUM,* AND PETER H. FORSHAM,** San Francisco, Calif.

DPH (25 μg/ml) inhibits insulin secretory responses to glucose and arginine in vitro in the perfused rat pancreas. Whereas only the late phase of glucose-induced insulin secretion is inhibited by DPH, both early and late arginine-induced secretion are inhibited by the drug. In vivo tests were (*a*) 100 g oral glucose tolerance (OGTT), and (*b*) intravenous arginine (ARG) 30 min after intravenous glucose (GL). Tests were done on separate days, before and after 4 days of DPH ingestion by eight lean nondiabetic normals (NL), seven lean early diabetics (D), six obese nondiabetics (OND), and four obese diabetics (OD). DPH serum levels attained were similar to those in epileptics taking the drug (mean 15.1 μg/ml). In NL, mean per cent change in total insulin response in OGTT after DPH was ↑ 30%. In contrast, in the other groups, these values (and P value versus NL) were: D ↓ 30% ($P < 0.02$), OND ↓ 33% ($P < 0.01$), OD ↓ 53% ($P < 0.005$). In NL, mean per cent change, after DPH, in total insulin response to intravenous ARG after intravenous GL was ↓ 11%. Alterations seen in the other groups (and P values versus NL) were: D ↓ 59% ($P < 0.005$), OND ↓ 53% ($P < 0.05$), OD ↓ 31% (NS). With OGTT these insulin changes were accompanied by minimal changes in glucose levels in NL, glucose rises in D and in the obese. Thus, nonobese diabetics and obese subjects appear more sensitive than normals to DPH-induced reduction of insulin secretion. In the diabetics, this may reflect an effect on β-cells with limited capacity. In obesity, this may represent increased suppressibility of a highly activated secretory mechanism. (Research supported by grants from NIH and L. J. Skaggs Foundation.)

185. Influence of Water Diuresis on Ampicillin Treatment of Enterococcal Pyelonephritis. SANDRA P. LEVISON* AND DONALD KAYE, Philadelphia, Pa.

Treatment with ampicillin for 2 wk has been shown to reduce but not eradicate bacteria from kidneys of rats with enterococcal pyelonephritis. Water diuresis produced by offering rats 5% dextrose in tap water (D/W) has been shown to have a variable effect in decreasing renal titers of enterococci. In the present studies, the effect of ampicillin (40 mg intramuscularly twice a day) in combination with water diuresis was determined on renal titers of enterococci after intravenous inoculation. Ampicillin injections with or without diuresis were started 4 or 21 days after initiation of infection and continued for 7 or 14 days. In comparison to controls (saline injections in rats drinking tap water), diuresis plus saline injections did not lower renal titers of enterococci (log median titers 5.0). Injection of ampicillin in nondiuresing rats somewhat reduced renal titers of enterococci after both 7 and 14 days of treatment started 4 or 21 days after initiation of infection. Diuresis plus ampicillin reduced renal titers significantly as compared to nondiuresing rats receiving ampicillin. Log median titers in diuresing rats receiving ampicillin starting 4 days after infection were 1.0 and 1.8, respectively, after 7 and 14 days of therapy as compared with 5.0 and 4.3 in nondiuresing rats receiving ampicillin. In diuresing rats receiving ampicillin starting 21 days after infection, log median titers were 1.0 after both 7 and 14 days of therapy as compared with 4.2 and 2.5 in nondiuresing rats receiving ampicillin. These studies demonstrate that diuresis resulting from administration of D/W plus ampicillin starting 4 or 21 days after intravenous injection of enterococci reduces renal titers more than ampicillin or diuresis alone.