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ABSTRACTS

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ABSTRACTS

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

1. Dextran-Maleimide: an Oriented Probe of Membrane Structure and Function. RICHARD E. ABBOTT * AND DAVID SCHACHTER, New York.

Molecules having the general structure R-a-X, where R = a water-soluble grouping highly impermeable to cell membranes, a = a connecting group, and X = a reactive group specific for certain membrane components, can serve as oriented probes of membrane structure and function. Dextran-maleimide, the first of a series of such reagents being prepared for systematic studies of cell membranes, has been synthesized with R = a dextran moiety of mol wt 10,000, a = the grouping -O-CH_2-, and X = maleimide. The reagent binds -SH groups of proteins covalently. In a typical procedure, 1.0 g of dextran T-10 (Pharmacia) dissolved in 3.0 ml of water was mixed with 1.0 g of *N*-chloromethylmaleimide, and the resultant slurry was stirred for 64–88 hr at room temperature. Thereafter, 7 ml of water was added, insoluble precipitate centrifuged off (30,000 rpm, 10 min, 5°C), the clear aqueous solution loaded on a Sephadex G-25 column, and the high molecular weight product eluted with water and freeze-dried. The maleimide content of the product was estimated by reaction with excess cysteine, using Elman's reagent to quantify unreacted -SH. In various batches the product has contained one to two maleimide residues per dextran molecule. In the same manner [^{3}H]-dextran-maleimide was prepared from [^{3}H]-dextran (mol wt 15,000–17,000, 1 mCi/2.4 mg, New England Nuclear Corp.). Initial experiments demonstrate that dextran-maleimide inactivates the purified soluble enzyme papain. Studies with the reagents are in progress to quantify cell surface -SH groups, to localize surface enzymes, and to label cell membranes for isolation procedures. (Supported by NIH grants AM-01483 and AM-04407.)

2. Uric Acid Transport in Rat Kidney. RUTH G. ABRAMSON,* JAY H. KATZ,* JOHN K. MAESAKA,* AND MARVIN F. LEVITT,** New York.

Clearance and free-flow micropuncture experiments were utilized to study uric acid (Ur) transport in rat kidney during hydropenia and subsequent saline-induced volume expansion. In all experiments, a prime and constant infusion of [2-^{14}C]Ur and [*methoxy*-^{3}H]inulin were administered. Since infused [2-^{14}C]Ur is largely converted to [2-^{14}C]allantoin in vivo, a new column chromatographic technique was developed in which simple stepwise elution was used to separate these compounds. This method permitted the radioassay of [2-^{14}C]Ur in plasma (P), urine (U), and nanoliter quantities of tubular fluid (TF). In nine control clearance experiments U/P_{Ur} relative to U/P_{In} ($[U/P_{Ur}]/[U/P_{In}]$) increased from a mean of 0.85 in hydropenia to 0.95 as volume was expanded to 7% of body weight. In seven other studies in which a prime (100 mg/kg) and constant infusion (100 mg/kg per h) of pyrazinamide were administered, $(U/P_{Ur})/(U/P_{In})$ increased in parallel fashion from a mean of 0.58 in hydropenia to 0.74 during volume expansion. These findings suggested that, with or without pyrazinamide, Ur reabsorption decreases as volume is expanded and that pyrazinamide reduces $(U/P_{Ur})/(U/P_{In})$ by inhibiting Ur secretion. To ascertain the site(s) of these transport processes, micropuncture collections were obtained from either early or late proximal tubules (PT) in nine rats. The $(TF/P_{Ur})/(TF/P_{In})$ in the early PT was consistently lower than the simultaneously obtained $(U/P_{Ur})/(U/P_{In})$. When the late PT $(TF/P_{Ur})/(TF/P_{In})$ was compared to the $(U/P_{Ur})/(U/P_{In})$, however, the ratios were found to be approximately equal in both hydropenia and volume expansion. These findings indicate that in the rat (*a*) net reabsorption of Ur occurs within the early PT, (*b*) Ur secretion becomes evident in the late PT, and (*c*) virtually no net Ur transport occurs within the distal nephron. (Research supported by grant from the NIH.)

3. Increased Renal Ammonia Production in Metabolic Acidosis: a Consequence of Enhanced Mitochondrial Glutamine Transport. WILLIAM ADAM * AND DAVID P. SIMPSON,* Seattle, Wash. (introduced by Seymour Klebanoff).

Chronic metabolic acidosis is accompanied by increased renal ammonia production from glutamine resulting in greatly increased NH_4^+ excretion. Renal NH_3 formation occurs chiefly from the conversion of glutamine to glutamate by glutaminase I which is located in the mitochondrial matrix space. When mitochondria are incubated with [^{14}C]glutamine and rapidly separated from the incubation medium, [^{14}C]glutamine cannot be detected in the matrix space, over 85% of the accumulated counts being present in glutamate. Hence, when glutaminase levels are constant, the rate of glutamine transport across the inner mitochondrial membrane can be estimated by measuring total ^{14}C or [^{14}C]glutamate accumulation in the matrix space. In six pairs of chronically acidotic or alkalotic litter mate dogs, glutamate accumulation in the matrix space was 26–75% (mean 52±6.7 SEM; $P < 0.005$) greater in the acidotic than in the alkalotic animals; glutaminase levels did not differ between the two groups. In 21 experiments in rats 3 h after induction of acidosis, glutaminase levels were unchanged; total ^{14}C accumulation in acidotic mitochondria was 37%±4.5 SEM greater than in nonacidotic controls ($P < 0.001$). In chronically acidotic rats K_m for NH_3 formation in mitochondria was the same (0.36 mM) as in controls (0.32 mM); the rate of NH_3 formation was four times greater in the acidotic group. These results suggest that chronic metabolic acidosis causes an increase in the number of transport sites for glutamine in the inner membrane of mitochondria from renal cortex. The resulting increase in delivery of glutamine to the matrix space enables glutaminase to form more NH_3 from this substrate. (Research supported by grants from NIH and Federal Health Program Services.)

4. Regulation of Cyclic Erythropoiesis in the Grey Collie. JOHN W. ADAMSON,* DAVID DALE,* AND RONALD ELIN,* Seattle, Wash., and Bethesda, Md. (introduced by Clement A. Finch **).

Grey collie dogs have 12-day cyclical fluctuations of blood neutrophils, monocytes, reticulocytes, and platelets as a consequence of periodic hypoplasia of marrow precursors. Their hematocrits average 30% (normal: 42.5%) and reticulocyte counts cycle from 0.0–1.0% to 2.5–3.2% (normal: 1.2%). Possible mechanisms for this phenomenon include abnor-

malities of stem-cell function, cycling of long-range humoral stimulators, exaggerated feedback control, or cycling of marrow inhibitors. To examine marrow regulation, both erythropoietic inhibitors and stimulators have been assessed in this setting. Inhibitors of erythropoietin (ESF) or hemoglobin synthesis were sought by culturing normal dog bone marrow cells in grey collie sera (9–82% serum concentration by volume) and measuring ^{59}Fe incorporation into hemoglobin. No inhibition of basal or ESF-dependent hemoglobin synthesis was observed. Daily reticulocyte counts and serum ESF activity (measured by both bioassay and in vitro) were used to monitor physiologic regulation through a minimum of two cycles and in response to phlebotomy in two normal and two grey collies, and after hypertransfusion in two grey collies. ESF, unmeasurable in normal dog serum, cycled in grey collies from undetectable levels to 0.2–0.6 U/ml, with maximum activity observed just before marrow regeneration. A normal physiologic response followed phlebotomy, with reticulocytes and ESF rising to peak values of $> 4.5\%$ and > 1.4 U/ml, respectively; continued cycling was seen at the increased levels. With hypertransfusion (hematocrit 46–52%) reticulocytes disappeared from circulation, reappearing as the hematocrit fell to pretransfusion levels. Neither bleeding nor hypertransfusion altered the underlying periodicity of the neutrophil or reticulocyte cycles. Thus, these data suggest that cyclic erythropoiesis in the grey collie results primarily from the cyclic production of ESF. (Supported by NIH and VA research funds.)

5. Dopamine As a Mediator of Salt-Induced Natriuresis in Man. R. W. ALEXANDER,* J. R. GILL, JR., H. YAMABE,* W. LOVENBERG,* AND H. R. KEISER,* Bethesda, Md.

Dopamine (DA), a potent natriuretic substance, probably acts through specific dopaminergic receptors in the kidney. The effects of acute and chronic salt loading on DA metabolism were determined. Seven normal subjects were infused with 5% dextrose solution, 3 ml/min, for 80 min (control), followed by normal saline, 15 ml/min, for 160 min. Urinary DA, norepinephrine plus epinephrine (NE-E), plasma dopamine-β-hydroxylase (DβH), hematocrit (Hct), and clearances of inulin (C_{In}) and p-aminohippurate (C_{PAH}) were determined (20-min periods). Seven other subjects were placed on ad lib. salt (162±16 [SE] meq/day) intake (5 days) and then on 259 meq Na^+ (10 days). 24-h urines were collected for DA, norepinephrine (NE), and Na^+. Control urinary DA, 2.18±0.22 μg per period, and Na^+, 107±14 μeq/min, increased during saline infusion to 2.79±0.19 μg per period ($P < 0.01$) and 359±47 μeq/min ($P < 0.01$), respectively. Plasma DβH decreased 11% ($P < 0.001$) after correcting for an 8% decrease in Hct. NE-E did not change significantly (0.52±0.05 to 0.44±0.06 μg per period). C_{In} and C_{PAH} showed small and insignificant ($P > 0.2$) changes (86± 4 to 91±4 ml/min and 453±21 to 489±28 ml/min, respectively); filtration fraction did not change (0.19 vs. 0.19). When dietary Na^+ was increased, DA also increased (26.1± 7.9%, $P < 0.025$) without significant changes in NE. The results suggest that salt loading may decrease adrenergic activity and increase DA in the kidney. DA may contribute to natriuresis by decreasing the tubular reabsorption of Na^+, possibly by a direct tubular effect, as suggested by the absence of significant renal hemodynamic changes.

6. Morphological Studies of Hand-Dissected Islets of Langerhans. H. ALEYASSINE,* ROBERT J. GARDINER,* AND W. P. DUGUID,* Montreal, Quebec, Canada (introduced by D. G. Cameron **).

We have recently reported a method of visualization of the islets of Langerhans which permits visual identification during all the steps of isolation. After ligation of the portal vein and subsequent injection of buffered salt solution into the common bile duct, a certain number of red blood cells are trapped within the islets of the distended pancreas and give a characteristic red color to the tissue. Islets from collagenase-treated tissue respond normally to glucose, and no evidence of morphological damage to the islet cells can be detected. In the present investigation we have used this method of identification of the islets *in situ* to permit us to bypass the step of collagenase digestion. Islets of Langerhans may be isolated without collagenase treatment which might alter their metabolic activity. After visualization of the islets, the pancreas is removed and placed in cold Hanks' solution. Under direct vision the individual islets may then be dissected out by hand into the same solution and processed for light and electron microscopy. If fixative solution is used at this step in place of Hanks' solution, direct fixation can occur immediately after removal of the pancreas. Ultrastructural examination of the tissue indicates that the cell architecture of the islets so obtained is unaffected by the presence of the red blood cells in the capillaries surrounding the islet. This method of visualization of the endocrine pancreas provides a major advance in handling islets for metabolic studies and for both light and electron microscopy. In addition, islets may be selected visually by size or by position within the pancreas, and large numbers of islets may be obtained from each tissue. As the method obviates the need for collagenase or other treatment, any effects on morphology or metabolism resulting from treatment during isolation are eliminated. Islets so obtained may be suitable for transplantation.

7. Ethanol Inhibition of Hemoglobin Synthesis: In Vitro Evidence for a Heme-Correctable Defect in Normal Subjects and in Alcoholics. M. A. M. ALI,* M. C. BRAIN, AND G. D. SWEENEY,* Hamilton, Ontario, Canada.

Transient sideroblastic erythropoiesis is a recognized complication of prolonged ethanol ingestion. Impaired in vitro synthesis of globin chains by reticulocytes from patients with congenital and acquired (nonalcoholic) sideroblastic anemia has been demonstrated by us. The addition of heme, and in some patients the addition of 5-aminolevulinic acid (5ALA), significantly increased globin synthesis. To investigate ethanolic sideroblastosis, we have studied the effects of heme, 5ALA, and pyridoxine hydrochloride (PHC) on globin-chain synthesis by normal bone marrow cells and reticulocytes in the presence of ethanol, and by reticulocytes of five patients with acute alcoholism. The addition of ethanol (0.05–0.66 M) to aliquots of four normal bone marrow suspensions and three reticulocyte-enriched normal blood samples resulted in a marked dose-dependent inhibition of [^{3}H] leucine incorporation into the α- and β-peptide chains of globin. The 20 and 40% inhibition produced by 0.1 and 0.33 M ethanol, respectively, was prevented by heme in all samples, in five out of seven by PHC, but in only one sample by ALA. The addition of heme to reticulocyte-enriched blood obtained from acute alcoholics increased globin-chain synthesis by 20%, whereas PHC and ALA were without effect, and none of the additions increased globin synthesis by normal reticulocytes. Further evidence of a disturbance of the heme synthetic pathway due to ethanol was the finding of raised erythrocyte coproporphyrin and protoporphyrin levels (means 34 and 335 μg/100 ml, respectively) in 11 of 26 alcoholics with sideroblastic erythropoiesis, whereas normal values were found in the 15 alcoholic patients with normoblastic erythropoiesis. (Research supported by MRC Canada.)

8. Decreased Oxygen Consumption and Myocardial Cellular Hypoxia in Alloxan Diabetic Rats. TRENTON B. ALLISON,* STEPHEN P. BRUTTIG,* JOSEPH C. SHIPP, ROBERT S. ELIOT,* AND MAURICE F. CRASS III,* Omaha, Nebr.

Myocardial high energy phosphates (ATP and creatine phosphate) are reduced in acutely diabetic rats, findings suggesting acute myocardial hypoxia (1972. *Circulation.* **46** (Suppl. II) :II–225). The purpose of these studies was to determine (*a*) whether oxygen delivery was altered in acute diabetes, and (*b*) whether insulin administration to diabetic rats would normalize high energy phosphates in heart and blood oxygen release. Arterial (aorta) blood 2,3-diphosphoglycerate (DPG), pH, P_{O_2}, O_2 content, and k_c (the overall rate constant for oxygen dissociation from whole blood) and venous (vena cava) blood pH, P_{O_2}, and O_2 content were measured. Systemic arteriovenous O_2 content difference in diabetic rats was 0.5 vol/100 ml, approximately 15% of the nondiabetic control value (4.0 vol/100 ml). Furthermore, arterial P_{O_2} rose from 80 to 108 mm Hg, pH decreased from 7.38±0.01 to 7.11±0.03, DPG fell from 7.0±0.4 to 4.0±0.4 mM/liter RBC and k_c decreased from 5.8±0.2 to 4.5±0.2 sec^{-1}; venous P_{O_2} increased from 52 to 79 mm Hg. Insulin administration to diabetic rats for 10 days restored heart energy phosphates and blood DPG and k_c to control values. Although adequate arterial oxygen tension and acidosis (which facilitates oxygen release from hemoglobin) was observed in acute diabetic rats, oxygen consumption was decreased. These observations reflect an impaired dissociation of oxygen from blood. DPG regulates oxygen release from blood; low DPG reduces and elevated DPG enhances the rate of release, or k_c (1971. [*Fed. Eur. Biochem. Soc.*]) (*Febs*) *Lett.* **16**: 257–261). Thus, in acute diabetes characterized by reduced DPG and acidosis, tissue hypoxia is due to a decreased release of oxygen from blood with DPG being one rate-limiting factor. (Research supported by NIH grant AM-14986-03.)

9. Isometric Inspiratory Contraction Force and Electromyographic Activity of the Diaphragm as Measures of CO_2 Sensitivity in Man. MURRAY ALTOSE,* STEVEN KELSEN,* NIGEL STANLEY,* AND NEIL CHERNIACK, Philadelphia, Pa.

Hyposensitivity of the medullary chemoreceptor contributes in some patients to the hypercapnia that develops with obstructive lung disease. However, this hyposensitivity is difficult to detect by conventional tests of CO_2 response since ventilation is depressed by airway obstruction even when the medullary chemoreceptor is normal. In the present study, we examined the usefulness of the diaphragmatic EMG (E_D) and the pressure generated by isometric contraction of the inspiratory muscles (Occ P_m) as indices of CO_2 response in anesthetized dogs and conscious man. The mouth pressure generated during complete obstruction to inspiration was used as a measure of the isometric contraction. In dogs, progressive hypercapnia during rebreathing increased both Occ P_m and E_D. The application of graded inspiratory flow resistive loads increased the *absolute* value of Occ P_m and E_D at any given P_{CO_2} but left unaffected the ratios of ΔOcc $P_m/\Delta P_{CO_2}$ and $\Delta E_D/\Delta P_{CO_2}$. In 15 normal volunteers and two patients with idiopathic hypoventilation despite normal lungs, the conventional ventilatory test of CO_2 sensitivity ($\Delta V/\Delta P_{CO_2}$) was directly related to the ΔOcc $P_m/\Delta P_{CO_2}$. In contrast, in two patients with chronic obstructive lung disease, the $\Delta V/\Delta P_{CO_2}$ was depressed, whereas the ΔOcc $P_m/\Delta P_{CO_2}$ was normal. It is concluded that in subjects with normal lungs, both the isometric inspiratory contraction force and the electromyographic activity accurately measure CO_2 sensitivity—even in the presence of ventilatory loads. Of the two indices, the contraction force is more easily quantitated and standardized. In addition, the increase in E_D or Occ P_m at any given P_{CO_2} may be a useful measure of neuromuscular adjustments to mechanical loading of the respiratory muscles. (Supported by NIH grant HL-08805.)

10. Lymphocyte-Mediated Cytotoxicity in Autoimmune Thyroid Disease and Thyroid Cancer. NOBUYUKI AMINO,* AND LESLIE J. DEGROOT, Chicago, Ill.

Cell-mediated immunity (CMI) is believed to be important in tissue damage of autoimmunity and in natural defense to neoplasia. We investigated CMI in patients with thyroid disease by assay of lymphocyte cytotoxicity. Peripheral blood lymphocytes were incubated with or without antigens or phytohemagglutinin (PHA), and supernatants of these cultures were incubated on target L-cell micromonolayers. Cytotoxicity was calculated as fractional survival rate of target cells incubated with supernatants of antigen or PHA-activated lymphocytes, relative to that of nonactivated lymphocytes. Cytotoxicity indices (mean±SD) obtained by PHA activation in Graves' disease (n = 12) (0.15±0.09), chronic thyroiditis (n = 4) (0.14±0.16), and primary hypothyroidism (n = 5) (0.18±0.07) were not different from normal subjects (n = 14) (0.19±0.09). When homogenate of Graves' thyroid gland was used as organ specific antigens, primary hypothyroid patients (0.62±0.14) responded intensely compared to Graves' disease (0.88±0.17) and chronic thyroiditis (0.84± 0.23), and were significantly different from normals (1.00± 0.11). The 2/12 Graves' disease-positive responses occurred in patients who had been previously treated with radioiodine and had developed hypothyroidism, although they were euthyroid on therapy when studied. Thyroid cancer patients (n = 9) had PHA-induced cytotoxicity similar to normals (0.23 vs. 0.19). When homogenates of cancer tissues were used as antigens, 2/9 showed cytotoxicity. One positive responder had received multiple doses of ^{131}I for ablation of metastases; the second had received intracutaneous immunization with autochthonous tumor. The latter patient responded intensely against allogeneic tumor as well as autochthonous tumor, but not to Graves' disease gland homogenate. Lymphocyte responsivity to PHA is normal in patients with autoimmune thyroid disease and thyroid cancer at time of initial diagnosis. Lymphocyte-mediated cytotoxicity, presumably induced by "lymphotoxin," may play a role in autoimmune thyroid disease and probably is involved in the progress of the disease to hypothyroidism. The data also indicate the presence of tumor specific antigens in thyroid cancer, suggesting that ^{131}I therapy may induce immunity and that immunization may enhance antitumor immunity. (Research supported by grants from NIH and ACS.)

11. Effect of Four Proteolytic Enzymes on Coagulant Properties and Molecular Structure of Human Factor VIII. JUDITH C. ANDERSEN * AND PATRICK A. MCKEE,* Durham, N. C. (introduced by R. Wayne Rundles).

Highly purified human factor VIII was incubated with varying concentrations of human thrombin, human plasmin, trypsin, and α-chymotrypsin to compare alterations in coagulant activity with effects on the molecular structure of factor VIII. Human factor VIII, purified by a modification of the method of Johnson and associates and reduced with β-mercaptoethanol, appears as a single protein band with mol wt 195,000 on SDS-gel electrophoresis. Factor VIII activity during incubation was monitored by the one-stage partial thromboplastin time and structural changes in the factor VIII molecule by SDS-gel electrophoresis. At low enzyme

concentrations, thrombin and trypsin rapidly activated and, more gradually, inactivated factor VIII, whereas at higher concentrations, both enzymes inactivated factor VIII. Even trace quantities of plasmin rapidly inactivated factor VIII without evidence of early activation. In low concentration, α-chymotrypsin had little effect on factor VIII activity; at higher concentrations, it rapidly inactivated factor VIII. Activation by thrombin and trysin, and inactivation by thrombin, trypsin, plasmin, and α-chymotrypsin all occurred without detectable change in subunit molecular weight. At higher concentrations of trypsin, plasmin, and α-chymotrypsin, or after prolonged incubation with low concentrations of these enzymes, degradation of the factor VIII subunit into lower molecular weight species was observed. Thus the activity of human factor VIII is exquisitely sensitive to minor proteolytic modification; and the sites of enzymatic cleavage resulting in both activation and inactivation may be within 10,000 daltons or 100 amino acid residues of one of the terminal ends of the factor VIII subunit, the limits of resolution using SDS-gel electrophoresis. (Research supported by grants from National Hemophilia Foundation, VA, and NIH.)

12. Ascending Urinary Infections After Ureteral Reimplantation in Rats. NORMAN ANDERSON,* PATRICIA CHARACHE,* AND ROBERT WYLLIE,* Baltimore, Md. (introduced by Jack Levin).

The role of chronic ureteral reflux in ascending pyelonephritis was evaluated in three groups of Wistar rats. In group I, spontaneous infection followed ureteral reflux, produced by surgical reimplantation of one ureter. In group II, urinary infections were induced by direct bladder injections with *S. aureus*. Infections in group III were made by infecting *S. aureus* into incised and sutured rat bladders. Infections were evaluated by sequential urine cultures and histologic studies. In group I, positive urine cultures occurred in 70 of 82 rats killed at intervals ranging from 3 to 112 days; 36 of 70 grew mixed pathogens. Bacteria cultured included: *S. aureus* in 50% and *S. epidermidis, P. mirabilis, S. fecalis,* and *E. coli* in 25% each. Mg NH_4PO_4 bladder stones developed in 39 of 54 rats killed after 7 days and progressively increased in size from 15 mg to 1430 mg by 16 wk. Urease-producing (U^+) bacteria were identified in 36 of 39 stone formers, and U^+ *S. aureus* contributed to 27 of these infections. Histologic signs of progressive infection appeared after 7 days in 54 of 54 kidneys with reimplanted ureters. Changes in kidneys with unoperated ureters were milder and appeared after 3 wk in 37 of 37 rats. In group II, bladder injections with 10^8 U^+ *S. aureus* caused persistent bacteruria in 9 of 27 rats, but only three developed bladder stones and sereve pyelitis. Injections with 10^8 U^+ *S. aureus* in group III caused chronic bacteruria, bladder stones, and pyelitis in 19 of 20 animals. In both groups, injections with non-urease-producing *S. aureus* produced transient infections without stones. Thus, ureteral reflux induced by surgery or stone formation contributed to renal involvement in ascending urinary infections. Further, U^+ *S. aureus* produced bladder stones and increased severity of infection when compared to non-urease producers. (Research supported by grants from NIH.)

13. Treatment of Hypercholesterolemia with Low-Dose Oral Neomycin. CARL S. APSTEIN,* P. K. GEORGE,* GORDON S. MYERS,* MARTHA MCCLUSKEY,* AND ROBERT S. LEES, Cambridge, Mass.

Neomycin is not widely used for treatment of hypercholesterolemia despite its relatively low cost and published evidence of its efficacy. We have evaluated neomycin therapy in 42 hyperlipidemic patients (35 type II and 7 type IV), at constant body weight and diet. Oral neomycin (0.5–2 g daily) reduced serum cholesterol concentration in all 42 patients. The absolute decrease was related to the daily dose and pretreatment cholesterol concentration. However, the percentage decrease was related only to the daily dose and was independent of the initial cholesterol concentration. Thus, in patients matched for pretreatment concentrations (240–350 mg/dl), 0.5 g/day of neomycin reduced cholesterol concentration by 14% ($n = 9$, $P < 0.01$); 1.0 g/day resulted in a decrease of 18% ($n = 6$, $P < 0.01$). The difference between the two dosages was probably significant ($P = 0.1$). Other patients, matched for cholesterol > 340 mg/dl, demonstrated a decline of 17% ($n = 9$, $P < 0.01$) for a dose of 1.0 g/day, and a decrease of 28% ($n = 4$, $P < 0.01$) for a dosage of 1.5 or 2.0 g/day. These responses differed significantly ($P < 0.05$). The addition of neomycin (1 g/day) to clofibrate or β-sitosterol therapy decreased cholesterol concentration by an additional 12% ($n = 13$, $P < 0.01$) or 19% ($n = 4$, $P < 0.05$), respectively. Addition of neomycin to Colestipol therapy had no consistent effect. Side effects related to neomycin were minimal. Monthly audiograms, performed in all patients, showed no change. Nausea was the most common complaint and was usually relieved by giving neomycin with meals. Diarrhea necessitated discontinuing the drug in two cases. These results indicate that neomycin is an effective cholesterol-lowering drug of low toxicity when used alone or in combination with clofibrate or β-sitosterol.

14. Insulin Receptor Deficiency States in Man: Two Clinical Forms. JUANITA A. ARCHER,* PHILLIP GORDEN,* C. RONALD KAHN,* JAMES R. GAVIN III,* DAVID M. NEVILLE, JR.,* MALCOM M. MARTIN, AND JESSE ROTH, Bethesda, Md., and Washington, D. C.

Recently, specific methods have been developed for direct study of polypeptide hormone receptors on target cells. Using biologically active [^{125}I]insulin, we demonstrated elsewhere that the specific insulin receptors in leukocytes are indistinguishable from those in adipocytes and hepatocytes, and that glucose-intolerant, insulin-resistant obese mice have a severe deficiency in insulin receptors in fat and liver that is reflected to a comparable degree in their lymphocytes. In the persent study, using circulating lymphocytes freshly isolated from patients, we found that cells from obese hyperglycemic insulin-resistant patients bind only about half as much of a tracer of [^{125}I]insulin (0.1 ng/ml) as cells of nonobese people and display a shallower curve of displacement on addition of physiological amounts of unlabeled insulin. Serial studies in six obese patients showed that calorie restriction produced amelioration not only of the glucose intolerance and insulin resistance, but of the defective insulin binding. In contrast to the acquired, reversible receptor defect in obesity, one patient with severe insulin resistance was found in whom the deficiency of insulin receptors appeared to be in the primary defect. This 16-yr-old nonobese female was noted on routine screening to have severe glucose intolerance, modest hyperinsulinism, and mild ketonuria that was minimally improved by astronomical doses (50,000 U) of insulin. Intravenous insulin (*one* U/kg) lowered blood glucose by only 20% in 1 h. Other tests, including antibodies and hormone studies, failed to explain the extraordinary hormone resistance. However, her peripheral lymphocytes had markedly reduced insulin binding, consistent with the degree of insulin resistance. These are the first examples of disease states in man in which the resistance to peptide hormones can be pinpointed to defects in specific hormone receptors.

15. Amino Terminal Human Parathyroid Hormone (PTH 1-34): Biological Properties and Specific Radioimmunoassay. CLAUDE D. ARNAUD, THOMAS DOUSA,* GLEN W. SIZEMORE,* WERNER RITTEL,* THOMAS FAIRWELL,* ROSEMARY RONAN,* AND H. BRYAN BREWER,* Rochester, Minn., Bethesda, Md., and Basel, Switzerland.

The amino acid sequence of the amino-terminal region (1-34) of the human parathyroid hormone (PTH 1-34) has been reported (Brewer et al. 1972. *Proc. Nat. Acad. Sci. U.S.A.* **69**: 3585). Its primary structure differs from that of bovine PTH 1-34 in six amino acid substitutions and from porcine PTH 1-34 in five amino acid substitutions. Human PTH 1-34 has been synthesized by classical techniques based on this structural information (Andreatta et al. 1973. *Helv. Chim. Acta.* **56**). This synthetic peptide migrates as a single component on disc-gel electrophoresis and in several thin-layer chromatographic systems. It produces hypercalcemia (6–11 mg/100 ml), hyperphosphaturia (0.3.2.4 mg P per h), and hypocalciuria (0.3–0.1 mg Ca per h) in the perfused, conscious, thyroparathyroidectomized rat, and stimulates adenylate cyclase (8-fold) in human renal cortical membranes. A high specific activity, "damage-free" preparation of ^{131}I-labeled human PTH 1-34 has been repeatedly produced by presumed labeling of histidine residues. It binds to antibody populations in two antisera developed against bovine (CH14M) and porcine (GP1M) PTH which have a high affinity for human PTH 1-84. This has made possible the development of specific radioimmunoassays of human PTH 1-34 which are capable of measuring as little as 50 pg of the synthetic peptide. The substantive differences in the structures of the various species of the biologically active regions of PTH probably explain some of the recently reported discordant immunological observations on the concentration of biologically active circulating PTH in hyperparathyroid patients using antisera directed against nonhuman species of PTH. The availability of a synthetic preparation of human PTH 1-34 now permits an investigation of this problem with species specific immune systems.

16. Serum Parathyroid Hormone (iPTH) in X-Linked Hypophosphatemic Rickets: Effect of Age and Vitamin D Toxicity. SARA ARNAUD,* RALPH GOLDSMITH,* GUNNAR B. STICKLER,* AND CLAUDE D. ARNAUD, Rochester, Minn. (introduced by Robert J. Ryan).

Low, normal, and increased serum parathyroid hormone (iPTH) has been reported in hypophosphatemic rickets (HR) patients by different laboratories. Using an immunoassay which measures the total complement of iPTH, we have observed age-dependent variation in serum iPTH [as well as serum calcium (Ca) and phosphorus (P)] in a normal population of 120 children and 31 adults. The pattern of change with age in normal age-matched controls in mean serum iPTH shows a nadir (13 μleq/ml) from 6 to 12 yr and similar values for 1- to 6-yr-olds (21 μleq/ml) and adults (23 μleq/ml). Mean serum Ca is highest (10.03 mg/100 ml) in 1–6 yr age group, plateaus from 6–12 yr (9.74 mg/100 ml), and then decreases to adult values (9.50 mg/100 ml). Mean serum P decreases from 5.56 mg/100 ml in the youngest age group (1–6 yr) to 3.35 mg/100 ml in adults. The pattern of change with age was the same for serum iPTH and Ca in both untreated HR patients (8) and in those without complications of prior vitamin D treatment (11). The only significant differences from normal were a higher mean iPTH ($P < 0.05$) in the 1–6 yr age group and a lower mean serum Ca ($P < 0.001$) in age group 6–12 yr. Four HR patients had significant elevations of iPTH. Three had prior D intoxication but normal creatinine clearance despite persistent hypertension and abnormal urinary sediment. One was untreated. The data indicate (*a*) appropriate steady-state relationship between serum Ca and iPTH in HR, (*b*) that hyperparathyroidism is not a regular feature of this disease or of sufficient magnitude to be a primary cause of the low serum P, and (*c*) abnormal persistence of hyperparathyroidism after past transient renal insufficiency. (Research supported by grants from NIH AM-12302 and RR 585.)

17. Genetic Polymorphism of Proline-Rich Human Salivary Proteins. EDWIN A. AZEN * AND FRANK G. OPPENHEIM,* Madison, Wis., and Boston, Mass. (introduced by Robert F. Schilling **).

A common genetic polymorphism was found among four proline-rich proteins previously purified and characterized from pooled human parotid saliva. In a study of randomly collected parotid saliva samples from 120 Caucasions, 79 Blacks, and 40 Chinese, three phenotypes were observed by electrophoresis in slab polyacrylamide gels using a 3,3′-dimethoxybenzidine-hydrogen peroxide stain. Assuming autosomal codominant inheritance of two alleles ($\underline{Pr^1}$ and $\underline{Pr^2}$), application of the Hardy-Weinberg rule gave good agreement between observed and expected values, indicating excellent fit by the Chi^2 test ($P = 0.89$). Gene frequencies were: for Caucasians, $\underline{Pr^1} = 0.27$, $\underline{Pr^2} = 0.73$; for Blacks, $\underline{Pr^1} = 0.20$, $\underline{Pr^2} = 0.80$; and for Chinese, $\underline{Pr^1} = 0.16$, $\underline{Pr^2} = 0.84$. Family data including 24 families and 57 children were in complete agreement with the genetic hypothesis. The salivary proteins exhibiting genetic polymorphism were shown to be identical to four small, proline-rich proteins previously isolated and characterized from pooled saliva. First, the electrophoretic mobilities of the purified proline-rich proteins were found to be identical with the proteins exhibiting genetic polymorphism. Second, after samples of parotid saliva from individuals with the three putative phenotypes were subjected to Sephadex G-50 fractionation, only the expected purified proline-rich proteins consistent with each phenotype could be detected in the elution profiles. The high frequency of the polymorphism, relative stability of the proteins, and ease in typing indicate that it may be useful for other types of genetic research, especially linkage studies. The proline-rich proteins share certain interesting chemical similarities to enamel protein, but their functions are currently unknown. (Research supported by grants DEO 3658-08 and DEO 3433-01 from the NIH.)

18. Production by Granulocytes of Superoxide, a Possible Bactericidal Agent. BERNARD M. BABIOR,* JOHN T. CURNUTTE,* WILLIAM E. HULL,* AND RUBY S. KIPNES,* Boston, Mass. (introduced by Marshall M. Kaplan).

Studies with granulocytes from normal patients (normal cells) and from patients with chronic granulomatous disease (CGD cells) have provided evidence that superoxide (O_2^-) may be involved in bacterial killing by these cells. Superoxide is a powerful reducing agent produced in biological systems by the one electron reduction of oxygen. Its participation in a biological process is indicated when the process is specifically inhibited by superoxide dismutase, an enzyme which catalyzes the conversion of O_2^- to H_2O_2 and oxygen. By this criterion, cytochrome c reduction by granulocytes has been shown to be mediated by O_2^- (*J. Clin. Invest.* In press). The present communication reports further studies of O_2^--dependent processes in normal and defective granulocytes. In normal cells, the rate of O_2^--mediated cytochrome c reduction was constant over 40 min and was proportional to

cell concentration to 3000 cells per mm^3. At a cytochrome c concentration of 20 μM, the rate was 15.1±3.0 pmoles/min per 10^6 cells. The true rate of O_2^- production must have exceeded this, however, since cytochrome c reduction was proportional to cytochrome c concentration up to 60 μM. NBT reduction by granulocytes appeared not to be mediated by O_2^-, even though O_2^- reduces NBT in other systems. In contrast to normal cells, CGD cells, which show defective bacterial killing, failed to carry out O_2^--mediated cytochrome c reduction (−2.2±2.2, representing net cytochrome c *oxidation* by these cells, compared with normal values of 15.1± 3.0; $P < 0.01$). The failure of CGD cells to produce detectable O_2^- supports the hypothesis that O_2^- participates in bacterial killing by granulocytes. (Supported by grants from NIH and the Medical Foundation.)

19. The Coronary Vascular Response to Ischemia. ROBERT J. BACHE,* F. R. COBB,* AND J. C. GREENFIELD, JR., Durham, N. C.

Transient coronary artery occlusion evokes vasodilation resulting in a reactive hyperemic response during which excess inflow of arterial blood ("debt repayment") is 300–600% of the deficit incurred during the ischemia. Studies were performed in 17 unanesthetized dogs with electromagnetic flowmeter probes and a pneumatic occluder on the left circumflex coronary artery to determine whether this marked overpayment is actually essential for restoration of coronary vascular tone. A 10-sec total coronary artery occlusion resulted in reactive hyperemia with 417±32% debt repayment. Subtotal occlusions resulted in similar debt repayments of 375±39%. When two 10-sec total occlusions were performed with an interval between occlusions of 4 sec, the first occlusion was 120±20% repaid during the interval, and the reactive hyperemia after the second occlusion was identical with that after an isolated 10-sec occlusion (debt repayment = 426±44%). Thus, only 120% repayment of the initial deficit restored normal reactivity to a second test occlusion. To further test whether coronary vascular tone could be regained without the usual overpayment of the deficit, 10-sec occlusions were produced and the occluder briefly released to allow the initial portion of the reactive hyperemia, then partially reoccluded to reduce inflow to the control level for a duration equal to a control-reactive hyperemic response, and then completely released. When a period of reactive hyperemia was allowed to produce 138±15% debt repayment (mean duration = 5.5 sec), no additional hyperemia occurred when the restriction was totally released. Thus, although coronary reactive hyperemia normally results in marked overpayment of the blood deficit, under controlled circumstances the excess arterial inflow actually necessary to restore coronary vascular tone represents only slightly more than 100% of the preceding deficit. (Supported by NIH grant.)

20. Ethanol Damages the Small Intestine and Changes Its Cell Population. ENRIQUE BARAONA,* ROMANO C. PIROLA,* AND CHARLES S. LIEBER, New York.

Alcoholics frequently develop diarrhea and malabsorption. This prompted us to study the effects of acute and chronic ethanol administration on rat intestinal morphology and "marker" enzymes of villi (lactase) and crypts (thymidine kinase). Acute intragastric ethanol administration (3 g/kg), in concentrations found in common alcoholic beverages (5–35 g/100 ml), produced intestinal damage manifested by hemorrhagic erosions of jejunal villi, already visible within 10 min. After 1 h, ethanol 35 g/100 ml produced striking lesions, whereas 5 g/100 ml provoked minimal alterations. The latter were associated with decreased activity of jejunal lactase (−33%, $P < 0.02$), but not of thymidine kinase; in vitro, ethanol (5 g/100 ml) did not affect these enzyme activities. Furthermore, in jejunal slices obtained from these rats and incubated without ethanol, oxygen consumption was depressed (−12%, $P < 0.01$). Chronic drinking of a diet containing ethanol (5 g/100 ml) for 3–4 wk also decreased jejunal lactase activity (−58%, $P < 0.01$), compared to that of controls pair-fed isocaloric dextrin-maltose instead of ethanol. The activity of another villous enzyme, alkaline phosphatase, was also reduced in the jejunal wall (13.6±2.7 U/g vs. 37.4±9.0, $P < 0.05$) but increased intraluminally (3.9±0.8 vs. 0.9±0.3, $P < 0.02$), presumably reflecting accelerated villous desquamation. Furthermore, jejunal villi appeared shorter and had fewer cells (−24%, $P < 0.01$). By contrast, jejunal crypts appeared deeper with increased cell count (+28%, $P < 0.01$) and thymidine kinase activity (+25%, $P < 0.01$), suggesting accelerated mucosal renewal. This was supported by the demonstration of increased incorporation of [^{3}H]thymidine into ileal DNA (+85%, $P < 0.02$). Villous changes predominated in the jejunum and crypt abnormalities in the ileum, where thymidine kinase activity increased by 169% ($P < 0.02$). Thus, ethanol ingestion produces enzyme changes and alterations of intestinal cell population, reflecting tissue damage and increased regeneration. (Supported by the USPHS and VA.)

21. Interaction of 2,3-Diphosphoglycerate (DPG) with Hemoglobin Little Rock (HbLR), β143 (H21) His → Gln, a Variant with an Altered DPG Binding Site. GEORGE BARE,* JAMES ALBEN,* STANLEY BALCERZAK,* AND PHILIP BROMBERG,* Columbus, Ohio (introduced by C. A. Doan**).

The β143 (H21) histidine residue of hemoglobin is a coulombic binding site for 2,3-diphosphoglycerate (DPG), a potent physiologic allosteric effector of oxygen affinity. A variety of hemoglobins lacking this residue have been reported to exhibit reduced interaction with DPG. We previously reported (*Nature (Lond)*. In press) that HbLR in 0.1 M phosphate had 3-fold higher oxygen affinity than HbA with normal Bohr effect and Hill's "n." We now have studied the oxygen equilibrium of "stripped" HbLR and its titration with DPG. In 0.05 M bis-Tris/0.1 M chloride, the log $p_{50}O_2$ for stripped HbLR was 0.53 U less than for stripped HbA. At DPG concentrations of 1.3 and 6.2×10^{-4} M the increment in log $p_{50}O_2$ for HbA was greater than that for HbLR. However, more complete titration curves showed the total DPG-induced increment in log $p_{50}O_2$ for the two hemoglobins to be equal. Calculation of binding constants of DPG to HbLR and HbA from the titration curves revealed a 2½-fold reduction in affinity of DPG for deoxy HbLR compared with deoxy HbA, and at least as great a reduction in DPG affinity for the respective oxyhemoglobins. These data indicate (*a*) that the increased oxygen affinity of HbLR is not principally attributable to selectively impaired DPG binding to the deoxy conformation; (*b*) that the allosteric effect of DPG on hemoglobin–oxygen equilibrium depends on the relative affinities for DPG of the deoxy and oxy conformations in addition to the absolute values of the binding constants; and (*c*) that DPG in sufficient concentration can exert its full allosteric effect on a hemoglobin molecule lacking the βH21 binding site. (Research supported by grant from the AHA.)

22. Serum Antibodies to Non-Type-Specific Moieties of Streptococcal M Protein in Rheumatic Fever. EDWIN H. BEACHEY,* ITZHAK OFEK,* AND ALAN BISNO,* Memphis, Tenn. (introduced by Gene H. Stollerman**).

Immunity to group A streptococcal infections is dependent upon development of type-specific M protein antibodies. It has recently been recognized that many human sera also contain antibodies to non-type-specific moiety(ies) closely associated with M protein (NTSM). Such moieties are present even in highly purified M protein vaccines. Antibodies to NTSM were assayed by the complement fixation (CF) test, using as antigen purified preparations of M30, a rare human serotype. Serum anti-NTSM titers were higher in acute rheumatic fever (ARF) patients than in subjects with uncomplicated streptococcal sore throat. To investigate the antigenic nature of NTSM, absorptions of high CF titer ARF sera were made. Streptococcal protoplast membranes of all serotypes tested absorbed anti-NTSM antibodies from all ARF sera. Furthermore, these antibodies also were partially absorbed by the fraction of M protein which binds to washed O erythrocytes (so-called "sensitizing factor") and which is thought to be teichoic acid. Using these sensitized erythrocytes, a wide range of passive hemagglutination titers (1:20 to 1:320) were observed in ARF sera. These results show that M protein vaccines contain at least two non-type-specific antigens which are also present in streptococcal protoplast membranes and to which rheumatic fever patients are hyperimmune. (Research supported by grants from NIH and VA.)

23. A Comparison of the In Vitro and In Vivo Behavior of [^{114m}I]- and [^{59}Fe]Chloride in the Rat. M. R. BEAMISH * AND E. B. BROWN,** St. Louis, Mo.

Isotopes of indium have been widely used as blood pool-scanning agents and for tumor detection and bone marrow imaging. Indium binds specifically to transferrin when incubated with plasma in vitro or when injected in vivo and has been reported to behave similarly to iron on the basis of reticulocyte uptake and heme incorporation studies. Tracer amounts of [^{114m}I]- and [^{59}Fe]chloride were incubated with rat plasma at 37°C for 30 min to insure complete binding to transferrin. The [^{114m}I] transferrin was then incubated with rat reticulocytes. The reticulocyte uptake of ^{114m}I was consistently about 10% that of ^{59}Fe. There was no ^{114m}I activity in the hemoglobin fraction obtained after osmotic lysis and Sephadex G-150 chromatography of the supernatant fraction. Groups of male Sprague-Dawley rats were given [^{114m}I]- and [^{59}Fe]chloride in 0.2 N HCl intravenously and were killed at daily intervals. The percentage incorporation for each isotope and the I:Fe ratios were obtained from whole liver and isolated parenchymal and Kupffer cell fractions after liver perfusion. Additional samples of blood, red marrow, spleen, kidney, and thigh muscle were analyzed. The percentage uptake into red cells, liver, and marrow at 49 h was:

	Red cells	Marrow	Liver
^{114m}I	1.02	0.322	10.32
^{59}Fe	54.5	1.17	7.7

The I:Fe ratios were consistently low in marrow and red cells and high in Kupffer cells, thigh muscle, and kidney. These observations suggest that indium bound to transferrin behaves differently from transferrin-bound iron as judged by both the in vivo and in vitro results. The significantly increased uptake of indium by Kupffer cells, kidney, and thigh muscle indicate that nonerythroid tissues play a major role in its localization.

24. Sodium-Independent Active Potassium Reabsorption in Proximal Tubule of the Dog. LAURENCE H. BECK,* DOROTHY SENESKY,* AND MARTIN GOLDBERG, Philadelphia, Pa.

Prior studies of proximal tubule (PT) reabsorption have failed to distinguish conclusively between a separate active K^+ transport system or K^+ movement linked to Na^+ reabsorption. To attempt to dissociate movement of K^+ from Na^+ and Ca^{++}, recollection micropuncture experiments were performed in PT of intact and parathyroidectomized (PTX) dogs under two different conditions known to inhibit Na^+ reabsorption: saline expansion, 5% body weight; and acetazolamide (ACTZ), 5 mg/kg. A control hydropenic group was also studied. Tubular concentrations of K^+, Na^+, and Ca^{++} were measured by electron probe analysis. During initial collections, mean±SEM tubular fluid/plasma (TF/P)K^+ was 1.07±0.05, 1.05±0.05, and 1.00±0.03 in intact hydropenic ($n=7$), saline ($n=6$), and ACTZ ($n=8$) groups; fractional reabsorption (FR) of K^+ in PT was 0.35, 0.39, and 0.31, respectively. After saline, (TF/P) inulin fell from 1.81 to 1.34 ($P<0.01$); (TF/P)K^+, (TF/P)Na^+ and tubular fluid/ultrafiltrate (TF/UF)Ca^{++} did not change, so that FR of all three ions fell proportionately. After ACTZ, however, despite a 24% inhibition of FR of Na^+ and Ca^{++}, (TF/P)K^+ fell to 0.85±0.04 ($P<0.005$) so that FR of K^+ was unchanged at 0.34. In three corresponding groups of PTX dogs, similar results were obtained; saline ($n=6$) caused parallel inhibition of FR of all three ions as (TF/P)K^+, (TF/P)Na^+, and (TF/UF)Ca^{++} were unchanged. ACTZ ($n=7$) inhibited FR of Na^+ and Ca^{++} by 41%, but (TF/P)K^+ fell from 1.03±0.03 to 0.89±0.04 ($P<0.005$) so that FR of K^+ was unchanged (0.36–0.34). A separate uphill transport system for K^+ in PT is therefore unmasked by ACTZ, a drug which selectively inhibits Na^+ (and Ca^{++}) reabsorption. Saline, on the other hand, inhibits net reabsorption of all three ions, probably by increasing passive backflux via intercellular channels. (Supported by grants from NIH.)

25. Parathyroid Hormone (PTH) Binding Receptor in Renal Cortex. NAMA BECK,* SANFORD BARSKY,* BERNARD B. DAVIS,* AND H. V. MURDAUGH, Pittsburgh, Pa.

To evaluate the possibility of a binding receptor for parathyroid hormone (PTH) in renal cortex which participates in the renal action of that hormone, homogenates (preparation similar to that for adenylate cyclase) and plasma membranes (prepared by sucrose-gradient centrifugation) were tested for binding affinity to [^{131}I]PTH. In homogenates at 0°C, pH 4, with 1 pg [^{131}I]PTH added, maximum binding occurred at 10 min. There was a logarithmic dose-response relationship between protein concentration in the homogenate and PTH binding, within limits of 0.5 mg protein (2.5±SE 0.8% added PTH) and 25 mg protein (14.6±0.7). Binding was not demonstrable in renal medulla, heart, liver, spleen, or brain. Bovine [^{131}I]PTH bound significantly to dog 14.5± SE 1.0%, and rat 14.0±1%, but significantly more to bovine renal cortex 18.7±1.4%, $P<0.05$. There was no binding of [^{131}I]ACTH, [^{131}I]TSH, or [^{131}I]insulin to renal cortex. Bound [^{131}I]PTH could not be displaced by ACTH, TSH, or insulin but was displaced in a dose-response fashion by cold PTH: 12.2% displacement by 25 pmoles PTH, 69.2% by 50, and 97.4% by 100. [^{131}I]PTH was also displaced by synthetic amino-terminal 36 amino acid PTH fragment. In plasma membranes, binding was 1000 times greater per milligram protein than homogenate. The data indicate that there is a PTH binding receptor, hormonally and tissue specific, and they suggest that the receptor is located in the plasma membrane. They are consistent with the receptor being related to adenylate cyclase. It is postulated from these data that binding to a specific molecule in the plasma membrane is the initial step in the biological action of PTH in the kidney.

26. Highly Purified Lipoprotein Lipase from Human and Pig Adipose Tissue. A. BENSADOUN,* C. EHNHOLM,* D. STEINBERG,** AND W. V. BROWN,* La Jolla, Calif. (introduced by John Ross, Jr.).

Lipoprotein lipase (LPL) has been purified 1,000- to 2,000-fold from acetone powders of pig and human subcutaneous fat. Yields of enzyme were substantially increased by extracting with concentrated NaCl or with heparin at 4°C. Aliquots of the same acetone powders first extracted with 0.05 M NH_4OH, pH 8.6, were subsequently extracted with graded levels of NaCl in Na Veronal, pH 7.4. There was a linear increase in the additional LPL released up to 1.7 M NaCl, which extracted more than 6 times the activity extracted by NH_4OH. At a concentration of 500 U/ml, heparin solutions extracted more than 8 times the activity extracted by NH_4OH. 1 M glucose was ineffective in eluting additional LPL activity after 0.05 NH_4OH extraction. Heparin-extracted and NaCl-extracted lipolytic activities were serum dependent and salt inhibited (1 M NaCl) to the same extent as the 0.05 M NH_4OH-extracted enzyme. Both the pig and human adipose tissue LPL extracted with 1.5 M NaCl were purified by affinity chromatography on Sepharose 4B containing covalently linked heparin. The preparations obtained were purified 2750- and 920-fold, respectively. The pig LPL was further purified by isoelectric focusing and showed a single band of activity at pH 4.0. The purified enzyme showed activation by heparin (37% at 6 μg/ml) and by $CaCl_2$ (40% at 10 mM). The purified human LPL showed 100% stimulation at 10 μg/ml of heparin and complete dependence on serum activator for activity. Apo Lp-glu prepared from human very low density lipoprotein gave 50% of maximal activation at a concentration of 2 μg/ml. (Research supported by NIH grant HL-14197.)

27. Decreased Systolic Blood Pressure in Hypertensive Subjects Who Practiced Meditation. HERBERT BENSON,* BERNARD A. ROSNER,* AND BARBARA R. MARZETTA,* Boston, Mass. (introduced by Walter H. Abelmann **).

A prospective investigation was designed to test whether the regular practice of a relaxation technique might lower blood pressure in hypertensive subjects. Resting, sitting blood pressures were measured by a Random-Zero Sphygmomanometer to eliminate observer bias in 30 hypertensive subjects (mean age 53.5 yr) who did not alter their antihypertensive medications. The 629 measurements during the first 6 wk constituted those of the control period. The control measurements were made on 6–8 separate days. On each day, measurements were repeated every 5 min until both systolic and diastolic pressures did not change more than 5 mm Hg from the preceding measurement. Subsequently, each subject was trained to practice a relaxation technique called transcendental meditation and returned for similar blood pressure measurements at 2- to 3-wk intervals. The 254 measurements over the next 9 wk during nonmeditational periods of the day constituted those of the experimental period. During the control period, blood pressures did not change significantly from day to day and averaged 150.2±18.9 mm Hg systolic (mean± 1 SD) and 92.5±12.6 diastolic. During the experimental period, systolic blood pressure decreased to 142.1±20.4, 140.1± 22.9, and 135.2±19.9 at 3, 6, and 9 wk, respectively ($P < 0.05$ by paired t test compared to pressures on the last control day). Diastolic pressure was not changed significantly. Nine of these subjects later stopped the regular practice of meditation and their systolic blood pressure returned to control levels within 4 wk after stopping. These data are quantitatively comparable to those obtained employing operant conditioning techniques. (Research supported by grants from NIH and the General Service Foundation.)

28. Phlebotomy for Type I Congenital Nonhemolytic Jaundice (CNJ). PAUL D. BERK,* STEVEN C. WHITE,* AND BRUCE SCHARSCHMIDT,* Bethesda, Md. (introduced by Nathaniel I. Berlin**).

Congenital nonhemolytic jaundice (CNJ) is a rare, recessive disorder in which severe unconjugated hyperbilirubinemia terminates in kernicterus and death. Therapy aimed at accelerating bilirubin removal has been largely unsuccessful. The following studies indicate that plasma bilirubin in CNJ can be reduced by decreasing bilirubin production (BRP). A mathematical model was developed in which erythropoiesis, the circulating RBC mass, and catabolism of RBC's to bilirubin was described by 21 compartments, 15 of which represent circulating RBC's of successively greater age. It was assumed that, in the absence of hemolysis, only RBC's in the oldest compartment are converted to bilirubin. When simulated by computer, the model was found to be compatible with isotopic studies of iron, bilirubin, and RBC kinetics in more than 100 patients with various disorders. Using data for RBC mass, RBC life span, and RBP from an untreated patient with CNJ, steady-state pool sizes and the rate of RBC synthesis were calculated from the model by computer. This steady state was then perturbed by the simulated removal of 1/6 of the circulating RBC's weekly, under the contraint that increased red cell synthesis maintain the circulating RBC mass. The simulated new steady state was characterized by a marked skewing of RBC age distribution toward younger cells, producing a fall in BRP from RBC's to 30% and total BRP to 38% of base line, and a corresponding fall in plasma bilirubin from 30.0 to 11.4 mg/100 ml. This required a 2.8-fold increase in RBC synthesis, which should be readily attainable by a normal marrow if iron stores are maintained parenterally. These studies suggest that phlebotomy represents a potential treatment for CNJ which is deserving of a trial by physicians caring for such patients.

29. Mechanism of Suppression of Vasopressin (ADH) by Norepinephrine (NE). T. BERL,* J. A. HARBOTTLE,* P. CADNAPAPHORNCHAI,* AND R. W. SCHRIER, Denver, Colo.

Recent studies have demonstrated that the water diuresis associated with the intravenous infusion of norepinephrine (NE) is mediated primarily by suppression of vasopressin (ADH) release. To investigate whether the increase in cerebral perfusion pressure (CPP) with intravenous NE (0.5 μg/kg per min) is directly responsible for suppression of ADH release, the carotid circulation of hydropenic dogs was pump perfused to selectively increase CPP. In six experiments, a mean increase in CPP of 30 mm Hg did not cause a decrease in mean urinary osmolality (Uosm) (786 to 905 mOsm/kg). The possibility was also examined that NE exerts a direct central effect to suppress ADH release. In seven experiments NE was infused into the carotid artery in a subpressor dose (0.12 μg/kg per min) estimated to equal the amount of the catecholamine reaching the cerebral circulation with intravenous NE. The Uosm was not significantly altered with intracarotid NE (920 to 909 mOsm/kg). The possiblity was also examined that changes in autonomic neural tone from arterial baroreceptors is responsible for suppression of ADH release with intravenous NE. In intact animals intravenous NE diminished Uosm from 919 to 117 mOsm ($P < 0.001$), while in animals with denervated arterial baroreceptors intravenous NE was not associated with a significant alteration in Uosm (1251 to

1191 mOsm/kg). The results therefore demonstrate that NE primarily suppresses ADH release by altering autonomic baroreceptor tone rather than by a direct central or pressor effect of the catecholamine. This same mechanism may be the primary pathway for other nonosmotic influences on ADH release.

30. Stimulation of the Hexose Monophosphate Shunt (HMP) by Pyruvate: Mechanism aid Possible Significance. E. BEUTLER AND E. GUINTO,* Duarte, Calif.

Oxidation of the 1-carbon of glucose to CO_2 in mature RBC occurs in the hexose monophosphate shunt (HMP), which is regulated by the availability of NADP. The HMP is known to be strongly stimulated by 10 mM pyruvate, but the mechanism of stimulation has remained obscure. The three possibilities which deserve consideration are (*a*) NADPH-NAD transhydrogenation; (*b*) the utilization of NAD in the place of NADP by enzymes of the HMP; and (*c*) oxidation of NADPH by pyruvate. Measurement of the disappearance of hexose-6-P from dialyzed hemolysates supplied with NADPH, NADPH + NAD, or NAD showed that hexose-6-P was consumed only when NADPH and pyruvate were present. RBC LDH catalyzed the reduction of pyruvate by NADPH. The high K_m values at pH 7.4 were much lower at pH 6.9. NAD was a strong competitive inhibitor. Calculations suggest that the rate of NADPH oxidation by pyruvate through LDH is adequate to explain the increase of HMP activity observed. It is negligible at physiologic pH and pyruvate concentrations, but could become important when the RBC pH falls to 6.9, especially if plasma pyruvate levels are elevated. This might be sufficient to cause hemolysis of red cells with a defective NADP-reducing system, as in G-6-PD deficiency, where hemolysis is known to occur during diabetic acidosis.

31. β-Sitosterolemia and Xanthomatosis: a Newly Described Lipid Storage Disease in Two Sisters. ASHIM K. BHATTACHARYYA* AND WILLIAM E. CONNOR,** Iowa City, Iowa.

Although the diet may contain 150–200 mg of plant sterols, only trace amounts have heretofore been found in human blood and tissues. We now report elevated plasma plant sterol levels, especially β-sitosterol, in two sisters (L and R, aged 23 and 21 yr) with tendon xanthomas; their cholesterol levels were 203 and 206 mg/100 ml. Repeatedly, L and R had 27.2±2 (SD) and 17.7±2 mg/100 ml of sitosterol, 9.7±2 and 8.2±0.5 mg/100 ml of campesterol, and 0.5±0.6 and 0.5±0.4 mg/100 ml of stigmasterol in plasma. These were 15.6 and 11.3% of total plasma sterols. About 60% of plasma β-sitosterol and campesterol was esterified; stigmasterol was entirely unesterified. 70–82% of β-sitosterol was carried by low density lipoproteins. Plant sterols in red blood cells totaled 13.65 mg/100 ml packed cells. Neither parent had detectable plasma plant sterols. Two tendon xanthoma biopsies contained 36.73 and 3.96 mg/g dry wt of plant sterols, largely sitosterol and all unesterified. Plant sterols were found in adipose tissue (0.165 mg/g) and in skin surface lipids (3.24 mg/g of lipid). The intestinal absorption of sitosterol, by two techniques, was 65% after [4-^{14}C]sitosterol and 62 and 58% after 2010 mg sitosterol, given as a test breakfast (normal absorption being less than 5%). One mechanism for the development of sitosterolemia was greatly enhanced absorption of dietary sitosterol. The presence of plant sterols in red blood cells, adipose tissue, and xanthomas in the same ratios as that of plasma suggests a ready exchange of plant sterols between plasma and tissues. In some way plant sterols initiated the development of xanthomas with otherwise normal serum cholesterol levels. (Supported by research grants MO1-FR-59 and HL-14230 from NHLI.)

32. Hepatic Enzyme Induction After Cutaneous Application of Polychlorinated Biphenyls. D. BICKERS,* L. HARBER,** A. ALVARES,* AND A. KAPPAS,** New York.

Polychlorinated biphenyls (PCB) are ubiquitous environmental pollutants found in human and animal tissues and are potent inducers of the hepatic drug metabolizing enzyme complex. Cytochrome P-450 functions as the terminal oxidase for the liver drug metabolizing system. This study was designed to evaluate topical application of PCB and its effects on hepatic P-450 and drug metabolism. Rats received daily topical applications of a PCB (AROCLOR 1254) on the shaved nuchal area (25 mg/kg per day × 6 in 0.1 ml acetone). Animals were restrained to prevent any other contact with the applied chemical. Hepatic microsomes were prepared and P-450 content measured by the CO-difference spectrum. The CO-difference spectrum of microsomes from control rats had an absorption maximum at 450 nm. After application of PCB, there was a shift in this peak to 448 nm and a greater than 3-fold induction of P-448 content. In addition there was a similar increase in ethylmorphine *N*-demethylase activity and a 50% increase in aniline hydroxylation. The in vitro enhancement of microsomal oxidation was accompanied by decreased pharmacologic actions of hexobarbital and zoxazolamine in vivo. Cutaneous exposure to PCB has significant effects upon drug metabolism in the liver. The induction of P-448 by PCB is similar to the microsomal heme protein alteration induced by the carcinogenic polycyclic hydrocarbons, but the enhanced ethylmorphine oxidation resembles the effects of phenobarbital. PCB are a new class of inducers having properties of both these types of inducing agents. (Research supported by grants from NIH and the Council for Tobacco Research.)

33. Ionic Control of 1,25(OH)$_2$D$_3$ Production in the Chick Kidney Tubule. DANIEL D. BIKLE* AND HOWARD RASMUSSEN,** Philadelphia, Pa.

The production of 1,25(OH)$_2$D$_3$, the most potent metabolite of vitamin D$_3$, has been shown to occur exclusively in the kidney. Control of this reaction appears to be the critical step in vitamin D metabolism. Previous evidence derived from renal homogenates and mitochondrial preparations or from measurements of metabolite levels in the serum suggested that the rate of production of 1,25(OH)$_2$D$_3$ is inversely proportional to serum calcium concentrations. As serum calcium is increased, less 1,25(OH)$_2$D$_3$ is found in the blood and less is produced in vitro by renal homogenates or mitochondrial preparations from such animals. Another metabolite, 24,25(OH)$_2$D$_3$, appears to be produced instead. However, since neither of the above approaches directly evaluates the response in the intact cell, we decided to reinvestigate this mechanism using intact renal tubular suspensions from chicks. Our conclusions are somewhat different. By varying the extracellular calcium concentration of our in vitro incubation medium, we find a marked inhibition of 1,25(OH)$_2$D$_3$ production in the absence of calcium. The production increases as the extracellular calcium concentration is raised, reaching a maximum at 1 mM calcium and falling off slowly at higher concentrations. By raising chicks with different calcium contents in their diets and supplementing some with oral vitamin D in physiological doses, we can readily change serum Ca and P. Although D-deficient chicks on high-calcium diets consistently have a higher serum calcium than D-fed chicks on normal calcium diets, the tubule

preparations from these D-deficient chicks have a considerably greater production of $1,25(OH)_2D_3$. However, the D-deficient chicks on high-calcium diets do not have as much conversion as chicks on low-calcium diets, whether given vitamin D or not. The data suggest that a low serum phosphate can overcome the inhibition by a high-calcium serum content. Both ions appear to play a role in the regulation of $1,25(OH)_2D_3$ production. The inhibition of $1,25(OH)_2D_3$ production by vitamin D or high-calcium diets is not associated with a reciprocal increase in $24,25(OH)_2D_3$ production in renal tubule preparations to the extent seen in homogenates or mitochondrial preparations from the same batch of animals. Instead, the amount of the substrate $25OHD_3$ that undergoes any form of metabolism is markedly curtailed in those animals in which $1,25(OH)_2D_3$ production has been inhibited. (Research supported by NIAMDD 5 FO3 AM52376-02.)

34. Uremia and the Endoplasmic Reticulum of the Liver. MARTIN BLACK, MICHAEL LICHTER, LUIS BIEMPICA, STANLEY GROSSMAN, AND IRWIN M. ARIAS,** Bronx, N. Y.

The hepatic endoplasmic reticulum (ER) is important in the metabolism of proteins, lipids, carbohydrates, drugs, steroids, and sterols. We postulate that defective hepatic ER function may contribute to manifestations and pathogenesis of uremia. Rats were made azotemic by 70% surgical reduction of renal mass producing BUN of 45–100 mg/100 ml. Sham-operated rats served as controls. 4 wk later, uremic rats gained weight; hepatic microsomal protein, cytochrome P450 content, and benzphetamine demethylase activity were reduced ($P < 0.01$, < 0.001, and < 0.02, respectively), and plasma cholesterol and triglycerides were increased ($P < 0.001$). The defect in ER function was not generalized since UDP glucuronyl transferase, aminopyrine demethylase, glucose-6-phosphatase, and 5′-nucleotidase activities were unaffected. Malondialdehyde production was unchanged, suggesting that lipid peroxidation is not enhanced in uremia. Electron microscopy of liver revealed focal ER disruption and dilatation, increased autophagic vacuoles and cytoplasmic lipid, and swollen mitochondria with flattened christae. Cytochrome P450-dependent drug metabolism was assessed in vivo by measurement of zoxazolamine paralysis time. It was prolonged in uremic rats ($P < 0.001$) and correlated inversely with hepatic P450 content ($r = 0.73$); drug blood levels were similar in control and uremic rats on awakening. DDT administration increased hepatic P450 content and reduced zoxazolamine paralysis time in uremic rats to that seen in control rats similarly treated. In 13 chronically uremic patients, P450-dependent drug metabolism was studied in vivo using antipyrine. Plasma antipyrine $t_{\frac{1}{2}}$ was either normal or slightly shortened when compared with results in control subjects. Many patients were unavoidably taking drugs which theoretically could enhance antipyrine metabolism. Glutethimide administration further reduced plasma antipyrine $t_{\frac{1}{2}}$, and hemodialysis produced variable effects. The results indicate that a reversible hepatic ER defect occurs in experimental uremia and that this defect may be related to certain manifestations of uremia and their pathogenesis. (Research supported by grants from NIH.)

35. Separation of Growth Hormone Binding from Insulin Binding in Liver Plasma Membranes. RICHARD S. BOCKMAN,* NORBERT I. SWISLOCKI,* PETER FOSTER,* GEORGE R. HENDERSON,* AND MARTIN SONENBERG,** New York.

In an effort to understand the initial actions of peptide hormones, we have studied growth hormone (GH) and insulin binding to plasma membranes. ^{125}I-labeled GH or insulin was incubated in 1.0 mM $NaHCO_3$ with liver plasma membranes from hypophysectomized rats. The mixture was overlayed on a 10%/37%/50% stepped sucrose gradient and centrifuged at 600 *g* for 1 h. Membrane subfractions were visible as two discrete bands, one at the 37%/50% sucrose interface, peak I, and peak II at the 10%/37% sucrose interface. This gradient fractionation of the membranes was studied by assaying membrane-specific enzymes and by protein analysis. Free GH or insulin remained at the top of the gradient. Only GH or insulin that was tightly bound to membrane migrated in association with the membrane fractions. Membrane-bound [^{125}I]GH after dissociation with SDS-mercaptoethanol had identical electrophoretic behavior on SDS-polyacrylamide gels as the starting iodinated hormone. Peak I, the more dense fraction, bound 30×10^{-15} moles [^{125}I]GH per mg membrane protein and 1.74×10^{-15} moles [^{125}I]insulin per mg membrane protein. Peak II, the less dense fraction, bound 6.0×10^{-15} moles [^{125}I]GH per mg membrane protein and 3.9×10^{-15} moles [^{125}I]insulin per mg membrane protein. Addition of excess, nonlabeled GH to the incubation mix prevented the iodinated GH from binding to peak I but was less effective in blocking GH binding to peak II. Native insulin competed effectively with [^{125}I] insulin for peak II sites and less effectively for peak I sites. Native insulin was without effect on [^{125}I]GH binding to peak I. Of the two fractions, peak I had the higher ATPase specific activity and was more responsive to GH stimulation than peak II. These studies show that liver plasma membranes can be resolved into subfractions with different peptide hormone receptivity and possibly different physiological activity. (Research supported in part by grants CA08748 of the NIH, GB19797 of the NSF, and BC119 of the ACS.)

36. Homogeneous Rabbit 7S Rheumatoid Factor with Antibody Specificity for Peptidoglycan. VIKTOR A. BOKISCH,* DAVID BERNSTEIN,* AND RICHARD M. KRAUSE,** New York.

Rabbits immunized intravenously with streptococcal vaccine produce 19S and 7S anti-IgG's in addition to anti-streptococcal antibody. 7S anti-IgG was measured by an assay based on the ability of 7S anti-IgG to coprecipitate with antigen-antibody complexes. Four anti-group C antisera, containing more than 5 mg/ml of 7S anti-IgG, were selected for isolation of 7S anti-IgG by means of immunoabsorbent columns. The four proteins reacted with the Fc portion of γ-globulin. One of these, R3387, had in addition specificity for cell wall components as evidenced by its ability to agglutinate groups C and A streptococcal cell walls. Experiments with radiolabeled R3387 revealed that 70–80% bound to the cell walls. The possibility that this binding was caused by contamination with other antibodies was eliminated by the exceptional homogeneity of this anti-IgG. Analysis of the protein on urea polyacrylamide gels showed one major light-chain band. Agglutination of streptococcal cell walls could be inhibited by peptidoglycan of *Staphylococcus epidermidis,* but not with peptidoglycan of *Corynebacterium poinsettiae.* This specificity indicates that 7S anti-IgG R3387 is reacting with the peptide portion of peptidoglycan, since the peptidoglycans differ only in peptide moiety. Inhibition of coprecipitation of R3387 with antigen-antibody complexes by the pentapeptide of peptidoglycan and other synthetic tetrapeptides revealed that the C-terminal amino acid D-Ala was the immunodominant determinant of peptidoglycan in this reaction. Moreover, the precipitin reaction between R3387 and an idiotypic antiserum raised against it was inhibited by the pentapeptide, indicating that it is the antibody binding site of R3387 which reacts with the peptide

portion of peptidoglycan. (Research supported by grant AI-08429 from NIH.)

37. Insulin and Glucagon: Dynamic Hepatic Interaction in Normal and Diabetic Man. JAMES D. BOMBOY,* JOHN E. LILJENQUIST,* STEPHEN B. LEWIS,* BRUCE C. SINCLAIR-SMITH,* PHILIP W. FELTS,* WILLIAM W. LACY,* OSCAR B. CROFFORD, AND GRANT W. LIDDLE,** Nashville, Tenn.

Insulin antagonizes glucagon-induced hepatic glucose production in the perfused rat liver, but this has not been demonstrated in man. Accordingly, we examined the interaction of glucagon and insulin in the control of net splanchnic glucose production (NSGP) in eight normal and three diabetic men, by employing the hepatic venous catheter technique. Glucagon was infused intravenously at 5 ng/kg per min for 90 min. Resultant arterial glucagon levels (1×10^{-10} M) did not exceed the physiologic portal range. In four normal men, NSGP increased rapidly from 92±12 (SEM) to 212±32 mg/min. Despite constant arterial glucagon levels, NSGP declined to a basal rate by 45 min. No change in hepatic vein cyclic adenosine monophoshate (cyclic AMP) concentration was detected. In three insulin-dependent diabetic men, NSGP induced by glucagon remained elevated throughout the 90 min period. To study the effect of exogenous insulin on this glucagon-induced increase in NSGP, insulin was infused at 10 mU/kg per min in four normal men to achieve arterial IRI concentrations of 1500 μU/ml (1×10^{-8} M). Blood glucose was stabilized by varying the glucose infusion rate (glucose "clamp" technique). During these infusions, NSGP was reversed and there was net splanchnic glucose uptake. After 40 min of insulin pretreatment, a 5 ng/kg per min glucagon infusion was begun. With an arterial insulin: glucagon ratio of approximately 100, glucagon caused no increase in NSGP. When the glucagon infusion was increased to 15 ng/kg per min (arterial insulin: glucagon ratio of approximately 33), NSGP was no longer suppressed by insulin and increased markedly. We conclude that insulin can suppress glucagon-stimulated NSGP in normal man. It is postulated that the failure of diabetics to modulate glucagon-induced NSGP is attributable to their failure to secrete sufficient insulin. (Supported by NIH grant HL 08195 and VA Training Grant TR-T-2.)

38. Lymphokines and Granulomatous Hypersensitivity. D. L. BOROS,* R. P. PELLEY,* K. S. WARREN, AND A. B. STAVITSKY,* Cleveland, Ohio.

Lymphokines have been demonstrated to be mediators of cellular hypersensitivity in vitro, but little evidence has been gathered concerning their role in vivo and in immunopathological reactions. Cellular hypersensitivity has been more strongly implicated as a major factor in the pathogenesis of schistosomiasis than in any other granulomatous disease. Therefore, experiments were performed to determine whether lymphokines are involved in the cell-mediated granulomatous inflammation of schistosomiasis. Lymphokines (MIF) were produced in vitro by spleen cells and by intact liver granulomas from mice with schistosomiasis on stimulation with soluble schistosome egg antigens (SEA). This MIF, adsorbed to bentonite particles, elicited granulomas on injection into normal mice. 20 million spleen cells or 100 liver granulomas (isolated by gentle homogenization) from mice with schistosomiasis mansoni were incubated in medium RPMI-1640 with 10% human plasma, glutamine, and antibiotics (TCM) in the presence of 1 ng–10 μg of SEA. At 24 h TCM was changed using medium without plasma or antigen. At 48 h TCM was assayed from MIF by David's indirect technique, using normal guinea pig peritoneal exudate cells. Maximal migration inhibition occurred with TCM from spleen cell cultures stimulated with 100 ng SEA (~50%) and from granuloma cultures exposed to 1 μg SEA (~60%). TCM from lymph node cultures did not yield MIF consistently. Schistosome granulomas incubated with mycobacterial culture filtrate showed no MIF activity. MIF-containing TCM was concentrated, dialyzed, and adsorbed onto bentonite particles (65 μ). After injection into the pulmonary microvasculature of normal mice, stained sections revealed mononuclear granulomas measuring 94 μ at 24 h. Particles coated with TCM from antigen-free cultures elicited only scanty reactions. These data suggest that lymphokines participate in granulomatous hypersensitivity. (NIH grant AI08163.)

39. Estimates of Canalicular Bile Secretion and Its Determinants in Man Utilizing the Biliary Clearance of [^{14}C] Mannitol. JAMES L. BOYER,* JOSEPH R. BLOOMER,* AND WILLIS C. MADDREY,* New Haven, Conn., and Chicago, Ill. (introduced by J. B. Kirsner**).

50 μCi of [^{14}C] mannitol were administered intravenously to five postcholecystectomy patients with indwelling T tubes to evaluate the biliary clearance of [^{14}C] mannitol as a means of estimating canalicular bile flow in man. Mannitol appeared in bile within 2–25 min, rose to a maximum within 18.5–42 min, and thereafter paralleled the plasma [^{14}C] mannitol disappearance curve. During subsequent 30-min control periods, bile-plasma [^{14}C] mannitol ratios and mannitol clearance averaged 0.64±0.17 and 0.26±0.15 ml/min, respectively. After 5 cc of 20% sodium dehydrocholate (Decholin) intravenously in six studies, bile-plasma mannitol ratios remained constant (0.67±0.10), while bile flow (1.15±16 ml/min) and mannitol clearance (0.77±1.5 ml/min) increased. In contrast, after secretion administration in three studies, bile-plasma [^{14}C] mannitol ratios diminished (0.34±0.7) as bile flow increased so that mannitol clearance remained essentially unchanged (0.42±0.03 ml/min). These findings suggest that mannitol enters bile at the level of the hepatocyte rather than bile ductules, and that mannitol clearance may be utilized in man to estimate canalicular flow. When determinants of canalicular secretion were examined by correlating mannitol clearance and bile acid secretion rates, a significant fraction was observed to be independent of bile salt secretion. Nonbile salt-dependent canalicular flow averaged 187 ml/24 h when bile drained spontaneously and averaged 430 ml/24 h when bile was collected during Decholin infusions. Phenobarbitol treatment (180 mg q.i.d. for 4–7 days) resulted in small increments in canalicular flow in two of four patients (86.4 ml/24 h and 60.5 ml/24 h) and increased the bile salt-independent fraction in these two patients. These studies represent the first estimates of canalicular bile flow and its determinants in man and demonstrate that a significant fraction is linked to bile salt-independent mechanisms previously observed in animals. (Research supported by USPHS grant AM 5966–10.)

40. Simultaneous Episodic Secretion of Luteinizing Hormone and Testosterone During Sleep in Puberty. ROBERT M. BOYAR,* JORDAN FINKELSTEIN,* SHELDON KAPEN,* HOWARD ROFFWARG,* ELLIOT WEITZMAN,* AND LEON HELLMAN,** New York.

Plasma luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) were measured by specific radioimmunoassays every 20 min for 24 h in 10 normal children (ages 9–15 yr) in varying stages of sexual maturation and in six normal men (ages 21–45 yr). Poly-

graphic monitoring of sleep stages was carried out during normal nocturnal sleep, as well as during acutely reversed diurnal sleep. Three prepubertal boys showed plasma LH, FSH, and T concentrations which were low and relatively constant, both when awake and during sleep, throughout the 24 h (no episodic secretion). Three boys in very early puberty showed augmented LH secretion without a significant increase in T secretion during sleep. However, the four boys in advanced puberty showed a marked increase in plasma LH (150–250% above waking), and plasma T (200–400%) synchronous with actual sleep. Spontaneous awakenings were accompanied by a marked and rapid decline in LH and T. When sleep was shifted 180° in three subjects, the increases in both plasma LH and T occurred during the new diurnal sleep period. In six adult men, there was episodic secretion of T with plasma T varying 200–500% during the 24 h period. In the adults, LH and T secretion were not closely related. These data show (*a*) that, as has been demonstrated for other hormones, T is secreted episodically showing great variability during the 24 h sleep-wake cycle; (*b*) that during puberty there is a marked augmentation of testosterone secretion during sleep synchronous with the secretion of LH; and (*c*) that this LH-T secretory program in puberty is intimately linked to actual sleep since it changes acutely with sleep reversal.

41. Inhibition of Progesterone Synthesis by Human Corpus Luteum and Placenta. EDWIN D. BRANSOME, JR.,* DEAN P. EDWARDS,* AND JAMES L. O'CONNOR,* Augusta, Ga. (introduced by Alfred Jay Bollet).

Increased synthesis of progesterone by the enzyme complex of 3β-hydroxysteroid-dehydrogenase and Δ-5 isomerase is necessary for successful implantation of the fertilized ovum and for maintenance of pregnancy. Using new methods of purification, we have found these enzymes in placenta and corpus luteum to be quite similar. The K_m's for the substrate pregnenolone are 1.6×10^{-6} M (placenta) and 3.5×10^{-6} M (corpus luteum). Using an assay of the kinetics of [^{3}H] pregnenolone conversion to progesterone by the placenta enzyme, we have found that estradiol-17β is a competitive inhibitor ($K_I = 2.8 \times 10^{-6}$ M), but that diethylstilbestrol has no effect. Estranes, unsaturated in ring B, with trans-substitutions above ring D are excellent inhibitors of the enzymes but possess little or no "estrogenic" activity. Equilenin ($K_I = 4 \times 10^{-8}$ M) and some of its derivatives are particularly potent; they are in addition noncompetitive inhibitors difficult for the substrate to displace from the enzyme. Preliminary data suggest that such steroids may be effective in vivo at concentrations which might be attained after brief oral administration. Our findings, therefore, reveal a new structure-activity principle of estrogen-protein interaction and suggest a new pharmacologic approach to preventing implantation and interrupting pregnancy. (Research supported in part by contract AID/csd 2491 from the USDS to the Population Council.)

41a. Lipolysis in Human Adipose Tissue. G. A. BRAY, D. LUONG,* L. B. SALANS,* E. A. H SIMS,* AND R. FISER,* Torrance, Calif.

These studies compare the lipolytic response of adipose tissue from lean and obese subjects before and after a period of weight gain. 1800 cal/m^2 was fed to both groups before the first biopsy of subcutaneous fat. Five lean subjects gained 15–25% of body weight by overeating and then ate 2700 cal/m^2 before the second biopsy. The three fat subjects ate 3600 cal/m^2 for 3 wk, followed by 2700 cal/m^2 before obtaining fat at surgery. Adipose tissue was incubated in vitro, and the release of glycerol was measured. Lean subjects released less glycerol in response to isoproterenol before weight gain. After weight gain the sensitivity of fat from lean subjects to isoproterenol increased to equal that of the obese. The lipolytic effects of dibutyryl cyclic AMP were similar in the obese and lean and did not change with weight gain. These studies suggested that adipose cells from lean subjects might form less cyclic AMP. Isoproterenol stimulated a dose-related rise in cyclic AMP within 4 min with a peak by 40 min, but epinephrine had little effect. Adipose cells from lean patients responded as well as those from fat patients. These studies have shown that (*a*) adipose tissue from obese patients is more sensitive to isoproterenol than fat cells from lean subjects on an 1800 cal/m^2 diet; (*b*) weight gain enhanced the sensitivity to lipolysis in adipocytes from lean subjects but not in obese patients; (*c*) the response of adipose cells to dibutyryl cyclic AMP was unaltered by overfeeding; (*d*) the formation of cyclic AMP was stimulated by isoproterenol but not by epinephrine; and (*e*) cyclic AMP was formed similarly in adipose cells from lean and obese.

42. Glomerulotubular Balance Associated with Increments of Plasma Sodium. E. H. BRESLER * AND KRISTIN T. NIELSEN,* New Orleans, La. (introduced by C. Thorpe Ray **).

That the rate of renal tubular reabsorption of sodium (T_{Na}) is closely attuned to changes in filtered load of sodium (F_{Na}) due to changes in glomerular filtration rate (GFR) is firmly established. Whether changes in F_{Na} induced by raising plasma sodium concentration (P_{Na}) alter T_{Na} has apparently not been as definitively answered. At least with respect to the proximal tubule, there have been conflicting reports caused possibly by undesirable variability arising from comparisons between animals. The present study involves comparison in the same animal and experiment, thus tending to minimize variability. Anesthetized dogs given 1.25 mg ethacrynic acid per kg or more were infused at 10 ml/min with a sustaining solution containing 3.5 mEq K per liter of normal saline containing inulin, creatinine, and PAH. For one or two 15- to 20-min intervals the salinity of this solution was increased to 5%. 10- to 30-min clearance periods were obtained. The time course for T_{Na} showed no resemblance to that for P_{Na}. When correction for variation in GFR was made by plotting T_{Na} per unit GFR (T_{Na}/GFR) against time, a marked parallelism between T_{Na}/GFR and P_{Na} is revealed. Thus tubular reabsorption of sodium keeps pace with filtered load of sodium, whether this be increased by changes in GFR or increments in P_{Na}. Thus any proposed sodium transport system must not only react to vary sodium transport when volume of fluid presented to the tubules varies but must also increase transport when concentration of sodium in a given volume is increased. Since ethacrynic acid was given, it is probable that the observed behavior of the tubules is largely if not entirely related to proximal tubular transport mechanisms. (Research supported by VA.)

43. Cyclic-AMP-Regulated Cholesterol and Fatty Acid Synthesis in Liver: Its Deletion in Hepatomas. LEE A. BRICKER * AND GERALD S. LEVEY,* Miami, Fla. (introduced by Eric Reiss **).

Cholesterol synthesis is regulated in liver in part by a sensitive negative feedback system mediated through the enterohepatic circuit. However, alterations in serum lipids in several endocrine disorders also suggest a possible hormonal role in regulation of lipid metabolism. We therefore examined the effects of cyclic adenosine monophosphate (cAMP) and dibutyryl cAMP (DBC) on hepatic lipogene-

sis using liver slices from male rats. cAMP (5×10^{-3} M) and DBC (3×10^{-4} M) each inhibited the incorporation of [^{14}C] acetate into digitonin-precipitable sterol (DPS) by $> 70\%$, and into *de novo* fatty acid by $> 80\%$. Production of $^{14}CO_2$ was unaffected. When [^{14}C] octanoate was used as substrate, similar suppression of incorporation of isotope into DPS by the cAMP and DBC was observed. By contrast, these nucleotides did not affect incorporation by [^{14}C] mevalonate into DPS. Since cholesterogenesis in hepatomas is not under negative feedback control, we examined the effects of cAMP and DBC on lipogenesis in two varieties of rat hepatoma, the "minimal deviation" Morris 9121 and the undifferentiated Morris 3924A. Sterol and fatty acid syntheses were unaffected in either tumor. The data indicate that hepatic lipogenesis is significantly and specifically regulated by cAMP early in the metabolic utilization of acetyl CoA, at a discrete point distinct from that of the cholesterol feedback system. Further, the nucleotide-regulated system, as well as the cholesterol feedback mechanism, is deleted in hepatomas. (Research supported by NIH grant 5 R01 CA11969-02.)

44. Studies of Biologic Actions of 1,25-Dihydroxycholecalciferol in Man. A. S. Brickman,* J. W. Coburn, S. G. Massry, J. E. Bethune, H. E. Harrison,** and A. W. Norman,* Los Angeles and Riverside, Calif., and Baltimore, Md.

1,25-Dihydroxycholecalciferol (1,25-$(OH)_2D_3$) appears to be the active form of vitamin D. Preliminary studies suggested that small amounts of the metabolite are biologically highly active in patients with uremia (CRF). The present studies evaluate the actions of 1,25-$(OH)_2D_3$, 0.1–2.7 μg/day for 6–12 days, in six normal subjects, seven CRF, three patients with pseudohypoparathyroidism (PHP), and four with hypophosphatemic vitamin D-resistant rickets (HVDRR). In normals, urine Ca rose by 32–130% within 2 days; fractional intestinal ^{47}Ca absorption increased from 0.25±0.01 to 0.38±0.02. Serum Ca and P and urine P were unchanged. In PHP, serum Ca rose by 1.3–2.0 mg/100 ml, urinary Ca was augmented, and ^{47}Ca absorption increased in two of three patients. 1,25-$(OH)_2D_3$ did not improve responsiveness of PHP to parathyroid extract as evidenced by changes in serum Ca, urine P, or cyclic adenosine monophosphate (cyclic AMP). In HVDRR, serum Ca increased by 0.3–1.0 mg/100 ml, urine Ca by 20–400%, and fecal Ca fell by 63% in one balance study, without change in serum or urine P. In CRF, serum Ca rose by 1.4±0.5 mg/100 ml; ^{47}Ca absorption increased from 0.19±0.02 to 0.38±0.04, and fecal Ca fell by 18–71%. These studies demonstrate that 1,25-$(OH)_2D_3$ is highly potent in normal man and in patients with CRF, PHP, and HVDRR; however, 1,25-$(OH)_2D_3$ did not correct renal P wasting in HVDRR. Responses to doses as low as 0.1 μg/day underscore the marked potency of 1,25-$(OH)_2D_3$ in man. It may be inferred that equally small amounts of the metabolite are normally produced and conversion of 25(OH)-D_3 to 1,25$(OH)_2$-D_3 must be closely regulated. (Supported by NIH grant AM-14750 and contract PH 43-68-1040.)

45. Diminished Binding of Alkylating Agents by Lymphocytes in Chronic Lymphocytic Leukemia. Jerome I. Brody, Philadelphia, Pa.

The purpose of this investigation was to determine whether the lymphocyte in chronic lymphocytic leukemia (CLL), a cell with numerically diminished mitogen receptors, similarly demonstrates defective binding of clinically used alkylating agents. Leukemic and normal lymphocytes were placed in separate cultures in identical, duplicate sets, each consisting of two vials. The first culture of each group held cells, plasma, and media, and the second culture held phytohemagglutinin (PHA) in addition. After a growth period, ^{14}C-labeled nitrogen mustard and cyclophosphamide, in individual and sequential experiments, were added in concentrations analogous to those employed in vivo and shown in this laboratory to be cytotoxic in vitro. After suitable incubation times, the cells in the first set were washed and digested in toto, whereas polynucleotides were precipitated from the lymphocytes of the second culture group. Normally, the major portion of radioactivity was found on the whole cell digests with significantly fewer, but detectable, emissions from the nucleotides. PHA-stimulated lymphocytes showed an increase in radioactivity, especially and more consistently on the intact cell. The critical observation, however, was the strikingly depressed radioactivity in both forms of assays using leukemic lymphocytes when compared with their normal counterparts. These results infer that the principal and, perhaps, immediate manner of cell injury and lysis by alkylating compounds is through cell surface binding, with DNA complexing playing a secondary role. The often prompt, initial disappearance of lymphadenopathy after administration of these drugs is consistent with such a concept. Finally, therapeutic resistance in CLL and hematopoietic toxicity after aggressive treatment may be clinical expressions of an anomalous leukemic lymphocyte membrane, with decreased drug-binding potential, and a comparatively more susceptible non-B-type lymphoid cell. (Supported by NIH grants.)

46. An Improved Method for Subcellular Fractionation of Human Platelets. M. Johan Broekman,* Nelson P. Westmoreland,* and Phin Cohen, Boston Mass.

Washed platelet suspensions derived from fresh ACD blood were subjected to explosive decompression under nitrogen and ultracentrifuged on a sucrose gradient. Eight fractions, seven distinct bands and supernatant, were separately harvested for enzyme and electron microscopy studies. The level of pressure, time of exposure at a given pressure, number of decompressions, and loading and makeup of the gradient were systematically varied to retain morphological integrity of mitochondria and alpha granules, while achieving maximal peaking of enzyme activities in the various layers. The fractions from top to bottom of the gradient were morphologically (and enzymatically) characterized as follows: (1) supernatant (LDH); (2) light membranes (5′-nucleotidase); (3) heavier membranes, putatively microsomes (glucose-6-phosphatase); (4) mitochondria, 60% pure (succinate-cytochrome c reductase); (5) mixture of mitochondria, alpha granules, and elongated polymorphic electron-dense bodies (mixture of enzymatic activities); (6) alpha granules, 70% pure, intact, homogeneous matrix (four acid hydrolases); (7) alpha granules, 60% pure, some swollen, some with condensed matrix (four acid hydrolases); (8) cell fragments (mixture of above enzymes). The four acid hydrolases were asymmetrically distributed in that the *p*-nitrophenylphosphatase peaked more in fractions 5 and 6, whereas beta glycerophosphatase, *p*-nitrophenyl-*N*-acetylglucosaminidase, and *p*-nitrophenylgalactosidase peaked more in fractions 6 and 7. Microsomes, mitochondria, and alpha granules were further purified, morphologically and enzymatically, by combining the same layers of several tubes, and after pelleting and resuspension in 0.25 M sucrose, recentrifuging the harvest on a sucrose gradient. Parallel studies with Teflon pestle homogenization did not furnish as pure or as distinct layering of microsomes, mitochondria, or alpha granules. We conclude that our method improves upon previous techniques for platelet fractionation. (Supported by grant HE-13802

from NIH and contract DADA-17-70-C-0083 from the U.S. Army.)

47. Identical Properties of β-Hydroxy-β-Methylglutaryl Coenzyme A Reductase (HMG CoA Reductase) from Liver and Hepatoma. MICHAEL S. BROWN * AND MARVIN D. SIPERSTEIN,** Dallas, Tex. (introduced by Jean D. Wilson).

In rat liver the activity of microsomal β-hydroxy-β-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-limiting enzyme of cholesterol synthesis, declines when the animals consume a high-cholesterol diet, and consequently the rate of cholesterol synthesis falls. This regulatory system is absent in all hepatomas studied to date. To determine whether the defect in hepatomas results from a structural alteration of HMG CoA reductase, we have solubilized and purified 675-fold HMG CoA reductase from normal liver and a well differentiated transplantable hepatoma (9121) that has been shown previously to lack feedback inhibition of HMG CoA reductase activity. HMG CoA reductase was solubilized from washed microsomes of normal liver by three methods: phospholipase A digestion (90% yield), 4 M KCl washing (72%), and freezing followed by glycerol extraction (67%). The solubilized enzyme from liver underwent rapid inactivation at 0°C with a half-life of 10 min, but at 25°C it lost only 10% of its activity in 24 h. The enzyme was protected from cold inactivation by maintenance in 4 M KCl. The solubilized enzyme withstood heating to 65°C for 10 min. By gel filtration its apparent molecular weight was 200,000 and it had an isoelectric point of 6.6 by isoelectric focussing. The solubilized hepatoma enzyme was identical with the normal enzyme in all parameters tested, including substrate saturation curves for HMG CoA and TPNH, time-course of cold inactivation and complete protection by 4 M KCl, heat resistance, molecular weight, and migration on polyacrylamide-gel electrophoresis. It is concluded that the failure of hepatomas to respond to feedback inhibition of cholesterol synthesis is due to a defect other than a structural alteration in HMG CoA reductase.

48. Bactericidal Activity and Opsonization in Human Serum Chelated with Ethyleneglycol Tetraacetic Acid (EGTA) and MgEGTA. CHARLES S. BRYAN,* DANIEL G. COLLEY,* AND RODGER M. DES PREZ, Nashville, Tenn.

Previous studies from this laboratory have demonstrated that human serum chelated with ethyleneglycol tetraacetic acid (EGTA) permits complement (C) activation through the C3 shunt while inhibiting the C142 pathway (Fine et al. 1972. *J. Immunol.* **109**: 807). The C142 pathway is also inhibited by MgEGTA in appropriate concentrations in human serum (Marney and Des Prez. 1969. *J. Immunol.* **103**: 1044). The present studies have established that bactericidal activity against a C-sensitive strain of *E. coli* takes place in human serum chelated with 10 mM EGTA and with mM MgEGTA. Activity was greater in 10 mM MgEGTA serum than in 10 mM EGTA serum. However, in neither instance was the onset of bactericidal activity as rapid as in unchelated serum. These findings support previous conclusions that bactericidal activity can be mediated through the C3 shunt, but that an intact C142 pathway may be necessary for the full bactericidal activity of serum (Goetze and Müller-Eberhard. 1971. *J. Exp. Med.* **134**: 90s; Root et al. 1972. *J. Immunol.* **109**: 477). Phagocytosis studies utilizing a two-phase system in which a C-resistant strain of *E. coli* was opsonized in the presence of normal serum, 10 mM MgEGTA serum, or 10 mM EDTA serum, washed, and then exposed to human leukocytes in EDTA serum, indicated that opsonization also proceeds in the presence of MgEGTA. This confirms previous evidence that opsonic activity can be mediated through the C3 shunt. (Research supported by NIH grant HL-08399 and the VA.)

49. Immunity to Colon Cancer: Assessment by Antigen-Induced Inhibition of Mononuclear Cell Migration. DAVID M. BULL,* JON R. LEIBACH,* AND RICHARD A. HELMS,* Columbus, Ohio, and Boston, Mass. (introduced by Charles S. Davidson **).

Methods for in vitro assessment of human tumor immunity (lymphocyte-mediated cytotoxicity, lymphoblastic transformation, inhibition of leukocyte migration) yield inadequate clinical correlations and are of limited diagnostic and prognostic usefulness. In studies of tumor immunity using inhibition of leukocyte migration, we found that granulocytes, platelets, and contaminating erythrocytes led to tumor antigen unresponsiveness and unreliable migration. Elimination of these cell types by differential sedimentation in Ficoll-Hypaque followed by layering in sucrose gave a lymphocyte/monocyte population that migrated reliably and was reproducibly antigen responsive. These lymphocyte/monocyte mixtures from 24 of 27 patients with colon carcinoma responded to a 1:3000 saline extract of homologous colonic adenocarcinoma with migration inhibition, showing migration indices (M.I. = migration area with antigen/migration area without antigen) of 0.64±0.14 (SD). Of the three patients with M.I. > 0.80, one had a small adenocarcinoma found histologically in a segment of colon resected for diverticulitis, and another showed evidence by skin testing of carcinomatosis-induced diminution in overall cellular immune function. 37 cancer-free control subjects, as well as five patients clinically considered to have colon carcinoma but in whom nonmalignant disease was ultimately demonstrated, failed to show tumor antigen-induced migration inhibition (M.I. = 0.99±0.10; mean M.I.'s significantly different, $P << 0.001$). Nine patients with colonic adenocarcinoma who had undergone curative surgery (mean follow-up period 3.8 yr) also showed uninhibited migration patterns (M.I. = 1.06±0.07). These results indicate that assessment of tumor immunity with inhibition of lymphocyte/monocyte migration correlates well with clinical course in colon carcinoma and may have diagnostic and prognostic value in this condition.

50. Defective Androgen Receptors in Testicular Feminization. L. P. BULLOCK * AND C. W. BARDIN, Hershey, Pa.

Testicular feminization is an inherited disorder of man, rat (tfm), and mouse (tfm/y) in which androgen-dependent differentiation does not occur due to end-organ insensitivity. Studies of RNA and protein synthesis in tfm rodents suggested a pretranscriptional regulatory defect. We have, therefore, examined the initial events of testosterone-target tissue interaction to define the molecular basis of this abnormality. Since tfm animals lack reproductive tracts, preputial glands and kidneys were selected for study. In normal rat preputial gland, testosterone was metabolized to dihydrotestosterone (DHT). This latter steroid was bound to a 7.5s cytoplasmic protein with a high affinity for DHT (but not testosterone). The protein-steroid complex was transferred into the nucleus where it presumably facilitates RNA synthesis. In the tfm rat, however, no high-affinity cytoplasmic binder was demonstrated and androgen transfer to the nucleus was markedly reduced. In mouse kidney, both in vivo and in vitro studies indicated scant reduction of testosterone to DHT and testosterone per se was transferred to the nucleus. These observations suggested that testosterone rather than DHT may be the active intranuclear androgen in kidney. Studies of the

7.5s androgen receptor protein from this organ support this postulate. The receptor had a high affinity for testosterone (K_d of 1.7^{-9} M, 5.7^{-14} moles/mg protein) in normal male and female mice. By contrast, tfm/y mice had no specific 7.5s receptor protein and androgen transfer to the nucleus did not occur. In conclusion, studies of two rodent species with testicular feminization indicate decreased cytosol androgen receptor activity, which could account for the lowered nuclear androgen binding. This inability to concentrate androgens at their active site could explain the androgen insensitivity in the tfm rat and mouse and perhaps in man. (Research supported by NIH grant 5R01-HD-05276.)

51. Effect of Diuretics on the Thick Ascending Limb of Henle's Loop. MAURICE BURG AND NORDICA GREEN,* Bethesda, Md.

This tubule segment was dissected from rabbit kidneys and perfused in vitro. There was active Cl transport resulting in net NaCl reabsorption from the lumen with the electrical PD oriented lumen positive. Furosemide (10^{-5} M), mersalyl (3×10^{-5} M), and ethacrynic acid (10^{-3} M) in the tubule lumen each caused a large decrease ($\geq 50\%$) in the electrical PD and net chloride flux, indicating inhibition of the active chloride transport. The fall in PD induced by furosemide in the lumen was rapid (< 5 sec) and immediately reversible, whereas furosemide (10^{-4} M) in the bath had essentially no effect. A longer time (1–10 min) was required for ethacrynic acid and mersalyl to lower the PD and reversal was also slower. The effect of mersalyl, however, was immediately reversed by *p*-chloromercuribenzoate in the lumen (3×10^{-5} M), just as the diuresis caused by mersalyl is reversed by *p*-chloromercuribenzoate in vivo. A high concentration of mersalyl in the bath (10^{-4} M) also decreased the PD, but the effect was not reversible. Ethacrynic-cysteine complex (a urinary excretion product) inhibited active chloride transport at a lower concentration (3×10^{-6} M in the lumen) than did ethacrynic acid alone and may be the major active form of the drug. Ethacrynic-cysteine in the bath (10^{-5} M) had no effect. Acetazolamide (10^{-3} M), hydrochlorothiazide (10^{-3} M), and amiloride (10^{-4} M) had no effect on the PD whether placed in the lumen or bath. It is concluded that furosemide, mersalyl, and ethacrynic acid, when present in the lumen of the thick ascending limb of Henle's loop, induce diuresis in vivo by inhibiting active chloride transport.

52. The Hemodynamic Effects of Amyl Nitrite in Coronary Artery Disease. GARY W. BURGGRAF* AND JOHN O. PARKER, Kingston, Ontario, Canada.

Amyl nitrite (AN) was introduced more than a century ago for the treatment of angina pectoris and has been utilized recently as a stress test to detect coronary artery disease (CAD), but there is little information as to its hemodynamic effects in subjects with CAD. Accordingly the hemodynamic response to AN inhalation (60 sec) was studied in 27 patients with CAD and 6 normal subjects. During sinus rhythm, AN given to 15 patients in the CAD group resulted in abrupt decreases in brachial arterial pressure from 124/72±9/4 (mean±SEM) to 90/48±5/3 mm Hg and in left ventricular end-diastolic pressure from 13±2 to 7±1 mm Hg. Pulmonary arterial mean pressure fell from 15±1 to 12±1 mm Hg. Increases occurred in heart rate from 83±4 to 109±5 beats/min, cardiac index from 2.7±0.2 to 4.0±0.4 liters/min per m^2, and in LV dp/dt from 1229±56 to 1461±105 mm Hg/sec. Maximal changes occurred by 60–90 sec with return to control values within 5 min. Right ventricular pressure and tension time index showed no significant change. Similar changes occurred in the 6 normal subjects. In 12 patients known to develop angina during right atrial pacing, AN given 3 min before pacing did not prevent angina. When administered during pacing-induced angina, AN relieved chest pain in 4 of 12 patients and resulted in hemodynamic changes similar to those during sinus rhythm. AN produced a rise in coronary sinus P_{O_2} from 22±1 to 32±4 mm Hg at 30 sec with a return to control values at 3 min. The observed hemodynamic response to AN would have differing effects on the balance of myocardial oxygen and requirements, which may explain its unpredictable therapeutic results. (Work supported by grant OHF 2-13 from the Ontario Heart Foundation and grant MA-3062 from the Medical Research Council of Canada.)

53. A New Rubella Antigen: Appearance and Persistence in the Blood After Natural Infection or Vaccination. ROGER CAPPEL,* ANN SCHLUEDERBERG,* AND HARVEY LIEBHABER,* New Haven, Conn. (introduced by Dorothy M. Horstmann).

A precipitating antigen designated *rho* has been found in sera from persons infected with rubella virus. *Rho* is distinct from the precipitating antigens, *theta* and *iota*. It is identical with an antigen recognized by Liebhaber as a component of the nucleocapsid fraction of the rubella virus particle. Hyperimmune serum prepared against this purified fraction was used to demonstrate the presence of antigenemia after natural and vaccine-induced infection. Preliminary physicochemical investigations indicate that the antigen is associated with a small protein particle with sedimentation coefficient between 7S and 19S. Serial sera collected during a prospective study of an epidemic among military recruits were examined for *rho*. Preexposure and acute and convalescent specimens from two groups were tested: (*a*) 18 susceptibles with primary infection; and (*b*) 11 recent vaccinees who were HI antibody positive but developed inapparent reinfection on exposure to wild virus. Among the 18 susceptibles, 17 developed *rho;* it was detectable as early as 3 days after onset of disease. Seven of the vaccinees became *rho* positive as a result of immunization; three of the four remaining acquired it after natural reinfection. It is not known how long *rho* may circulate in the blood. It disappeared within 4–6 wk in the seven with clinical disease, but was still present in approximately one-third of those with inapparent infection 6 wk after the epidemic. It has been found as late as 1 yr after onset and was detectable in 5 of 21 "normal" rubella antibody-positive adults. The possible immunopathologic implications of chronic rubella antigenemia are yet to be determined. (Supported by grant PHS CC-00532-03.)

54. Activity of Antibodies to Cross-Reactive Antigens of Enterobacteria. PHILIP C. CARLING,* MARGARET A. JOHNS,* AND WILLIAM R. MCCABE, Boston, Mass.

Earlier studies have demonstrated that active or passive immunization of mice with an antigen (Re) to basal core lipopolysaccharide, shared by most Gram-negative bacilli, afforded significant protection against challenge with heterologous bacilli. Subsequent investigations showed that high titers of antibody to Re, but not O-specific antigen of the infecting organism, were associated with a decrease in the frequency of shock and death from Gram-negative bacteremia in man. The present studies were undertaken to identify the protective antigen of Re mutants more precisely and to evaluate the in vitro activity of antibody to Re against heterologous bacilli (*E. coli* and *K. pneumoniae*). Antibody to the lipid A portion of Re endotoxin was not required for protective activity. Re antisera without antibody to lipid A

afforded significant protection to mice ($P < 0.02$), and adsorption of Re antiserum with lipid A did not negate its protective activity. Bactericidal and opsonic activity of sera, free of O-specific antibody, from animals immunized with Re mutants of *S. minnesota,* was evaluated against heterologous smooth bacilli. Only equivocal bactericidal activity could be demonstrated, but Re antisera exhibited a significant opsonic effect against heterologous bacilli. Heterologous smooth bacilli opsonized with Re antisera were cleared significantly more effectively, in the mouse, after intraperitoneal or intravenous challenge than bacteria opsonized with normal control serum. Intraperitoneal clearance of *E. coli* opsonized with Re antiserum was 3-fold greater and intravenous clearance was 10-fold greater than after opsonization with control serum.

55. Clofibrate Reversal of Platelet Hypersensitivity in Hyperbetalipoproteinemia. ANGELINA CARVALHO,* RAYMOND VAILLANCOURT,* RAE CABRAL,* ROBERT W. COLMAN, AND ROBERT S. LEES, Boston, Mass.

Since familial hyperbetalipoproteinemia (type II) is characterized by early death from thrombotic complications of atherosclerosis, we evaluated platelet function in 24 type II patients. 12 were receiving clofibrate (Atromid-S). Aggregation, nucleotide release, antiheparin activity, [^{14}C] serotonin release, coagulant phospholipid, and clot retraction were measured. In 20 normal subjects, minimum concentrations for full aggregation response were: ADP 1.7±0.18 μM (mean± SEM), *l*-epinephrine 3.9±0.87 μM, collagen 149±12 μg/ml. Significantly increased sensitivity ($P < 0.01$) was noted in untreated patients: ADP (0.51±0.10), *l*-epinephrine (0.07± 0.04), and collagen (33±6.3). Treatment with clofibrate reverted aggregation to normal with ADP (1.7±0.38) and towards normal with *l*-epinephrine (1.8±1.0) and collagen (63±12). Nucleotide release in 12 normal individuals was: ADP (1.2±0.25), *l*-epinephrine (2.5±0.05), and collagen (2.7±0.43) μmoles/10^{11} platelets. Untreated patients had significantly increased nucleotide release: ADP 6.6±1.4 ($P < 0.01$), *l*-epinephrine 8.4±1.4 ($P < 0.01$), and collagen 11± 2.6 ($P < 0.05$). Clofibrate treatment returned nucleotide release towards normal with *l*-epinephrine and collagen ($P < 0.01$), but not with ADP. Three patients with increased platelet sensitivity and release were followed during clofibrate treatment. Platelet aggregation and nucleotide and [^{14}C] serotonin release reverted towards normal at 6 wk. No significant differences were found in treated or untreated patients in antiheparin activity, clot retraction, and coagulant phospholipid. Therapeutic concentrations of clofibrate (100–200 μg/ml) in vitro decreased sensitivity and [^{14}C] serotonin release of normal platelets and reduced type II platelet sensitivity towards normal. Heightened platelet sensitivity may be important in the pathogenesis of clinical complications of hyperbetalipoproteinemia. Platelet abnormalities are restored towards normal by clofibrate both in vivo and in vitro. These observations may explain the decrease in incidence of atherosclerotic complications in patients treated with clofibrate. (Research supported by NIH SCOR grant HL14209.)

56. Connective Tissue Activation: Progress in Isolation of a Connective Tissue-Stimulating Peptide. C. WILLIAM CASTOR,** Ann Arbor, Mich.

A connective tissue-activating peptide (CTAP) extracted from human cells induced increased metabolic activity (activation) in cultured human synovial cells resembling that in rheumatoid synovitis. The hypermetabolic behavior induced by the polypeptide material included accelerated formation of hyaluronic acid, formation of lower molecular weight hyaluronic acid, increased consumption of glucose, and increased production of lactic acid. Previous studies indicated that the active principle was protein in nature. Gel permeation chromatography suggested a molecular weight between 4000 and 10,000. While earlier studies utilized CTAP extracted with a thiol-rich neutral buffer, recent experiments have shown a significant advantage in extraction with 0.1 M glycine buffer, pH 2.2, containing 0.1% β-mercaptoethanol. The use of an acidic thiol-rich buffer not only extracts the biologically active principle, but excludes a large portion of inactive protein and leads to sharper fractionation on gel permeation columns. Treatment of the acidic extract with 2 vol of chloroform:methanol (2:1) removes approximately 80% of the protein without major loss of biologic activity. Application of CTAP preparations to Sephadex DEAE-A25 equilibrated with 0.02 M phosphate buffer, pH 6.5, containing 0.05 M NaCl and 0.1% β-mercaptoethanol, removes variable (50–80%) amounts of contaminating protein but does not bind CTAP activity. CTAP was tightly bound to columns of Sephadex CM-C25 and was eluted at 0.02 M phosphate, pH 7.4, containing 0.35 M NaCl. This treatment resulted in a 3-fold increase in the specific activity of the connective tissue-activating peptide. Examination of these preparations on polyacrylimide gel revealed six distinct fractions dominated by a cathodal protein staining band. (This study was supported by USPHS grant AM-10728.)

57. Bone Marrow Sinus Cell Packing As a Determinant of Reticulocyte Release. JACK K. CHAMBERLAIN,* LEONARD WEISS,* AND ROBERT I. WEED, Rochester, N. Y., and Baltimore, Md.

For blood cells to enter the general circulation, transit through the marrow sinusoidal wall must occur. This study was undertaken to investigate whether ultrastructural changes in the marrow accompany erythropoietin (EP)-induced erythroid proliferation. However, in comparing different assay animals it was found that hypertransfusion blunted the reticulocyte response induced by erythropoietin. Female white swiss mice, untransfused and hypertransfused with matched controls, were injected with erythropoietin (6 U per animal). Reticulocyte counts, corrected for differences in hematocrit, were obtained at intervals. Animals were sacrificed for electron microscopic study of the bone marrow at various times during the reticulocyte response. Reticulocyte counts (mean %±SE corrected to normal hematocrit) in hypertransfused animals were:

Time (*h*)	0	41	68	92
Control	0.0	0.0	0.02±0.02	0.0
EP	0.02±0.02	0.03±0.02	3.2±0.7	3.6±1.4

Reticulocyte counts in untransfused animals were:

Time (*h*)	0	27	51	75	98
Control	7.0±0.68	4.0±0.29	3.7±0.2	5.1±0.4	3.6±0.6
EP	6.8±0.45	4.0±0.12	13.4±1.4	18.7±1.4	19.1±2.8

In the hypertransfused EP-treated mice, electron microscopy of the marrow showed erythroid hyperplasia. However, there was a marked reduction in the number of reticulocytes in transit through the sinusoidal wall in comparison to untransfused EP-treated animals and the untransfused controls. Openings in the sinusoidal walls were associated with cells in passage and occurred rarely if at all without this association. Erythropoietin-induced reticulocytosis is significantly impaired in hypertransfusion. The combination of erythroid proliferation in the cords and packing of the sinusoids suggests that the effect of hypertransfusion on reticulocyte release is not via inhibition of erythroid proliferation, but

rather may be related to increased intrasinusoidal pressure from packing opposing the transmural movement of the reticulocytes. (Supported by USPHS research grant HE-06241.)

58. Isolation of the Messenger RNA for Steroid Hormone Response and Synthesis of Its DNA Complement. L. C. CHAN,* A. R. MEANS,* S. E. HARRIS,* AND B. W. O'MALLEY, Houston, Tex.

The chick has served as an excellent animal model for studying the mechanism of steroid hormone action since injections of estrogen and progesterone induce synthesis of the specific proteins ovalbumin and avidin, respectively. We have now isolated and partially purified the specific messenger RNA molecules for ovalbumin and avidin. These messenger RNA's were extracted and quantified using an in vitro heterologous assay system containing ribosomes and translation factors from rabbit reticulocytes. During estrogen-mediated growth of oviduct target tissue, production of ovalbumin was always preceded by prior induction of ovalbumin mRNA. Single injections of estrogen to chicks previously withdrawn from all hormone led to rapid increases (1–3 h) in ovalbumin mRNA, which preceded increases in the *rate* of ovalbumin synthesis. The half-life of the mRNA (~6–9 h) corresponded to the $t_{\frac{1}{2}}$ for cessation of ovalbumin synthesis. After partial purification (90%) of ovalbumin mRNA, reverse transcriptase enzyme (AMV) was utilized to make complementary [^{3}H] DNA copies from the messenger RNA. Hybridization experiments were then employed to determine the number of structural genes in chick DNA coding for ovalbumin. A limited number (possibly one) of such genes appeared to exist in each haploid genome. Thus, this single gene must be transcribed numerous times to account for the high rate of ovalbumin synthesis (> 60% of total protein) in oviduct. Additional experiments with another steroid hormone (progesterone) have confirmed the generality of this proposed mechanism in that progesterone inducton of the oviduct protein avidin also follows prior increases in specific avidin messenger RNA. In summary, results with both estrogen and progesterone indicated that the rate-limiting event in sex steroid hormone induction of specific target-cell proteins is the hormone-mediated intracellular accumulation of their specific mRNA's.

59. Hemoglobin Okaloosa (β48 (CD7) leu → arg): an Unstable Hemoglobin with Decreased Oxygen Affinity. SAMUEL CHARACHE, BERNADINE BRIMHALL,* PAUL F. MILNER,* ESTHER E. GAYLE,* AND LESLEY J. COBB,* Baltimore, Md., and Portland, Ore.

A Caucasion technologist discovered that she was a carrier of a slow-moving hemoglobin. She was not anemic, but her reticulocyte count was 3.6% and her red cell survival was shortened (72 days, normal 116). Oxygen affinity of her blood was somewhat decreased (p50 30 mm Hg), but red cell 2,3-DPG was normal (4.7 μmoles/ml RBC). Several family members also had elevated reticulocyte counts; the abnormal hemoglobin (Hb Okaloosa) comprised 32–36% of their hemolysates. Hb Okaloosa was more unstable than Hb Hasharon and less unstable than Hb Zurich after incubation in 0.1 M phosphate buffer at 55°C, or in 17% isopropanol at 37°C. Oxygen affinity of "stripped" Hb Okaloosa was slightly decreased (p50 0.93 mm Hg, Normal 0.68); this difference was exaggerated by the presence of 2,3-DPG (4×10^{-4} M DPG: Okaloosa 4.0 mm Hg, normal 2.3). Heme-heme interaction and the Bohr effect were normal. Position CD7 is occupied by leucine in the α-, β-, δ-, γ-, and myoglobin chains of man, and in the α- and β- chains of a wide variety of other vertebrates. Although the mechanism is unclear, replacement of leucine by arginine in this "invariant" portion of the molecule appears to produce minor, but widespread, derangement of function. (Research supported by NIH.)

60. Adenosine 3,5-Monophosphate in Pancreatic Islets: Effect of Glucose, Theophylline, and Tolbutamide. M. ARTHUR CHARLES,* JANUARY LAWECKI,* AND GEROLD M. GRODSKY,* San Francisco, Calif. (introduced by Alan Goldfien**).

Indirect studies have strongly implicated cAMP as an important intermediate involving glucose-induced insulin release. However, several reports have indicated that glucose does not alter islet cAMP. Total cAMP can be estimated in islets after perifusion, thus permitting simultaneous evaluation of insulin release patterns. Using this technique, a 2.5-fold elevation of islet cAMP, after a 2 or 20 min stimulation by 16.7 mM glucose, is observed. During 20-min stimulations, maximal insulin release is achieved at 16.7 mM glucose, although cAMP levels are further elevated by 27 mM glucose. When 10 mM theophylline is added to maximal glucose-induced insulin release, secretion and cAMP are markedly augmented when compared to glucose alone, yet cAMP values are not different from theophylline alone. Theophylline alone induces only small amounts of insulin release, yet cAMP is elevated 4-fold. Tolbutamide alone also elevates islet cAMP levels at both 2 and 20 min, but in addition induces first-phase insulin release, whereas theophylline does not. The effect of tolbutamide on second-phase glucose-induced insulin release is also less striking than with theophylline. These studies support the following hypotheses. (*a*) cAMP is not a direct mediator of glucose-induced insulin release, and at a glucose levels exceeding 16.7 mM cAMP is distinctly dissociated from release. (*b*) Total islet cAMP appears to be a regulatory metabolite influencing glucose-induced insulin release both at submaximal and maximal glucose levels. (*c*) Theophylline and tolbutamide probably stimulate secretory processes by different mechanisms, since they induce different patterns of release even though they both elevate cAMP. (Grant aid from NIH, Kroc Foundation, and Hoechst Co.)

61. Renal Perfusion in Acute Renal Failure: Angiotensin-Mediated Cortical Vasoconstriction Reversed by a Competitive Antagonist. WILLIAM R. CHENITZ,* ALBERT MIMRAN,* NORMAN K. HOLLENBERG,* AND JOHN P. MERRILL,** Boston, Mass. (introduced by Kendall Emerson, Jr.**).

Several observations suggest a role for the renin-angiotensin system in the pathogenesis of acute renal failure (ARF). We assessed this possibility with a specific competitive antagonist to angiotensin (1-sar-8-ala angiotensin II) in a rat ARF model. Normal renal blood flow, assessed with the labeled microsphere method in 10 rats was 3.8±0.3 ml/g per min, representing 14.4±0.9% of cardiac output. Microspheres were confined to the renal cortex. 24 h after $HgCl_2$ (5 mg/kg subcutaneously) the BUN had risen to 88±4.1 mg/100 ml in nine rats. Renal blood flow was reduced to 2.4±0.4 ml/g per min ($P < 0.01$), representing 11.0±1.4% of the cardiac output. Within 20 min of initiating the infusion of the antagonist (1 μg/kg per min intravenously), renal blood flow rose to 3.1±0.3 ml/g per min (n = 8), not significantly lower than control. The percentage of cardiac output to the kidneys (15.8±1.3) exceeded control. More prolonged infusions will be required to assess the functional consequences of improved cortical perfusion, but

the studies suggest that angiotensin mediates the cortical ischemia of ARF.

62. Salmonella Septicemias in New York City, 1962–1971. CHARLES E. CHERUBIN,* HAROLD C. NEU, PASCAL IMPERATO,* AND NEIL BELLEN,* New York.

Clinical observation of repeated episodes of septicemia due to different Salmonella serotypes in several patients prompted us to review the natural history of this disease. All bacteriologically proven cases of Salmonella septicemia (412 cases) in New York City from 1962 through 1971 were analyzed with regard to age distribution, underlying disease, association of specific serotypes with certain diseases, clinical presentation, and fatality. *S. typhimurium* was the serotype most frequently encountered for the entire period, but it has declined significantly in the last few years. *S. enteritidis* now accounts for 35–40% of septicemias, *S. heidelberg* for 5%, and *S. choleraesuis* occurred only five times in the decade. *S. typhimurium* and *S. heidelberg* showed bimodal distributions with age, whereas *S. enteritidis* has no consistent pattern of age distribution. Minor serotypes occurred most in children. More than half the cases had no underlying disease. The underlying diseases were alcholic liver disease, carcinoma, lymphoma, and systemic lupus erythematosus. *S. typhimurium* was the serotype most commonly seen. *S. enteritidis* was more common in sickle-cell patients. There were 55 deaths (13.3%). In seven the septicemia was clearly the cause of death, and in 40 (9.7%) sepsis was a contributing factor. This study documents that the majority of Salmonella septicemias occur in individuals without underlying disease. *S. choleraesuis* infection is rare. Repeated episodes of septicemia over several years were well tolerated by individuals with severe underlying disease, indicating that excessive attention has been given to the rare fatal cases. Salmonella septicemia in patients with ultimately or rapidly fatal disease has a low fatality rate compared with septicemia due to other Gram-negative organisms.

63. Interrelationship of Colony-Stimulating Factor (CSF) and Colony-Forming Cells (CFC) in Blood and Marrow During Marrow Regeneration. PAUL A. CHERVENICK, Pittsburgh, Pa.

Colonies of granulocytes and mononuclear cells can be grown in vitro from blood and marrow of animals and man in the presence of a colony-stimulating factor (CSF). CSF, considered a regulator of granulopoiesis, is present low levels in normal plasma and is increased in animals injected with vinblastine. This report describes the interrelationship of changing plasma CSF levels and blood and marrow CFC during marrow regeneration. Adult dogs were injected intravenously with 0.15 mg/kg vinblastine. Blood and marrow were cultured daily thereafter for determining CFC. Nucleated cells, suspended in 1.5% methylcellulose, fetal calf serum, and McCoy's 5A medium, were incubated for 7 days at 37°C in 7.5% CO_2. Stimulation of colony formation was by a feeder layer of human leukocytes. Plasma CSF was measured at daily (or more frequent) intervals by determining its ability to stimulate colony formation from murine marrow. All animals became neutropenic. Increased CSF levels, ranging between 600 and 1200% of controls, were present 4–7 days after vinblastine. CFC in blood increased from between 10 and 80/ml in controls to 600–1440/ml between days 4 and 7 and closely followed the increase in CSF. Marrow CFC decreased from $30\pm2.7/10^5$ nucleated cells in controls to $3.5\pm0.8/10^5$ cells by day 2. This was followed by a marked increase of 300–800% of controls between days 5 and 7, followed by a significant decrease below normal through day 13. The increase in plasma CSF and its close relationship to marrow CFC and subsequent blood neutrophilia suggests that CSF and marrow CFC are intimately related. The delayed increase in blood CFC suggests that it is in direct response to the increase in CSF. The decrease in CFC during neutrophila suggests this compartment is under physiologic regulation.

64. Return of Leukocyte Alkaline Phosphatase in Chronic Myelocytic Leukemia Marrow Cells Cultured in a Diffusion Chamber System. G. CHIKKAPPA,* W. R. BOECKER,* A. L. CARSTEN,* E. P. CRONKITE,** AND S. OHL,* Upton, N. Y.

Bone marrow from chronic myelocytic leukemia (CML) patients was cultured in diffusion chambers (DC) implanted in murine peritoneum. Cultured cells were positive for Phl chromosomes. Murine hosts received whole body radiation (LD 100/30). Cellularity, leukocyte alkaline phosphatase (LAP) of the segmented neutrophils (PMN's), and chromosome karyotyping were determined from serially removed chambers' contents. Total cellularity rose by 1.8–2.25 by days 3–5. From peak levels, the cell count decreased by day 20 to 0.1–0.48 of time zero. Differential counts showed an orderly maturation in neutrophilic cells commencing in myeloblasts and terminating in PMN's. LAP was absent or low ($< 4\%$ cells were positive) in the inoculate. The enzyme rose ($> 80\%$ cells became positive) above normal level and fell with the rise and fall of the PMN counts in cultures. These results indicate that the CML marrow neutrophilic cells proliferate and mature in an orderly fashion. The leukemic cells are capable of LAP synthesis under the culture conditions. It is unlikely that only the LAP-positive normal and not the enzyme-negative leukemic cells proliferated in the cultures, because the Phl chromosome, a marker for CML cells, persisted in the cultures for 21 days. A diffusible factor released from the host might be responsible for neutrophil growth as well as LAP synthesis in the cultures. Persistence of the chromosome in cultured cells strongly implies that the abnormality is maintained in the stem cell and transmitted to discernible neutrophilic cells during differentiation. (Research supported by the U. S. Atomic Energy Commission and the Leukemia Society of America, Inc.)

65. Estrogen-Androgen Imbalance in Men with Hepatic Cirrhosis. INDER J. CHOPRA,* DAN TULCHINSKY,* AND FRANK L. GREENWAY,* Los Angeles, Calif. (introduced by D. H. Solomon **).

We studied, by radioimmunoassay, the serum concentrations of estradiol-17β (E2), testosterone (T), dihydrotestosterone (DHT), and gonadotropins in 13 men with hepatic cirrhosis, 6 of whom had gynecomastia. The mean ($\pm$SE) serum T of 234 ± 50 ng/dl in cirrhotics was significantly lower than that of 568 ± 51 in normal men of comparable age. The concentration of unbound T was subnormal in 12 of 13 patients. Serum DHT levels were diminished in 5 of 6 patients in whom it was measured. The serum E2 was elevated in 60% of cirrhotics; the mean value of 4.7 ± 0.49 ng/dl was significantly higher than the normal mean of 2.7 ± 0.21. The unbound E2 was increased in 10 patients. The mean serum LH was significantly higher in cirrhosis than in the normal men, but FSH was not significantly elevated. The values of serum T, E2, and gonadotropins in cirrhotics with gynecomastia were not significantly different from those in patients without gynecomastia. The most striking abnormality and the one that was uniformly present in cirrhosis was a supranormal ratio of serum unbound E2 to unbound T.

This ratio was 3- to 51-fold (mean 19) greater than normal in the presence of gynecomastia and 2- to 12-fold (mean 6) greater than normal in its absence. These data indicate that gynecomastia in cirrhosis may be related to hypoandrogenization as well as to true feminization. (Research supported by grants from NIH.)

66. Myoneural Specialization at the Esophagogastric Junction in Three Species. JAMES CHRISTENSEN, JEFFREY CONKLIN,* AND BARRY FREEMAN,* Iowa City, Iowa.

Length-tension curves and motor responses to electrical field stimulation were examined in vitro in serial 2-mm-wide transverse muscle strips from the esophagogastric junctional region in cat, rhesus, and opossum. Stimuli were rectangular pulses at 200 ma, 0.5 msec, and 10 cps in 10-sec trains. A region at the junction (10.4 mm long in five cats, 14.4 mm in five rhesus, 7.2 mm in five opossums) relaxed during stimulation. Distal to that region, strips contracted during stimulation; proximal to the relaxing region, all strips contracted briefly after the stimulus train (the "off-response"). Tetrodotoxin, 10^{-7} M, abolished all responses. Length-tension curves of strips from the junctional zone were steeper than those from above and below it. Slopes were not affected by tetrodotoxin and correlated poorly with muscle mass per unit length ($r = -0.23$ to $+0.91$, $n = 5$). An inhibitory innervation and a steep length-tension curve characterize the mammalian esophagogastric junction. These properties may define the lower esophageal sphincter. (Research supported by grant from NIH.)

67. Elevated Plasma Myoinositol in Uremia and in Experimental Neuropathy. REX S. CLEMENTS, JR.,* P. V. DE JESUS,* AND ALBERT I. WINEGRAD,** Philadelphia, Pa.

Myoinositol is a normal, low molecular weight, dialyzable plasma constituent, and is a substrate for membrane phosphoinositide synthesis. The kidney is the major site of myoinositol catabolism in rats, and nephrectomy abolishes its oxidation to CO_2. In nomal human adults, fasting plasma myoinositol averaged 21.6±1.1 μM by gas-liquid chromatographic analysis, whereas in uremics (BUN > 100 mg/100 ml) it was 423±27.2 μM ($P < 0.001$). In 76 normals and uremics there was a linear correlation between plasma myoinositol and BUN ($r = +0.94$). A dialyzable plasma component has been postulated to contribute to the pathogenesis of uremic neuropathy since efficient hemodialysis may prevent its progression. Decreased sensory and motor nerve conduction velocity (MNCV) may preceed clinical neuropathy. Feeding experiments have been successfully employed to demonstrate the effects of elevated plasma levels of D-galactose and D-xylose in rats. Therefore, normal rats were fed diets containing 35% myoinositol to examine the effects of elevated plasma levels of this cyclic polyol. Plasma myoinositol increased from 62.3±8.2 to 566.3±75.6 μM after 1 wk, and sciatic nerve MNCV decreased from 62.9±1.8 to 38.6±1.5 m/sec ($P < 0.001$). This decrease is much more rapid than that observed with vitamin-deficient diets. Rats fed myoinositol exhibited reduced weight gain; however, similar growth curves occurred in rats fed 35% sorbitol whose MNCV remained normal (62.7±1.8 m/sec). 4 wk of myoinositol feeding resulted in signs of clinical neuropathy, including tail drop and lower extremity muscle wasting. These observations suggest that myoinositol deserves serious consideration as the putative plasma factor in the pathogenesis of uremic neuropathy. (Research supported by grants from NIH.)

68. The Effect of Human Light (Kappa) Chains on Function and Morphology of the Rat Kidney. D. S. CLYNE,* E. BJENSTRÜP,* M. R. FIRST,* A. J. PESCE,* M. E. LEVY,* AND V. E. POLLAK,** Chicago, Ill., and Cincinnati, Ohio.

Bence Jones proteins may cause renal disease in man and have been shown to affect the metabolism of kidney tissue slices. We report in vivo studies designed to explore the effect of Bence Jones proteins on renal function and morphology. After two 24-h control periods, 300 mg of human kappa chain, isolated from urine of a patient with IgA myeloma and renal failure, was injected intraperitoneally into 12 female Sprague-Dawley rats. Urine total protein, human kappa chain, rat albumin (radioimmunoassay), alpha amino nitrogen, sodium, potassium, chloride, phosphate, glucose, and osmolality were measured on urine collected at 6, 12, and 24 h after injection, and daily thereafter. Pairs of rats were sacrificed at periods from 6 to 7 days after injection. Renal cortical tissue was studied by light and electron microscopy and by immunofluorescence using freeze substituted, 0.5-μ sections. Of the injected kappa chain, 3% was excreted in 6 h, 13% in 12 h, and 22% in 24 h; excretion was unmeasurable thereafter. Rat albumin excretion increased ($P < 0.01$) in the first 24 h. Total protein excretion increased, but the other parameters measured were unchanged. Numerous kappa chain droplets were found in proximal tubular cells of all rats. With increase in time after injection they became fewer, larger, and more basal in position. By electron microscopy many cytosomes, containing electron dense crystalline material, were observed in the cells of the convoluted and straight proximal tubules. These results indicate that kappa chains crystallize within proximal tubular cells without producing obvious impairment of function. They also suggest that competitive protein reabsorption may occur. (Research supported by NIH grants AM-10314 and AM-12330.)

69. Preservation of Myocardial Function by Antegrade and Retrograde Flow in Collaterals. MICHAEL V. COHEN,* JAMES M. DOWNEY,* EDMUND H. SONNENBLICK, AND EDWARD S. KIRK,* Boston, Mass.

Coronary collaterals developing to supply ischemic myocardium transform the coronary system from an end-arterial to an anastomotic network. Thus the effect of progression of coronary artery disease and its surgical treatment will be influenced by preexisting collaterals. We explored the characteristics of the collateralized coronary bed in dogs 3–12 wk after occlusion of the left anterior descending (LAD) coronary artery. The main left coronary artery (LCA) and LAD distal to the occlusion were cannulated. Contractile force gauges were sewn to areas perfused by LAD and left circumflex (LCf) arteries. After acute coronary occlusion in animals without collaterals, the ischemic area rapidly became noncontractile. In contrast, contractility distal to a chronic occlusion was normal and could be decreased only by altering the balance between myocardial oxygen delivery and utilization, e.g., by decreasing LCA flow. Contractility depressed in this manner could be restored by reestablishing flow in the occluded vessel. After reestablishing LAD flow, subsequent acute occlusion of the LCA reduced, but did not eliminate, contractility in the LCf region. Retrograde flow in collaterals (LAD → LCf) sustained the LCf region so that the LAD now supplied the entire myocardium. With either antegrade (LCf → LAD) or retrograde collateral flow, intracoronary nitroglycerin improved ventricular performance and augmented peripheral coronary pressure and contractile force in the ischemic area. We conclude that nor-

mal antegrade as well as retrograde collateral perfusion can limit the extent of regional ischemia and preserve myocardial function. In a heart with collaterals, revascularization procedures would thus be expected to favorably affect function in local as well as adjacent vascular beds. (Research supported by grants from NIH.)

70. Decreased Synthesis of C3 in Membranoproliferative Glomerulonephritis. HARVEY R. COLTEN,* RAPHAEL H. LEVEY,* FRED S. ROSEN, AND CHESTER A. ALPER, Boston, Mass.

Metabolic studies of the third component of complement (C3) in patients with membranoproliferative glomerulonephritis (MPGN) have suggested that decreased C3 biosynthesis often contributes to depressed serum levels of C3. Other authors have claimed that the decrease in serum C3 associated with MPGN is solely a consequence of increased C3 catabolism. The present study was undertaken in an attempt to resolve this controversy. Recent advances now permit a direct study of C3 biosynthesis in vitro. These studies have confirmed that the liver is a principal site of C3 biosynthesis in that short-term cultures of liver produced biologically active C3, production of C3 was temperature dependent and reversibly inhibited by cycloheximide, and [^{14}C]amino acids were incorporated into C3 protein (β_{1c}). Liver biopsies were obtained from patients with MPGN, patients with other hypocomplementemic renal diseases, and controls (patients without renal disease). In general, rates of biosynthesis of C3 by liver specimens in vitro corresponded to synthesis rates calculated from metabolic turnover studies. Liver samples from two patients with MPGN failed to produce detectable C3 in vitro, although they were capable of synthesizing C2 and C5. Studies of the metabolic turnover of radiolabeled C3 in these two patients also indicated a depression of C3 biosynthesis. The rate of C3 synthesis did not correlate with the serum concentration of C3. Preliminary data suggest two possible mechanisms for decreased C3 biosynthesis in MPGN: a relative deficiency of a normal heat-stable serum factor that stimulates C3 biosynthesis, and an inability of the liver from patients with MPGN to respond to this C3-stimulating factor. (Supported by USPHS grants HD-05916, AI-05877, AM-13855, and AM-16392.)

71. Effect of Changes in Plasma Potassium on Plasma Aldosterone Concentration in the Absence of Changes in Potassium Balance. C. ROBERT COOKE,* JOHN S. HORVATH,* MICHAEL A. MOORE,* AND W. GORDON WALKER,** Baltimore, Md.

The regulatory mechanism responsible for the recently demonstrated correlation between plasma aldosterone (PA) and plasma [K^+] in anephric individuals when K^+ is altered by hemodialysis or by K^+ accumulation between dialyses remains unclarified. To determine whether changes in plasma [K^+] could effect such changes in plasma aldosterone in the absence of changes in K balance, we studied seven anephric patients during infusions of glucose and insuin before hemodialysis. Changes in plasma aldosterone and plasma [K^+] measured before and at 60, 120, and 180 min after infusions of glucose and insulin were as follows:

	Before	60 min	120 min	180 min
[K^+]	5.9±0.3	4.6±0.4	4.2±0.5	4.5±0.4
PA ng/100 ml	4.8±1.2	2.6±1.0	1.6±0.4	2.2±0.5

Changes in plasma aldosterone and plasma [K^+] were significantly correlated ($r = 0.516$; $P < 0.01$), with no significant change in plasma [Na^+]. Differences observed at 60 min after beginning the glucose and insulin infusion were significant for both K and aldosterone ($P < 0.005$ and $P < 0.01$, respectively). These studies demonstrate an exquisitely sensitive control mechanism for the regulation of plasma aldosterone in anephric patients which responds to changes in plasma [K^+] in the absence of changes in K^+ or Na^+ balance. The decline in plasma [K^+] in these studies reflects net transfer of K^+ into cells, but it cannot be inferred that the intracellular K^+ concentration within the cells of the adrenal cortex necessarily increases. The prompt modulation of plasma aldosterone in these studies suggests a homeostatic mechanism that is capable of responding rapidly to changes in plasma [K^+]. (Supported in part by NIH grants HL3303 and RR35.)

72. Serum Lipoproteins Alter Red Cell Membrane Structure. RICHARD A. COOPER,* ELIZABETH C. ARNER,* AND JAMES S. WILEY,* Philadelphia, Pa. (introduced by Arnold S. Relman **).

Serum lipoproteins with an increased mole ratio of free cholesterol to phospholipid (FC/PL) occur in patients with cholesterol-rich "spur" red cells and liver disease. To test the hypothesis that lipoprotein FC/PL composition is responsible for abnormalities of membrane structure, normal lipoproteins, whose lipid composition was altered with sonified free cholesterol-dipalmitoyl lecithin dispersions, were incubated for 24 h with normal red cells. In low FC/PL environments (FC/PL = 0.1) membrane FC selectively decreased (−60%), and in high FC/PL environments (FC/PL = 2.2) it was selectively increased (= 110%), leading to membrane FC/PL mole ratios of 0.4–2.0 (normal = 0.95). Cholesterol-depleted cells appeared spheroidal in wet preparation with cell diameters decreased to 6.5 μ (normal = 7.5 μ) but no change in cell volume. Their surface area (calculated from mean hemolytic volume) was decreased 25% and their deformability (filterability through 8-μ Millipore filters) was markedly impaired. In contrast, the periphery of most cholesterol-rich cells appeared folded (in wet preparations) or spiculated (on dried smears), and nondistorted cells were flat with diameters of 12.0 μ. Their surface area was increased 23%; however, their deformability was decreased. Osmotic fragility, an indirect measure of surface area, correlated closely with membrane FC/PL over the entire range studied ($r = 0.93$). Na influx in cholesterol-depleted cells was twice normal, resulting in a doubling of intracellular Na concentration and a 50–75% increase in both active Na efflux and active K influx. In contrast, the Na concentration and the rates of Na and K flux in cholesterol-rich cells were normal. These studies demonstrate that lipoprotein composition directly affects red cell membrane structure, shape, and permeability, and they support the concept that abnormal lipoprotein composition plays an etiologic role in the genesis of cholesterol-rich spur cells in liver disease.

73. Antigen-Binding Cells in Immune and Tolerant Mice. SIDNEY R. COOPERBAND AND MARY JANE BENFARI,* Boston, Mass.

It is now generally accepted that antigen recognition by lymphocytes occurs at the cell surface by antigen-binding receptors. Using a system which allows equilibrium conditions, we have attempted to study the interaction of antigen with cell surface receptors during immunization and tolerance induction. This system utilizes 10^8 murine node or spleen lymphocytes in suspension and varying concentrations of radioactive soluble antigen. The procedure involves sampling first a given cell suspension and then, after sedimentation, the cell-free supernatant. The cell-bound antigen is calculated

from the difference between the radioactivity of the cell suspension and supernatant samples. Kinetic studies demonstrate that equilibrium is achieved within minutes of antigen addition. Metabolic studies at 4°C and at 37°C demonstrate binding at both temperatures; however, a turnover of antigen occurs at 37°C with 70–90% loss of antigen from the cells within 18 hr. Experiments with increasing antigen concentration permit an estimate of average antigen-binding avidity and maximal quantity of antigen bound per cell. We have followed these characteristics of antigen-binding cells during immunization to bovine serum albumin (BSA). Antigen-binding cells first appear in node and spleen 4 days after antigen administration; maximum antigen binding was found 11 days later. Binding cells were detected in large numbers for longer than 6 months. The kinetics of disappearance of antigen-binding cells suggest at least two different cell populations with different half-lives. Similar studies have been performed with mice rendered tolerant to high doses of BSA. Lymphoid cells from these animals develop antigen-binding capabilities similar to immune mice despite an absence of antibody formation. (Supported by grants from the NIH.)

74. Evaluation of Crystalline Subunit Adenovirus Vaccines. ROBERT B. COUCH, JULIUS A. KASEL,* HELIO G. PEREIRA,* ASHLEY T. HAASE,* AND VERNON KNIGHT, Houston, Tex.

Recent crystallization of hexon and fiber antigens of adenovirus provided the opportunity to examine the immunogenicity in man of subunits of the virion. 18 normal adult volunteers were given two intramuscular doses of 100 μg of adenovirus type 5 hexon, 12 were given 200 and then 100 μg of fiber, and 19 were given saline. Vaccines were essentially nonreactogenic. Serum antibody responses occurred in 60% of men given hexon and 100% of those given fiber. 42 of the 49 vaccinees were challenged intranasally with live adenovirus type 5. 16 of 19 controls, 11 of 15 hexon vaccinees, and 4 of 8 fiber vaccinees exhibited virus shedding and/or antibody response. 10 of 19 controls developed febrile pharyngitis, whereas none of the fiber vaccinees developed illness ($P < 0.02$). 3 of 15 hexon vaccinees developed illness; only one had fever. Two of the three had no detectable serum antibody at the time of challenge. Febrile illness in hexon vaccinees with preexisting antibody were significantly less than in controls ($P = 0.01$). These studies show that adenovirus type 5 crystalline subunit vaccines are essentially nonreactogenic, antigenic, and capable of producing significant protection against live virus challenge. Such preparations have significant advantages in that they should not be tumorigenic and are free of proteins not needed for production of immunity. Further studies of these vaccines are indicated, particularly in pediatric age groups, for the possibility of general use.

75. Essential Amino Acids in Plasma During Administrations of Their α-Keto-Analogues in Renal Failure and in Portal-Systemic Encephalopathy. A. WILL COULTER,* WILLIS C. MADDREY,* AND MACKENZIE WALSER,** Baltimore, Md.

Keto-analogues of essential amino acids can partially substitute for dietary protein in chronic renal failure (CRF) (1973. *J. Clin. Invest.* **52**: 678), and their use has been proposed in portal-systemic encephalopathy (PSE). In order to determine their effect on plasma amino acids, repeated measurements were made in ten patients with severe CRF and four with PSE. A mixture of five keto-acids (analogues of valine, leucine, isoleucine, methionine, and phenylalanine) plus lysine, threonine, histidine, and tryptophan was given orally in CRF and intravenously in PSE. Initial measurements in CRF revealed significantly reduced valine and leucine, normal isoleucine, methionine, and phenylalanine, but increased phenylalanine:tyrosine ratio. Alloisoleucine was usually present though absent in controls. During keto-acid supplementation, alloisoleucine invariably rose (to ca. 20 μM), even though pure S-(-)-α-keto-β-methylvalerate was given. The probable explanation is spontaneous racemization of this compound, which proceeds slowly at pH 7.4 in vitro. Isoleucine tended to fall. Valine rose towards normal only when N balance was strongly positive. Leucine remained low and phenylalanine:tyrosine ratio high, even when amino acids were given instead of their keto-analogues. In PSE, initial values for valine and leucine were also low but methionine was increased and phenylalanine:tyrosine reduced; alloisoleucine was again usually present. At the end of the 3 h infusion, methionine and phenylalanine rose markedly, and valine, leucine, and isoleucine usually rose too. Alloisoleucine reached high levels, but subsequently subsided. The results show that oral keto-acids fail to normalize the aminogram in CRF, despite clinical improvement; intravenous keto-acids in PSE give rise to the corresponding amino acids plus (transiently) alloisoleucine.

76. Effects of Volume Expansion (VE), Purified Parathyroid Extract (PTE), and Ca^{++} on Renal Bicarbonate Reabsorption ($RHCO_3$) in the Dog. CHARLES K. CRUMB,* MANUEL MARTINEZ-MALDONADO,* GARABED EKNOYAN,* ANDRE N. MINUTH,* AND WADI N. SUKI, Houston, Tex.

Metabolic acidosis is associated with hyperparathyroidism and alkalosis with hypercalcemia. To investigate the role of parathyroid hormone (PTH) and Ca^{++} in the regulation of $RHCO_3$, studies were performed on HCO_3-loaded dogs. Volume expansion (VE) lowered $RHCO_3$ in both intact (23.7 to 21.6 mmoles/liter GFR, $P < 0.005$) and thyroparathyroidectomized (TPTX, 28.8 to 23.4 mmoles/liter GFR, $P < 0.02$) dogs; glomerular filtration rate (GFR), renal blood flow (RBF), fractional clearance of sodium (FCNa), and fractional clearance of chloride (FCCl) did not change significantly. Infusion of C^{++} raised ultrafilterable Ca^{++} from 5.3 to 8.5 mg/100 ml in intact and from 4.9 to 7.0 mg/100 ml in TPTX dogs; $RHCO_3$ increased from 23.0 to 27.1 mmoles/liter GFR ($P < 0.05$) in intact and from 26.6 to 28.3 mmoles/liter GFR ($P < 0.05$) in TPTX dogs. The FCNa, FCCl, and $FCCa^{++}$ did not change in either group. GFR fell in TPTX dogs (35.4 to 29.4 ml/min, $P < 0.05$) without a change in RBF. We conclude that (*a*) the lowering of $RHCO_3$ by VE does not depend on PTH; (*b*) PTE acts directly on the renal tubules to lower $RHCO_3$; (*c*) Ca^{++} enhances $RHCO_3$ and this effect is exerted in the absence of PTH and calcitonin; and (*d*) neither the effects of Ca^{++} nor those of PTH appear to be mediated by altered hemodynamics, although this cannot be excluded in Ca^{++}-infused TPTX dogs. (Research supported by grants HL 12209, HL 5963, and RR 5425 from USPHS.)

77. Collagen Synthesis in the Mammalian Lung. RONALD G. CRYSTAL,* Bethesda, Md. (introducted by Robert W. Berliner **).

The composition and synthesis of lung collagen was studied in newborn and adult rabbits and in 10- to 16-wk human fetuses. Partially purified lung collagen can be separated by carboxymethylcellulose (CMC) or polyacrylamide-gel chromatography into at least two chains (α_1, α_2) with approximate mol wt 100,000 for each. In a modified Dulbecco's medium (37°C, 95% O_2, 5% CO_2) the incorporation

of [^{3}H] proline or [^{3}H] glycine into collagen by lung slices was linear for more than 4 h. After incubation, slices homogenized in 0.5 M acetic acid were analyzed for labeled and unlabeled hydroxyproline, and collagen chains were separated by CMC or gel chromatography. Both human and rabbit lung slices actively synthesized α_1- and α_2-chains. A precursor of the α_1-chain, pro-α_1, converted to α_1 with longer incubations. The α_1- and α_2-chains synthesized contain [^{3}H] hydroxyproline and were selectively degraded by collagenase. The hydroxylation of [^{3}H] proline in lung collagen was completely inhibited by α,α-dipyridyl in concentrations which only partially inhibited noncollagen protein synthesis. Whereas newborn lung contains 50% less collagen than adult lung (per unit weight), the newborn lung synthesized collagen at a rate 10 times faster than adult lung. The ratio of collagen synthesis to noncollagen protein synthesis in the newborn was 5 times that in the adult. It is hoped that these techniques can be utilized to study the control of lung collagen synthesis in normal growth and in disease.

78. Membrane Receptors and the Mechanism of Action of Cholera Toxin. PEDRO CUATRECASAS, Baltimore, Md.

Highly radioactive and biologically active [^{125}I] cholera toxin has been used to study in detail the nature of the interaction of this toxin with intestinal and liver cell membranes and with intact adipose tissue cells. The toxin binds very rapidly, and the dissociation constant of the complex is estimated to be near 10^{-9} M. A maximum of 2×10^4 molecules of toxin can bind per fat cell in a saturation plateau which corresponds to the concentration range which is biologically active in these cells. The toxin has specificity for complex carbohydrate determinants, as illustrated by the ability to bind to certain glycoproteins and to gangliosides. The greatest affinity occurs with ganglioside G_{M1}, which is probably the natural membrane receptor. Exogenous ganglioside G_{M1} can be incorporated spontaneously into intact fat cells, and the ability of these cells to bind toxin and to generate lipolytic processes in response to toxin are greatly enhanced. This constitutes an experimental reconstitution of a modulator-receptor system and indicates that gangliosides are the true receptors for cholera toxin. The magnitude of the biological response is related to the number of toxin-ganglioside complexes at the cell surface. Other studies suggest that a major relocation or reorganization of the toxin-receptor complex within the structure of the membrane must occur before the toxin can perturb adenylate cyclase. Insoluble agarose-ganglioside derivatives can be used to purify cholera toxin by affinity chromatography. These adsorbents, as well as large, soluble polyaminoacid-ganglioside derivatives, can effectively extract free cholera toxin from solution and thus can act to prevent the effects of cholera toxin on cells. (Research supported by grants from NSF, NIH, and ACS.)

79. Chronic Idiopathic Neutropenia (CIN): Response to Prednisone Therapy. DAVID C. DALE,* DUPONT GUERRY IV,* AND SHELDON M. WOLFF, Bethesda, Md.

21 patients (aged 2–58 yr; 4 male, 17 female) with CIN (duration 4 months to 20 yr) had mean neutrophil counts of 527±92/mm^3 (range 25–1800) without anemia, thrombocytopenia, monocytopenia, lymphopenia, or splenomegaly. The patients had reduced marrow mature neutrophils ("granulocyte maturation arrest"). Bone marrow granulocyte reserve responses to endotoxin, cortisol, and etiocholanolone, as well as neutrophil accumulation in the cutaneous inflammatory responses, were reduced proportional to the blood neutrophil counts. DF^{32}P-labeled neutrophil half-lives were normal in the patients with the highest counts but were uninterpretable in most patients with counts less than 500/mm^3. Infections occurred rarely in these patients and generally no therapy was indicated. However, the two patients with the lowest neutrophil counts (frequently 0–50 neutrophils/mm^3) had intermittent fever and pyogenic infections. They responded to alternate-day prednisone therapy (50–100 mg) by a shift to normal marrow morphology, increased marrow reserve responses (mean before = 451±305, after = 1665±265). In the one patient studied, urinary colony-stimulating factor as measured by in vitro myeloid colony formation markedly increased with prednisone therapy. Compared to pretreatment, the cutaneous inflammatory responses improved both the day "on" and "off" prednisone. DF^{32}P-labeled neutrophil half-lives were normal or slightly prolonged, studied both days "on" and "off" prednisone (mean = 9.1±2.2 h, four studies). With prednisone treatment neither patient has had further fever or infection in 2 yr and 2 months follow-up, respectively. These studies indicate that (*a*) CIN is usually a benign disorder even with blood neutrophils of 100–500/mm^3; (*b*) alternate-day prednisone therapy can increase neutrophil counts in some of these patients; and (*c*) such patients may benefit clinically from the prednisone-induced increase in neutrophils.

80. Direct Assessment of Surface Glomerular Capillary Dynamics in Experimental Post-Ischemic Acute Renal Failure. T. M. DAUGHARTY,* I. F. UEKI,* AND B. M. BRENNER, San Francisco, Calif.

Using Wistar rats with surface glomeruli, we examined the mechanisms responsible for the fall in GFR in acute renal failure. Values for mean arterial pressure ($\overline{AP}$), single nephron (SN) GFR, filtration fraction (SNFF), and glomerular plasma flow (GPF), mean glomerular capillary ($\overline{P}_G$), proximal tubule (P_T) and peritubule capillary (P_C) hydrostatic pressures, afferent (π_A, R_A) and efferent (π_E, R_E) arteriolar oncotic pressures and resistances were obtained before (C) and after (E) 3 h of nearly complete occlusion of the left renal artery. Mean values during C, and mean differences (C − E), for 10 rats were: $\overline{AP}$ 110±4 SE and +0.3±3 mm Hg; SNGFR 28±2 and −11±1 nl/min ($P < 0.001$); GPF 74±7 and −27±2 nl/min ($P < 0.001$); $\overline{P}_G$ 46±1 and +1±2 mm Hg ($P < 0.5$); P_T 12±0.4 and +1±0.7 mm Hg ($P < 0.2$); π_A 18±0.5 and −1±0.6 mm Hg ($P < 0.2$); R_A 3.8±0.5 and $+2.6\pm0.6 \times 10^{10}$ dyne·sec·cm^{-5} ($P < 0.005$); and R_E 2.6±0.3 and $+2.2\pm0.7 \times 10^{10}$ dyne·sec·cm^{-5} ($P < 0.025$). Ischemic injury resulted in a fall in SNGFR averaging 41%. SNFF remained unchanged; therefore, the fall in SNGFR was accompanied by a proportional decline in GPF. $\overline{P}_G$, P_T, and π_A remained unchanged and $\pi_E/(\overline{P}_G - P_T)$ closely approximated unity, indicating filtration pressure equilibrium by the efferent end of the glomerular capillary during C and E. We conclude the following. (*a*) SNGFR fell solely as a consequence of the fall in GPF. (*b*) Since SNGFR equals ultrafiltration coefficient × mean ultrafiltration pressure, this product must also have fallen in proportion to ΔGPF. (*c*) GPF, but not $\overline{P}_G$, fell as a result of large increases in both R_A and R_E. (*d*) These changes in R_A and R_E play a fundamental role in the pathogenesis of this form of acute renal failure. (Supported by VA and NIH.)

81. Acute Effects of Insulin on Glucose Metabolism by Rat Liver Slices: Independence from Glucagon. MAYER B. DAVIDSON* AND JUDITH A. BERLINER,* Los Angeles, Calif. (introduced by J. Brown **).

Although the mechanism of the acute insulin effect on hepatic carbohydrate metabolism is largely unknown, it has

recently been postulated that insulin acts on the liver by competing with glucagon to influence cyclic-AMP levels. Insulin can depress hepatic glucose output by (*a*) stimulating glucose utilization, (*b*) inhibiting gluconeogenesis, (*c*) increasing glycogen synthesis, and/or (*d*) blocking glycogenolysis. We have studied insulin effects on these four separate pathways simultaneously in a simple liver slice system. High K^+ buffer (130 mM) was used in the glycogen studies and physiological K^+ buffer (5 mM) for the other two pathways. Basal glucose production and recovery of [^{14}C] glucose in CO_2 and glycogen were linear for at least 2 h. Insulin (1.0 U/ml) increased [^{14}C] glucose recovery in glycogen (50%), decreased glycogenolysis (23%), and had no effect on glucose oxidation to CO_2. Insulin also inhibited gluconeogenesis (μg glucose produced per 100 mg tissue per 2 h) from alanine (28%), glycerol (25%), and fructose (26%). Glucagon (10 μg/ml) neither affected basal or glycerol-mediated glucose production nor blocked insulin's ability to inhibit glycerol-stimulated gluconeogenesis. Therefore these data suggest that at least part of the acute insulin effect on liver does not involve competition with glucagon to influence cyclic-AMP levels. Liver slices from starved rats can provide a simple reproducible system to study the acute effects of insulin in hepatic carbohydrate metabolism. (Supported by grant from Diabetes Association of Southern California.)

82. Demonstration of Dual A-V Nodal Pathways with Paroxysmal Supraventricular Tachycardia. PABLO DENES,* DELON WU,* RAMESH DHINGRA,* AND KENNETH ROSEN,** Chicago, Ill. (introduced by Morton D. Bogdonoff **).

Dual A-V nodal pathways have been postulated to explain the occurrence of A-V nodal reentry in paroxysmal supraventricular tachycardia (PSVT). We have obtained evidence of this in three patients with PSVT, utilizing His bundle recording (H) and atrial stimulation. A_1 and H_1 were the atrial and H responses of basic beats. A_2 and H_2 were the responses to an atrial extra-stimulus inserted at decreasing coupling intervals. Plotting of H_1-H_2 responses against A_1-A_2 coupling intervals revealed similar curves in all patients. As A_1-A_2 decreased, H_1-H_2 decreased appropriately. At a critical A_1-A_2, a sudden marked increase in H_1-H_2 occurred, suggesting failure of a fast pathway (the fast pathway effective refractory period [ERP]). The shortest H_1-H_2 before sudden increase was the fast pathway functional refractory period (FRP). Further shortening of A_1-A_2 defined a second H_1-H_2 curve with flat or decreasing slope. The longest A_1-A_2 with no H_2 response was the slow pathway ERP. The shortest H_1-H_2 of the second curve was the slow pathway FRP. Fast pathway ERP and FRP were: 450 and 540 msec (patient 1); 305 and 415 msec (patient 2); and 280 and 325 msec (patient 3). Slow pathway ERP and FRP were, respectively: 340 and 820 msec, 265 and 495 msec, and 190 and 375 msec. All patients had echo zones which coincided with A_1-A_2 equal or less than the fast pathway ERP. These results provide the first direct demonstration of dual A-V nodal pathways in patients with PSVT, as manifest by dual A-V nodal conduction times and refractory periods. Antegrade failure of the fast pathway with subsequent availability for retrograde conduction allows A–V nodal reentry. (Supported by NIH contract.)

83. Antitrypsin Gene Frequencies in Healthy People. THOMAS A. DEW,* BIBBI ERADIO,* AND JOHN A. PIERCE,** St. Louis, Mo.

Antitrypsin deficiency is associated with emphysema and juvenile cirrhosis. The acid starch-gel-crossed immunoelectrophoretic technique of Fagerhol and Laurell identified a large number of phenotypes. This system has been called the proteinase inhibitor (Pi) system and is inherited as an autosomal codominant trait. Pi gene frequencies have been determined in Sweden and Norway but not in the United States. Confusion has existed because workers have determined phenotypes from measurement of antitrypsin concentrations and proteinase inhibitor capacities (a technique successful only for the classical ZZ deficiency). We have examined the serum of 2047 consecutive donors from the Barnes Hospital Blood Bank with a rocket immunoelectrophoresis to determine antitrypsin concentrations. In every case, acid starch-gel electrophoresis and crossed immunoelectrophoresis were performed to identify phenotypes. Old phenotypes were repeated at least once for confirmation. The gene frequency in this series was M 0.9480, S 0.0344, Z 0.0127, F 0.0027, I 0.0012. These results are similar to the 2830 Norwegians studied by Fagerhol for M (0.946), Z (0.016), and I (0.0012), but different for S (0.023) and F (0.0133). The F frequency is probably technical and unimportant. However, the difference in S alleles is significant. It may be a difference in the population studied, or could be an underestimation of the S alleles from the starch-gel inspection. Approximately 12% of the interpretations we made from careful inspection of the stained starch-gel proved inaccurate. 4% of the samples classified as MM contained antitrypsin concentrations less than 60% of our normal. While this study generally confirms the accuracy of previous works, it illustrates again the necessity for the two-stage technique in determining phenotypes. (Research supported by NIH grant NHLI 71-2218.)

84. In Vitro Synthesis and Secretion of Macromolecules by Human Fetal Intestine; Response to Viral Infection and Concanavalin A. R. M. DONALDSON, D. SERFILLIPI,* AND N. R. BLACKLOW,* Boston, Mass.

Direct examination of intestinal response to injury requires isolated tissue preparations which remain viable. Explants of human fetal intestine were maintained in organ culture for up to 3 wk with villous architecture intact and without fibroblastic degeneration. At intervals, explants were incubated with [^{3}H] thymidine, [^{14}C] leucine, or [^{14}C] glucosamine to measure incorporation into tissue DNA, protein, or glycoprotein, respectively. After cultures had been established for 72–96 h, incorporation of radioactive precursors and secretion of labeled protein and glycoprotein into the incubation medium was constant for 10–14 days. Preincubation with cycloheximide inhibited incorporation of [^{14}C] leucine and [^{14}C] glucosamine. The prolonged viability and steady synthetic activity of human fetal intestine in organ culture allowed us to examine the effects of (*a*) a virus known to infect intestinal mucosa and (*b*) a lectin known to agglutinate human fetal intestinal epithelial cells. As shown previously, infection of explants with echovirus 11 produced viral growth titers comparable to levels achieved with standard tissue cultures. Infected explants showed morphological deterioration and reduced incorporation of [^{3}H] thymidine and [^{14}C] leucine 4–7 days after inoculation. In contrast, [^{14}C] glucosamine incorporation into tissue and secreted glycoprotein was increased 2- to 3-fold. Concanavalin A, in concentrations which stimulate DNA synthesis in cultured lymphocytes, markedly suppressed DNA and protein synthesis by fetal intestine. Again, mucosal injury was associated with increased incorporation of [^{14}C] glucosamine to glycoprotein. These results demonstrate that in organ culture (*a*) human fetal intestine maintains morphologic integrity

and steadily synthesizes DNA, protein, and glycoprotein for 2–3 wk; (*b*) echovirus and concanavalin A suppress fetal intestinal growth and ultimately cause tissue damage; and (*c*) intestinal injury induced by two different agents is accompanied by diminished DNA and protein synthesis but by increased glycoprotein synthesis.

85. Histamine Responses of Guinea Pig Airways In Vivo and In Vitro. J. S. DOUGLAS,* A. J. LEWIS,* J. OREHEK,* AND A. BOUHUYS,** New Haven, Conn.

We have examined airway smooth muscle responses to histamine, in vivo in spontaneously breathing unanesthetized guinea pigs, and in vitro using spirally cut tracheas from the same animals. ED50 values to histamine in vivo varied 100-fold (0.01% w/v to 1% w/v histamine base) but varied only 4-fold in vitro (ED50 values 8.6×10^{-9} M to 3.5×10^{-8} M). This suggests that in vivo responses are modified by neural or humoral mechanisms, or both. Doses of isoproterenol which decreased histamine-induced muscle tension by 300 mg in vitro varied 1500-fold (8×10^{-14} M to 3.1×10^{-10} M) and were log normally distributed. The tracheas of animals sensitive to histamine in vivo were insensitive to isoproterenol in vitro and vice versa. Thus, variations in β-adrenergic responses appear to be, in part, responsible for variations in histamine responses in vivo. Histamine superfusion of spirally cut tracheas released a prostaglandin-like substance which contracted the rat stomach strip but not the rat colon (both treated with combined antagonists), suggesting PGE-like activity. Release of prostaglandin-like substances was inhibited by indomethacin (0.6 μg/ml). Indomethacin reduced responses to minimally effective doses of histamine and increased responses to submaximally effective doses. Thus, local release of prostaglandin-like substances may be an additional cause of variation in smooth muscle responses. In conclusion, variations of airway responses to bronchoconstrictor agents in vivo include effects of differences in (*a*) inherent sensitivity of airway smooth muscle cells to the agonist, (*b*) cholinergic/adrenergic balance, and (*c*) local release of prostaglandin-like substances. Modification of local humoral release or reduced β-adrenergic responses or both may produce a condition analogous to airway hypersensitivity in bronchial asthma. (This work was supported in part by USPHS grants HE-14534 and HE-14179.)

86. Reduced Activation of Vasopressin (ADH)-Sensitive Adenylate Cyclase in Mice with Hereditary Nephrogenic Diabetes Insipidus. THOMAS P. DOUSA* AND HEINZ VALTIN,** Rochester, Minn., and Hanover, N. H.

ADH-sensitive adenylate cyclase, cyclic AMP phosphodiesterase, and protein kinase were studied in vitro in a strain of mice with severe nephrogenic diabetes insipidus (so-called DI +/+ severe). Previous studies have suggested that the major defect in these mice lies in ADH-induced water permeability of the distal nephron (1972 *Kidney Int.* 1: 135). Adenylate cyclase activity was determined in 600 *g* sediments of homogenates of renal medullary tissue from 17 diseased mice and 23 controls (so-called VII +/+). Basal activity, as well as activity stimulated by various concentrations of ADH or of fluoride, was measured. There was no significant difference between diseased and control mice in either basal activity or the activity stimulated by fluoride. However, the adenylate cyclase activity stimulated by saturating concentrations of ADH was reduced by about 40% in diseased mice when compared to controls ($P < 0.05$). The two groups did not differ significantly in the concentration of ADH required to elicit a half-maximal response. Nor were there any significant differences in the activity of cyclic AMP phosphodiesterase or in the stimulation of protein kinase by cyclic AMP. These results suggest that mice of the DI +/+ severe strain with nephrogenic diabetes insipidus have an ADH receptor with normal affinity for the hormone. The decreased maximal stimulation by ADH in diseased mice *might* contribute to their unresponsiveness to ADH. However, it is still possible that the major defect lies not in the activation of adenylate cyclase but rather in a step subsequent to the formation of cyclic AMP or of protein kinase. (Supported by USPHS grants AM 16105, AM 08469, and 6-K3-GM 21,786.)

87. Humoral Mechanisms of Endotoxin Tolerance. BERNARD DUBUY,* EDWARD J. YOUNG,* AND SHELDON E. GREISMAN, Baltimore, Md.

Passive transfer of serum from animals rendered tolerant to Gram-negative bacterial endotoxin is known to protect against the effects of endotoxemia. The present studies explore the nature of these humoral protective factors. Acclimatized New Zealand rabbits were given antiserum obtained from donors immunized with endotoxins from (*a*) wild type Gram-negative bacteria possessing "O" antigenic side chains or (*b*) rough mutant Gram-negative bacteria unable to synthesize these "O" side chains. Employing test doses of endotoxins that evoked fever within the sensitive portion of the dose-response range, and varying volumes of antiserum, pyrogenic tolerance conferred with anti-wild type endotoxin serum was found directed primarily towards the homologous endotoxin. Only minimal protection could be transferred to endotoxins derived from heterologous Gram-negative bacteria. Indeed, only minimal protection could be transferred if the endotoxin was derived from a different strain of the immunizing Gram-negative bacteria. In contrast, antiserum to rough mutant Gram-negative bacteria lacking O antigenic units conveyed pyrogenic tolerance that was significantly more effective against heterologous endotoxins. The level of such cross protection, however, was significantly less than that conveyed by specific anti-wild type antiserum to its respective endotoxin. The protective serum factors were globulins whose activity was separable into both 2-mercaptoethanol resistant and sensitive fractions on pyrogen-free Sephadex G200 columns. The findings indicate that while O specific antiserum affords the greatest degree of passive protection against its homologous endotoxin, antiserum to rough mutant endotoxin preparations lacking O specific side chains offers the better approach to passive transfer of broad protection against endotoxemia. These findings are in agreement with passive protection reported for the local Shwartzman reaction by Braude and Douglas (1972 *J. Immunol.* **108**: 505) and presumably occur because the absence of the O specific side chains exposes common antigen(s) in the endotoxin molecule. (Research supported by grant from NIH.)

88. Properties of Soluble Gonadotropin Receptors Extracted from the Testis and Ovary. MARIA L. DUFAU,* EDUARDO H. CHARREAU,* AND KEVIN J. CATT,* Bethesda, Md. (introduced by Leonard Laster).

Extraction of gonadotropin receptors from particulate fractions of the rat testis and ovary with nonionic detergents revealed that 80–90% of the specific binding sites for luteinizing hormone (LH) and chorionic gonadotropin (hCG) were rendered soluble in 0.1–1% solutions of Triton X-100. After solubilization, quantitative studies with ^{125}I-labeled hCG and density gradient centrifugation were performed in 0.1% Triton. The soluble receptors retained hormonal specificity

for LH and hCG and displayed high affinity for labeled hCG ($Ka = 0.5–1 \times 10^{10}$ M^{-1}). The initial rate of hormone-receptor association was higher at 34°C than at 24°C and 4°C, but greater binding occurred at the lower temperatures due to inhibition of receptor degradation. Dissociation of the hormone-receptor complex at pH 7.4 was extremely slow, 50% of the bound hormone being released after incubation for 96 h at 4°C; below pH 4, specific binding was completely reversed within 1 min. Gel filtration on Sephadex G200 and Sepharose 6B revealed that the Stokes radius of the free receptor and the hormone-receptor complex was 64 Å. Density gradient centrifugation in 5–20% sucrose at 250,000 *g* for 16 h showed clear separation of the hormone-receptor complex (7.5 S) from hCG (2.9 S); the sedimentation coefficient of the free receptor was 6.5 S. The density of the hormone-receptor complex determined by centrifugation in cesium chloride was 1.289. The respective molecular weights of the free and combined forms of the receptor were calculated to be 194,000 and 224,000 daltons, and the fractional ratio of the complex indicated that the soluble receptor is an asymmetrical molecule with axial ratio (prolate) of 12. The high conformational stability of the gonadotropin receptor should permit more detailed analysis of the structural features of the hormone binding site.

89. Insulinotropic and Glucagonotropic Effects of Gastric Inhibitory Polypeptide. J. DUPRE, J. C. BROWN,* AND A. RABINOVITCH,* Montreal and Vancouver, Canada.

Insulinotropic and glucagonotropic effects of crude pancreozymin-cholecystokinin (PZ-CCK) have been attributed to PZ-CCK. Preparations previously used contained 10% PZ-CCK, with additional 15% recently identified gastric inhibitory polypeptide (GIP), and no secretin. We report studies with 10% PZ-CCK and highly purified PZ-CCK and GIP in rats and man. In urethane-anesthesized rats (200 *g* weight) intravenous 10% PZ-CCK 10 μg elevated plasma insulin (IRI) 2.6±0.30 ng/ml ($P < 0.01$) and glucagon (IRG) 58±16 pg/ml ($P < 0.02$), after 5 min. Pure PZ-CCK 1.0 μg elevated IRI less, 1.1±0.21 ng/ml ($P < 0.02$), with no change in IRG after 5 min. One-tenth dose 10% PZ-CCK (1 μg) elevated IRI 0.9±0.15 ng/ml ($P < 0.02$) but did not affect IRG, while corresponding dose pure PZ-CCK (0.1 μg) had no effect. GIP 2.0 μg elevated IRI 3.6± 0.60 ng/ml ($P < 0.02$) and IRG 50±13 pg/ml ($P < 0.02$) after 5 min. PZ-CCK and GIP showed no glucagon-like immuno-reactivity, and this was minimal in 10% PZ-CCK or crude gut extracts. Responses were independent of plasma glucose and did not occur in saline-treated rats. In seven normal volunteers given intravenous glucose 5 g over 10 min, 10% PZ-CCK 50 μg elevated plasma IRI 1.5±0.43 ng/ml ($P < 0.05$) after 10 min, but purified PZ-CCK 5–10 μg had no effect. Neither preparation affected IRG in these conditions. Thus insulinotropic effects in man and insulinotropic and glucagonotropic effects in rats, observed with 10% PZ-CCK, were not accounted for by PZ-CCK, but were reproduced in rats with GIP in amounts present in 10% PZ-CCK. It is suggested that GIP, a probable physiologic enterogastrone, may also contribute to humoral effects of the intestine on pancreatic endocrine function, and that close structural similarities among GIP, secretin, and glucagon may be related to common actions on the islets. (Research support by grants from Canadian MRC.)

90. Disturbance of the Ratio Between Blood Thymus-Dependent (T) and Bone Marrow-Derived (B) Lymphocytes in Leprosy. JOHN DWYER,* WARD BULLOCK, AND JAMES FIELDS,* New Haven, Conn., Lexington, Ky., and Staten Island, N. Y.

The abnormalities of cell-mediated immunity (CMI) associated with lepromatous leprosy suggested disturbed T-cell function. T:B lymphocyte ratios were determined in peripheral blood of 13 normal and 16 lepromatous subjects by marking B-cell immunoglobulin receptor sites with ^{125}I-labeled rabbit antihuman IgM serum. Simultaneously lymphocytes from these subjects were stimulated with phytohemaglutinin M (PHA) and pokeweed mitogen (PWM). In vitro studies were then correlated with clinical and histologic criteria of disease severity. Results indicate that B-cells constituted a significantly higher percentage of lymphocytes in lepromatous than in normal blood ($P < 0.05$). In 5 of 16 cases, the percentage of B-cells ranged from 40 to 80%, whereas the normal range was 20–33% of B-cells. PHA-induced DNA synthesis in lymphocytes from the five cases with highest B-cell percentages was reduced. Response to PWM was also low. Furthermore, the in vitro abnormalities of lymphocytes from these cases were concordant with the presence of lepromatous infection in its most severe clinical form. This association was not invariable as B:T cell ratios and responses to mitogens were normal in two patients with severe leprosy. It is concluded that the nonspecific disturbance of CMI in some patients with lepromatous leprosy may result from a reduction of circulating T-cells by destruction or impedance to recirculation rather than from immunosuppression of intrinsically normal T-cells. (Research supported by NIH grant AI 10094.)

91. Myocardial Cell Acidosis During Acute Ischemia. R. M. EFFROS,* P. O. ETTINGER,* K. MAROLD,* B. HAIDER,* H. A. OLDEWURTEL,* AND T. J. REGAN,** Newark, N. J.

Since altered cell pH has been thought to be an important determinant of the response to myocardial ischemia, an in vivo pH study has been undertaken using the indicator dilution technique of Effros and Chinard. Intact anesthetized dogs were mechanically ventilated and heparinized for catheter placement in the anterior descending coronary artery and coronary sinus. The former had a balloon for inflation to effect ischemia and a distal lumen for blood flow measurement (^{85}Kr) and delivery of indicators. 1.4 ml of an isotonic suspension containing vascular indicators (^{51}Cr-labeled red cells and [^{125}I] albumin), a pH indicator ([^{14}C] 5,5-dimethyl-1-2,4-oxazolidine-dione "DMO," or $^{14}CO_2$), an extracellular indicator (sucrose) and tritiated water were rapidly injected through the arterial catheter and blood was pumped from the venous catheter into tubes at 5-s intervals for 200 s. Cell pH was calculated from indicator mean transit times, plasma pH, and pKa of the pH indicator. Recirculation was corrected by deconvoluting arterial concentrations from venous levels, and by exponential extrapolation. Myocardial cell pH determined with DMO averaged 6.97±0.01 SE ($n = 12$) before ischemia. Decreased coronary perfusion (control = 134± 4 ml/min per 100 g, ischemic = 46±2 ml/min per 100 g), resulted in a fall of cell pH in the first hour to 6.75±0.01 ($n = 9$, $P < 0.001$) in each of six dogs. Arterial pH remained unchanged. Similar values were obtained with $^{14}CO_2$ (control = 7.02±0.01, ischemic = 6.70±0.03, $n = 4$). In each of three DMO studies, restoration of blood flow was followed by return of cell pH toward control values. Thus, substantial but reversible intracellular acidosis occurs early in the course of ischemia when major alterations of ventricular function appear. (Suggested by NHLI 72-2970.)

92. Separation and Partial Purification of Two Types of Triglyceride Lipase from Swine Postheparin Plasma. CHRISTIAN EHNHOLM,* ANDRE BENSADOUN,* AND W. VIRGIL BROWN,* La Jolla, Calif. (introduced by Scott Grundy.)

Recently a form of triglyceride lipase (TGL) isolated from human and dog postheparin plasma (PHP) has been shown to differ from the lipoprotein lipase (LpL) of adipose tissue by (*a*) not requiring an apolipoprotein cofactor for full activity and (*b*) showing activation rather than inhibition in high (0.65–1.0 M) NaCl. These properties are quite similar to those of a TGL in extracts of liver (L-TGL). The evaluation of postheparin lipolytic activity has thus been complicated by the presence of at least two TGL's. Techniques have now been developed which allow separation of two types of TGL from plasma in one chromatographic step. Postheparin plasma obtained from domestic swine was extracted by sequential treatment with acetone, heptane, ethanol/ether, and ether. The lipid-free powder (dried with N_2) was dissolved in barbital buffer, applied to a column of Sepharose containing covalently linked heparin, and eluted with a linear gradient of NaCl from 0.4 to 1.5 M (all steps at 4°C). The first TGL activity (peak 1) eluted at 0.65 M NaCl, was *not* inhibited by incubation at 28°C in 1 M NaCl and was *not* further activated by plasma apolipoproteins. A second TGL activity (peak 2) eluting at 1.2 M was inhibited by 1.0 M NaCl (> 90%) and activated (14-fold) by an apolipoprotein isolated from human very low density lipoprotein, apoLp-Glu). Peak 1 and peak 2 closely resemble L-TGL and LpL, respectively, in (*a*) the molarity of NaCl required for elution from the column and (*b*) in their responses to 1 M NaCl and to the apolipoprotein. The absence of peak 1 in postheparin plasma of hepatectomized swine gave further evidence for its identity with L-TGL. The roles of these two TGL's in lipoprotein clearance and their relevance to the hyperlipoproteinemias are under study. (This work was supported in part by NIH grant 7 FO5 TW1774-02, in part by NIH grant HL 54494-01, and in part by NIH grant HL 14197-02.)

93. BCG-Induced Suppression of Autoantibodies in New Zealand Mice. EDGAR G. ENGLEMAN,* MICHAEL DAUPHINEE,* AND NORMAN TALAL, San Francisco, Calif.

NZB/NZW F_1 mice develop an autoimmune disease similar to systemic lupus erythematosus in man. In these mice, cellular immune functions are depressed and humoral antibody responses are augmented. To investigate the effects of cellular immune stimulation on autoimmunity, NZB/NZW female mice aged 10, 16, and 28 wk received five intravenous injections of 1 mg BCG every fifth day, and levels of serum anti-DNA and anti-RNA were measured by a filter radioimmunoassay. 1 wk posttreatment, seven of nine 16-wk-old controls but only two of nine treated mice had anti-DNA and anti-RNA antibodies. This effect of BCG persisted for at least 2 months. Even 28-wk-old mice, with high pretreatment antibody levels, experienced a 60% reduction in anti-RNA and a 25% reduction in anti-DNA. To document that BCG treatment stimulated cellular immunity, thymus-derived effector cells were measured in a cytotoxicity assay. Equal numbers of spleen cells from treated and untreated 24- and 40-wk-old female mice were injected into lethally irradiated histoincompatible C57B1/6J recipients. 4½ days later, the recipient spleen cells were incubated with ^{51}Cr-labeled C57B1/6J lymphoma cells for 8 h and cytotoxicity assayed by ^{51}Cr release. BCG treatment doubled specific cell lysis in 24-wk-old mice (from 40 to 82%) and caused a 13-fold increase in 40-wk-old mice (from 4 to 52%). Anti-theta serum completely prevented cell lysis by both treated and control spleen cells. BCG-treated mouse spleen added directly to the target cells caused no lysis. Thus, BCG both suppressed autoantibodies and augmented a T-cell mediated immune response in NZB/NZW mice. These may be related phenomena, in that stimulation of a T-cell suppressor could inhibit autoantibody production by B-cells. Such augmentation of T-cell function might prove therapeutic in autoimmune disease. (Research supported by grant from VA.)

94. Association Between Cyclic AMP Excretion and Natriuresis in Uremia. CARLOS H. ESPINEL * AND HERSCHEL L. ESTEP, Richmond, Va. (introduced by John L. Patterson).

A natriuretic factor has been extracted from serum of patients with chronic uremia that increases the rate of cyclic AMP excretion in uremic rats. The purpose of this study was to investigate further the nature of this increased natriuresis per nephron in uremic rats. Daily balance studies of Na, K, P, Ca, and cyclic AMP excretion were performed in pair-fed uremic rats. Glomerular filtration rate (GFR) was estimated by the clearance of inulin. Decreased nephron population was produced by stepwise partial nephrectomy. In the experimental group, increased natriuresis per nephron was prevented by reducing dietary NaCl intake in direct proportion to the decrease in GFR (PRNa group). The control group had a constant dietary NaCl intake which produced a high rate of Na excretion per nephron. When compared at similar GFR's, there was no significant difference in the daily excretion of K, P, and Ca between the control and the PRNa groups of rats. On the other hand, a clear relationship between sodium excretion and urinary cyclic AMP was observed. The control group, in which the fraction of the filtered Na excreted (FENa) was 2.5%, excreted 129 nmoles of cyclic AMP per day corrected for GFR. In contrast, the PRNa group, which had a FENa of 0.3%, excreted 66 nmoles of cyclic AMP per day corrected for GFR. This finding suggests that the increased natriuresis per nephron in uremic rats is a result of intrarenal or humoral factors which may be mediated by cyclic AMP.

95. Serial Cardiodynamics in Malignant Hypertension. DONALD REESE EUBANKS,* THOMAS WILLIAM EADES,* AND JAMES GIBB JOHNSON,* Memphis, Tenn. (introduced by Charles E. Kossmann **).

Right heart catheterization was performed in eight untreated patients with malignant hypertension who displayed mean femoral artery pressures ranging from 127 to 206 mm Hg. Three had normal pulmonary artery pressures, while in five the mean pressure ranged from 22 to 34 mm Hg. These same five patients had elevated right ventricular systolic pressures and elevated pulmonary artery mean wedge pressures, the latter ranging from 11 to 37 mm Hg. Three patients had elevated right ventricular end-diastolic and mean right atrial pressures. Seven patients had low cardiac indices ranging from 1.6 to 2.4 liters/min per m^2. One patient with a cardiac index 3.6 liters/min per m^2 had a hematocrit of 19. All patients had an elevated systemic resistance ranging from 38 to 120 μ/m^2. None had an elevated pulmonary arteriolar resistance. Five patients had serial studies at 1 week, 3 months, and 6 months after the initial study. All were treated with antihypertensive agents, three with chronic hemodialysis, and one with bilateral nephrectomy. All demonstrated a fall in systemic resistance. Of the three patients with elevated pulmonary artery wedge pressures, these returned to normal in two. Although there was a dramatic decrease in heart size on the serial chest X-rays, the cardiac index remained unchanged in all but one patient. Of great interest was that the total blood volume was reduced during the initial study and

normal on subsequent studies when the blood pressure became normal. These studies (*a*) exclude increased cardiac output as a contributing factor to the elevated BP in malignant hypertension and confirm the presence of a low flow high resistance hypertension; (*b*) characterize the pulmonary flow and pressure patterns in malignant hypertension and document that pulmonary artery resistance is normal; (*c*) suggest an irreversible abnormality in myocardial function even after systemic resistance is lowered; and (*d*) reveal an enlarged heart at a time when circulating blood volume is less than normal and a decrease in heart size and increase in blood volume as the pressure is controlled. (Research supported by NIH grant HL 14242.)

96. Concomitant Effects of Insulin on Surface Membrane Conformation and Polysome Profiles of Serum-Starved 3T3 Fibroblasts. R. BLAIR EVANS,* VERA MORHENN,* ALBERT L. JONES, AND GORDON M. TOMKINS,** San Francisco, Calif.

By scanning and transmission electron microscopy, we have established that insulin rapidly reverses changes in surface membrane conformation and polysome profile induced by transfer of actively growing fibroblasts from serum-containing to serum-free medium. 90 micrographs of actively growing, serum-deprived, and insulin-treated cells were randomly selected for morphometric analysis from a pool of 700 individual cells. Unit cytoplasmic areas were randomly selected from each cell and 25,000 non-membrane-bound cytoplasmic (f)-ribosomes were evaluated. 94% of the total f-ribosomes of actively growing cells were aggregated as polyribosomes. This figure fell to 78% after 18 h of serum deprivation. A reduced number of f-ribosomes per unit of cytoplasm was noted. 1 h after insuin administration to serum-starved cells aggregation rose to 92% and f-ribosomes per unit area increased by 22%. Scanning microscopy of actively growing cells revealed an abundance of surface microvilli. Serum starvation promotes an almost complete disappearance of these structures. 2 h of insulin treatment restored the microvillous conformation characteristic of actively growing cells. Bundles of microfilaments parallel to the surface membrane project into the microvillous core. This study shows the combined and rapid effect of insulin on the regulation of polysome formation and the induction of a specific surface membrane conformation in cultured cells. The observations further indicate that insulin, acting on the surface membrane, can affect such parameters as membrane transport and the rates of protein and RNA synthesis. (Supported by the VA Hospital and USPHS grant GM17239.)

97. A New Assay for Riboflavin in Human Urine Based upon Competitive Protein Binding. ARPAD G. FAZEKAS,* CARLOS E. MENENDEZ,* AND RICHARD S. RIVLIN, New York.

Presently available methods for analysis of riboflavin (RF) in human urine by fluorometric, microbiological, or enzymatic techniques are laborious and time consuming. To circumvent these difficulties, a sensitive new assay has been developed utilizing the riboflavin binding protein from chicken egg white (Rhodes et al. 1959. *J. Biol. Chem.* **234**: 2054) which binds riboflavin with high affinity and high specificity. Chicken egg white is homogenized in water, filtered through cotton wool, and incubated at 3°C for 2 h with [2-^{14}C] riboflavin to form a protein-tracer complex. For preparation of the standard curve, 0–500 ng of nonradioactive riboflavin dissolved in water are equilibrated with the protein-tracer solution at 3°C for 1 h. Bound RF is separated from free RF by absorption of the latter by Florisil. After allowing florisil to settle, aliquots of the remaining solution are removed for counting of radioactivity. The standard curve is then constructed by plotting percent of total radioactivity bound against nanograms of RF per tube. For analysis of human urines, 5-ml aliquots are centrifuged, extracted with benzl alcohol, and evaporated to dryness. Residues are taken up in water and incubated with the protein-tracer solution as above. Recovery of riboflavin in urine is 83%, with mean variation between duplicate determinations of 4.9%. Riboflavin excretion by 41 normal adults, 21–65 yr old, averaged 1.02±0.07 mg/24 h, agreeing closely with previously published reports. This method is simple, specific, accurate, and quantitative, allowing 80–100 analyses to be performed per day, and is suited both for metabolic balance studies and for large scale nutritional surveys. (Supported by grants from NIH and the Stella and Charles Guttman Foundation.)

98. The Binding of Thrombin to the Surface of Human Platelets. JOHN R. FEAGLER,* DOUGLAS M. TOLLEFSEN,* AND PHILIP W. MAJERUS, St. Louis, Mo.

We have previously postulated that thrombin may initiate platelet aggregation and the release reaction by acting at the platelet surface. We have now demonstrated surface receptors for thrombin using highly purified bovine thrombin (2000 NIH U/mg) labeled with ^{125}I. Binding of [^{125}I] thrombin to intact washed human platelets was measured by Millipore filtration. A correction for nonspecific binding was made by addition of a 100-fold excess of unlabeled thrombin to duplicate reaction mixtures at each concentration of [^{125}I] thrombin. These experiments indicate that human platelets have approximately 50,000 thrombin receptor sites with an apparent dissociation constant of 1.25 U/ml (20 nM thrombin). The apparent "K_i" determined for unlabeled thrombin added to [^{125}I] thrombin was 1.18 U/ml, indicating that the iodinated thrombin was not denatured. Furthermore, diisopropyl fluorophosphate (DFP)-treated thrombin binds to platelets identically to active thrombin even though this molecule does not cause platelet aggregation or release. Experiments using high resolution electron microscopy-autoradiography established that the [^{125}I] thrombin was located on the cell surface. The mean position of 500 grains was 490±90 Å SEM outside of the platelet unit membrane. The median grain position was 340 Å outside the unit membrane. These distances suggest that thrombin is localized in the platelet surface "glycoprotein coat." Essentially identical localization was found for [^{125}I] lentil phytohemagglutinin (mean 180±90 Å SEM median 250 Å) which is known to bind to platelet surface glycoprotein. [^{125}I] thrombin and lentil-PHA were also found bound to the surface of the canalicular system. These experiments suggest that the platelet surface contains receptor sites of high affinity for thrombin, but that binding of thrombin is not sufficient to induce aggregation and the release reaction since DFP-thrombin binds normally. (Supported by grants from NIH, ACS.)

99. Studies on the Mechanism of the Altered Disease Induced by Reovirus Mutants. BERNARD N. FIELDS* AND CEDRIC S. RAINE,* Bronx, N. Y. (introduced by M. D. Scharff).

Temperature-sensitive mutants of viruses have classically been used for studies on viral genetics and biochemistry. Our prior demonstration that certain mutants induce on altered disease pattern in model animal systems allows the correlation of viral-induced pathology with specific viral gene products. Further studies have been performed on the reovirus core mutant-induced degeneration of nervous tissue

in young rats. Using the methods of electron microscopy and fluorescent antibody staining of infected brain tissue, the pathophysiology of the altered disease has been studied. These studies have established the fact that the core mutant, like wild type, is still neurontrophic but is capable of persisting 6 wk after infection. Antibody appears between 3 and 4 wk after infection. Wild-type virus-infected animals die between 1 and 2 wk after infection. Thus in the mutant infection, instead of an efficient acute destructive process, there is a subclinical indolent process whose ultimate affects occur over a prolonged period of time. The correlation of these pathologic findings with the biochemical lesion of the viral mutant offers the opportunity to precisely define the need of viral components in virus-induced diseases. (Supported by grants from the NIH, NSF, and ACS.)

100. Streptococcal M-Protein: a Molecule Composed of Subunits. VINCENT A. FISCHETTI,* EMIL C. GOTSCHLICH, GERALDINE M. SIVIGLIA,* AND JOHN B. ZABRISKIE, New York.

Hitherto M-protein has been dissociated from streptococcal cell walls by boiling in HCl or incubation with hydroxylamine at pH 10. The isolated protein is inhomogeneous and thus difficult to purify and characterize immunochemically. Using the nonionic detergent Igepal CA 630, M-protein has been extracted from type 6 and type 14 cell walls. Chromatography on CM cellulose followed by Sepharose 6B gel filtration has yielded serologically active preparations of M-protein. The yields have been approximately 1 mg/100 mg of cell walls. Upon electrophoresis this protein migrates as a single band in polyacrylamide gels containing SDS or Triton X 100. The molecular weight of the subunit is 30,000. Removal of the detergent causes association of the subunits to aggregates of different sizes with an average molecular weight of 90,000. Type 6 M-protein contains few aromatic amino acid residues, a single methionine, and no cysteine. Edman degradation indicates that the N-terminus is blocked. This protein has been labeled with ^{125}I. In a radioactive antigen-binding test employing 50% SAS to precipitate the immunoglobulins, less than 10% of the radioactivity was bound by fetal calf or normal rabbit serum. The binding activity of rabbit typing sera to all known M types was measured. At a 1/11 dilution of these sera, type 6 antiserum bound 90% and 11 other antisera bound between 20 and 60%, while the remaining 38 typing sera bound less than 10% of the antigen. Preliminary results suggest that the sensitivity is sufficient to measure human type-specific antibodies. (Research supported by grants from PHS HL-03919, AFEB, and NYHA.)

101. DNA Synthesis by Human Mammary Gland Cells In Vitro. B. ALLEN FLAXMAN,* Philadelphia, Pa. (introduced by Eugene J. Van Scott **).

Little is known experimentally about control of DNA synthesis in the human mammary gland. In the present study, pieces of normal human mammary gland were obtained from six patients whose breasts were removed because of associated carcinomas. The normal material, which was far removed from any neoplasm, was grown in organ culture in defined medium (Eagle's minimal essential) without hormones or serum for 16 days. In all cases, the epithelium of both large and small ducts showed excellent preservation under these conditions. Autoradiographic studies with tritiated thymidine demonstrated a significant level of DNA synthesis (labeling index of 34/1000 in 7-day cultures). Mitoses were also seen. Addition of insulin or prolactin (5 μg/ml in each case) caused a further increase in the labeling index (69/1000 and 64/1000, respectively). Serum had no significant effect. The results show that, in contrast with the mouse where neither epithelial maintenance nor DNA synthesis occurs in defined medium without added hormones or serum, epithelial maintenance and DNA synthesis in the human mammary gland are not entirely dependent on these factors. The importance of identifying species differences in controls of cell behavior in vitro is emphasized. (Research supported by grants from NIH.)

102. Quantitative Deficiency of Chain-Specific Globin Messenger RNA in the Thalassemia Syndromes. BERNARD G. FORGET,* DAVID HOUSMAN,* ARTHUR SKOULTCHI,* AND EDWARD J. BENZ, JR.,* Boston, Mass. (introduced by Sherman M. Weissman).

In the thalassemia (thal) syndromes, functional deficiency but not quantitative deficiency of globin messenger RNA (mRNA) has been demonstrated. A hydridization assay has been devised to specifically test for quantitative differences between alpha and beta mRNA content of globin mRNA. The assay utilizes as radioactive probes the synthetic DNA copies (cDNA) obtained by incubating AMV reverse transcriptase with purified rabbit mRNA shown to be 80–90% enriched in alpha- and beta-chain specific mRNA. Sufficient cross hybridization occurs between human mRNA and rabbit cDNA to obtain concentration curves showing saturation of hybridization at given RNA concentrations. Non-thal human globin mRNA shows saturation of hybridization against both alpha and beta cDNA's at the same RNA concentration. Alpha thal mRNA showed saturation against alpha cDNA at only 10 times the RNA concentration which gave saturation against beta cDNA, thus demonstrating a tenfold quantitative deficiency in alpha-chain mRNA relative to beta-chain mRNA. Because of the 90% content of beta mRNA in the alpha thal mRNA, the cDNA of this RNA was used as a probe to test for beta mRNA content in beta thal. Non-thal human mRNA gave saturation of hybridization against both rabbit alpha cDNA and human beta (alpha thal) cDNA at the same RNA concentration. Beta thal mRNA gave saturation against human beta cDNA at only 10 times the RNA concentration necessary for saturation against rabbit alpha cDNA, indicating a tenfold quantitative deficiency of beta mRNA relative to alpha mRNA. There exists, therefore, true quantitative deficiency of chain-specific globin mRNA in RNA obtained from reticulocytes of patients with both alpha and beta thal. (Supported by grants from the NIH.)

103. Evidence for Carrier-Mediated Transport of Urea. NICHOLAS FRANKI,* SHERMAN LEVINE,* AND RICHARD M. HAYS, New York.

It is not known whether urea moves across the tubule by passive diffusion or carrier-mediated transport. Recent studies have shown that phloretin inhibits the movement (K_{trans}) of urea, acetamide, propionamide, thiourea, and formaldehyde across the luminal membrane of the toad bladder, but does not affect water flow, sodium transport, K_{trans}ethanol, or ethylene glycol. While this suggests a specific pathway for amides and certain other solutes, it does not provide direct evidence for carrier-mediated transport. We have now obtained evidence for a saturable, vasopressin-sensitive carrier in the toad bladder epithelial cell. K_{trans} [^{14}C]acetamide was determined across paired vasopressin-treated bladder sacs, one in normal Ringer's solution, the other in Ringer's plus unlabeled acetamide in the inner and outer bathing medium. K_{trans} [^{14}C]acetamide was unaffected by up to 75 mM unlabeled acetamide; at 125 and 150 mM it was significantly depressed (19% and 29% respectively; $P < 0.02$ at both

levels). An identical (28%) depression of K_{trans} [^{14}C]urea also occurred at 150 mM acetamide. The permeability of tritiated water, ethanol, and sodium was unaffected by 150 mM acetamide, indicating that acetamide was competing with isotopic urea and acetamide for carrier sites, rather than generally depressing membrane permeability. We would conclude that (*a*) amides, notably urea, penetrate the cell membrane by carrier-mediated facilitated diffusion; (*b*) the carrier has a high capacity (K_m140 mM) and is responsive to vasopressin and inhibited by phloretin; (*c*) the affinity of solutes for the carrier may depend in part on hydrogen bonding; and (*d*) differences in urea permeability of various nephron segments may represent the presence or absence of this carrier system. (Research supported by grants from NIH.)

104. Regulation of Fatty Acid Synthesis in Vitamin B_{12} Deficiency. EUGENE P. FRENKEL,* R. L. KITCHENS,* AND JOHN M. JOHNSTON,* Dallas, Tex. (introduced by Elias Strauss **).

To determine the mechanism by which abnormal fatty acids are synthesized in B_{12} deficiency, the regulatory enzymes of fatty acid synthesis were studied in liver and brain cytosol of normal and B_{12}-deprived rats (with low serum and tissue B_{12} levels and decreased coenzyme activity). Fatty acid synthetase specific and total activity was 2- to 5-fold greater in B_{12} deprivation than normal. The presence of an activator in the B_{12}-deprived or an inhibitor in the normal was not found by crossover studies and partial purification of the enzyme. Since B_{12} deficiency is associated with an increase in intermediates (propionyl CoA and methylmalonyl CoA), the effect of these on synthetase activity was studied. Propionyl CoA was utilized as a substrate; however, methylmalonyl CoA markedly inhibited synthetase activity. Acetyl CoA carboxylase activity was also increased 2- to 4-fold in B_{12} deprivation. Gel filtration and crossover studies failed to demonstrate an inhibitor or an activator. Methylmalonyl CoA markedly inhibited acetyl CoA carboxylase activity. Propionyl CoA was shown to be a substrate for the enzyme competing with acetyl CoA, and the product synthesized was methylmalonyl CoA. Finally, total fatty acid synthesis measured by tritiated water was increased in B_{12} deprivation. Thus, fatty acid synthesis is altered in B_{12} deficiency by the increased level of intermediates that participate in synthesis with decreased incorporation of the normal precursor acetyl CoA. Propionyl CoA competes with acetyl CoA as substrate providing a mechanism for odd-chain fatty acid production, and its product, methylmalonyl CoA, is an inhibitor of the controlling enzymes of fatty acid synthesis.

105. A Direct Assay of Bacterial Killing. ARTHUR M. FRIEDLANDER,* San Diego, Calif. (introduced by John Vaughan).

Killing assays of micro-organisms rely upon enumeration of colony-forming units (CFU) or measurement of growth spectrophotometrically. These methods are slow, subject to error, and may only measure growth inhibition rather than killing. Release of radioactivity from isotopically labeled organisms has been used to study killing by serum and phagocytes. Workers using ^{32}P have shown some release of cellular DNA. However, all isotopes used previously label some cellular constituents whose release cannot necessarily be equated with cell death. This study describes a killing assay of bacteria labeled with radioactive thymidine (TdR). It assumes that release of cellular DNA is a *direct* measure of cell death. *E. coli* 0111:B4 ("serum sensitive") labeled for 90 min with [^{14}C] TdR was chased with nonradioactive TdR for 15 min. 0.05 ml washed bacteria plus 0.3 ml human serum ($1–5 \times 10^8$ bacteria per ml) were incubated at 37°C. Aliquots were taken to determine CFU and radioactivity released from bacteria. Radioactivity released from bacteria was calculated from percent radioactivity filtered through 0.4-μ filters. At 15 min, CFU were reduced by > 99% and 6% of the counts are filterable. By 120 min, 100% of the counts are released. No counts were released by heat-inactivated serum. Unheated serum also released slight amounts in 120 min (0–6.1%) from "serum-resistant" *Salmonella typhimurium,* but heated serum released none. Ampicillin caused a > 99% fall in CFU and ~80% release of counts in 120 min. Since DNA release accurately reflects microbial killing, this assay offers a rapid quantitative killing test free of plate count errors and nonspecific isotopic release. (Supported by a grant from NIH.)

106. Inherited Cystathioninuria Associated with Urinary Tract Calculi. GEORGE W. FRIMPTER, San Antonia, Tex.

Inherited cystathioninuria has been described in association with a variety of clinical states and in two young sibs without apparent disease. One of the previously reported patients, a child, had bilateral staghorn calculi. A 33-yr-old man presented with multiple urinary tract calculi beginning in his early twenties. Numerous studies of mineral metabolism had been fruitless; stone analyses revealed calcium carbonate and oxalate. Urine revealed a moderately positive cyanide-nitroprusside test for disulfide as has been found in some patients with cystathioninuria. (Cystathionine itself does not yield a positive cyanide-nitroprusside test.) 24 h excretion of cystathionine was 894, 794, and 823 mg and plasma concentration was elevated. Administration of 400 mg of pyridoxine hydrochloride daily was followed by marked reduction in excretion of cystathionine, negative cyanide-nitroprusside tests, and freedom from calculi. Casual urine pH determinations before pyridoxine hydrochloride were 5.89, 5.47, 5.49, and 6.53, although he achieved a urinary pH of 4.81 after ammonium chloride loading. During administration of pyridoxine hydrochloride, urine pH decreased to 5.28, 518, and 5.20, leading to the hypothesis that deviation of sulfur from sulfuric acid to cystathionine rendered the urine inadequately acidic to prevent stone formation. The amount of sulfate which could be produced by "unblocking" the metabolism of 800 mg of cystathionine would be over 300 mg. However, attempts to demonstrate increased urinary sulfate excretion during administration of pyridoxine hydrochloride were unsuccessful, suggesting pyridoxine stimulated another pathway. The unknown substance or substances giving the positive test for disulfide appeared to be in fractions between urea and aspartic acid on the 150 cm amino acid analyzer column. Although apparently a benign disorder compatible with normal life, this biochemical defect may predispose to disease initiated by acquired stress.

107. Autonomic Inhibition of Cardiac Function in Spontaneously Hypertensive Rats. EDWARD D. FROHLICH,* MARC A. PFEFFER,* AND JANICE M. PFEFFER,* Oklahoma City, Okla. (introduced by Leonard P. Eliel **).

To provide insight into neurohumoral influences in hypertension, pharmacological inhibition of cardiac functions in an experimental counterpart of essential hypertension was produced by parasympathetic (atropine, 1 mg/kg) and beta-adrenergic (sotalol, 40 mg/kg) inhibition in 40 normotensive (NR) and spontaneously hypertensive (SHR) Wistar rats (aged 12–24 wk). 10 pairs were studied with ether anesthesia (preparation I) in order to measure cardiac output (electromagnetic flowmeter; open-chest) and maximum aortic flow acceleration (dF/dt). Another 10 pairs were unanesthe-

tized for measurement of resting intraventricular (left) pressures (preparation II). Both perparations demonstrated faster pretreatment heart rates in the SHR (preparation I: 417±12 vs. 306±12; preparation II: 390±10 vs. 340±12 beats/min; $P < 0.001$). Both preparations demonstrated similar myocardial contractility in NR and SHR: dp/dt, 8264± 332 vs. 7431±401 mm Hg/sec; dF/dt, 278±12 vs. 307±14 ml/sec^3, SHR vs. NR, respectively. Autonomic inhibition markedly reduced heart rate, dp/dt, dF/dt, cardiac output, and arterial pressure of NR and SHR; nevertheless, arterial pressure remained higher in SHR ($P < 0.001$). Atropine alone increased heart rate of the NR to SHR levels; this effect was minimal (if at all) in SHR, suggesting less parasympathetic influences in SHR hearts. With addition of sotalol, heart rate fell to higher or similar levels in the NR (preparation I: 244±8 vs. 272±7, $P < 0.02$; preparation II: 311±6 vs. 309±6 beats/min, SHR vs. NR). These data provide strong evidence, supporting recent clinical observations in essential hypertensive man, that an autonomic imbalance exists in SHR hypertension which is manifested by a predominance of adrenergic activity, possibly mediated through reduced parasympathetic influences.

108. Effects of Hypophysectomy and Growth Hormone on Hypothalamic Catecholamine Metabolism in the Rat. LAWRENCE A. FROHMAN, ANTONIO GROPPETTI,* DANIELA COCCHI,* AND EUGENIO E. MULLER,* Milan, Italy, and Buffalo, N. Y.

Considerable evidence exists for the role of central catecholamines in the regulation of growth hormone (GH) secretion. The present studies were performed to evaluate possible effects of GH on hypothalamic catecholamine metabolism by determining the incorporation rate of [^{3}H] tyrosine (TY) into dopamine and norepinephrine (NE) in intact and hypophysectomized rats. [^{3}H] TY was injected into the lateral cerebral ventricle through a previously implanted catheter. Animals were killed at 15 min, when [^{3}H] NE specific activity was increasing at a nearly linear rate. Hypothalamic catecholamines were isolated, assayed, and their radioactivity determined. Both endogenous TY levels and [^{3}H] TY specific activity were increased after hypophysectomy. The incorporation of [^{3}H] TY into NE was decreased despite elevated endogenous NE levels. Treatment of hypophysectomized animals with corticosterone and thyroxine for 7 days corrected the alterations in TY metabolism and increased incorporation of [^{3}H] TY into NE without effecting endogenous NE levels. The addition of GH decreased [^{3}H] TY incorporation into NE, but NE levels remained unchanged. Treatment of normal animals with GH increased endogenous TY levels resulting in decreased [^{3}H] TY specific activity. Endogenous NE levels were also increased, but incorporation of [^{3}H] TY into NE was unaltered. Changes in incorporation of [^{3}H] TY into dopamine due to both hypophysectomy and hormone therapy were similar to those into NE. Central catecholamines have been reported to have a stimulatory effect on GH but an inhibitory effect on ACTH secretion. The alterations in hypothalamic catecholamine metabolism associated with changes in hormonal milieu suggest that the hormonal feedback mechanism may be mediated by modifying neurotransmitter metabolism, which in turn influences the secretion of appropriate releasing factors.

109. Mechanism of Activation of Bovine Factor X. B. C. FURIE,* B. FURIE,* A. J. GOTTLIEB,* AND W. J. WILLIAMS,** Philadelphia, Pa., and Syracuse, N. Y.

Structural change in bovine factor X (X) after activation by a peptidase-free preparation of coagulant protein of Russell's viper venom (CP) was studied. X was activated by incubation at 37°C for 10 min with CP in a molar ratio 400–1000:1 and 0.008 M $CaCl_2$. Reaction was stopped by the addition of solid urea to a concentration of 8 M. Complete conversion of X to activated factor X (Xa) occurred as determined by activity assays and disappearance of the X band with appearance of a new band on SDS-gel electrophoresis (SDS gel). The molecular weight of X is 56,000±3000 by equilibrium centrifugation (EC) in 6 M guanidine-HCl and 62,000 by SDS gel. SDS gel and EC of X reduced with mercaptoethanol demonstrated subunits with mol wt 42,000 and 19,000. X contains 2.3% sialic acid, 2.4% neutral sugar, and 3.9% amino sugar. The molecular weight of Xa in 6 M guanidine-HCl is 55,000±3000 by EC and 56,000 by SDS gel. Xa contains subunits of mol wt 35,000 and 19,000 by SDS gel in mercaptoethanol. Xa contains 1.5% sialic acid, 1.5% neutral sugar, and 3.8% amino sugar. X and Xa were closely similar in their elution volumes from Bio-Gel P-100 in 8 M urea, and amino acid analyses and N-terminal analyses failed to show significant differences between X and Xa. Both X and its heavy (carbohydrate-containing) subunit display increased anodal migration on SDS gel after activation. No activation peptide or glycopeptide could be demonstrated with 8½% or 10% SDS gel. However, a PAS-positive band was demonstrated employing 12½% SDS gels in urea. These data indicate that the activation of X by coagulant protein is due to release of a small glycopeptide or a polysaccharide from the heavy subunit of X.

110. Immunological Relatedness of a Human Leukemic Polymerase and Leukemia Virus Reverse Transcriptase. ROBERT C. GALLO AND GEORGE TODARO,* Bethesda, Md.

Previously, DNA polymerase with biochemical properties like reverse transcriptase of RNA tumor viruses was isolated from cytoplasmic particulate fractions from "blast" cells of patients with acute leukemia. Like virus enzyme, it catalyzes *endogenous RNA-dependent* DNA synthesis. Properties of *purified* enzyme were also like virus enzyme, distinguishing it from normal leukocyte major DNA polymerases. Antibodies (IgG) were prepared from sera of rabbits immunized with reverse transcriptases from avian, murine, and primate type-C leukemic viruses. By polymerase inhibition, they distinguished between polymerases from: (*a*) type-C vs. mammary tumor virus; (*b*) avian vs. mammalian type-C virus; (*c*) lower mamalian vs. primate type-C virus; and (*d*) viral vs. cellular. Comparative inhibition of polymerase from C-type viruses by antibody to polymerase from *mouse leukemia virus* was: mouse > cat > primate > avian. Human leukemic reverse transcriptase was inhibited to the same degree as the enzyme from primate virus (gibbon ape leukemia virus). Most significantly, antibody to polymerase from the newly isolated *primate (gibbon ape) leukemia virus* inhibited human leukemic enzyme comparably to gibbon virus polymerase itself, the enzyme which antibody was prepared against. Inhibition of polymerase from lower mammalian type-C virus was much less and avian virus not at all. Antibody to *avian leukemia virus* enzyme significantly inhibited only the avian virus enzyme. None of these antibodies inhibited the major DNA polymerases of normal human cells. The enzyme from two AML and one ALL patient were tested. So far, only the AML enzymes have shown the immunological relatedness to type-C viral enzyme. We conclude that some human leukemic cells contain reverse transcriptase immunologically related to enzyme

of C-type virus (particularly primate), and, therefore, may have one expression of type-C leukemia virus information.

111. Carbon Sources of Alanine and Glutamine Released by Skeletal Muscle. ALAN J. GARBER,* IRENE E. KARL,* AND DAVID M. KIPNIS, St. Louis, Mo.

If alanine release from skeletal muscle represents carbon derived primarily from glucose via pyruvate, as has been suggested, then the glucose-alanine cycle does not account for a net flow of carbon from protein to carbohydrate. The present study was undertaken, therefore, to define the carbon sources of alanine and glutamine, the two predominant amino acids released from muscle. An intact rat epitrochlaris muscle preparation was developed which maintained ATP, ADP, AMP, and phosphocreatine levels, as well as linear rates of glucose uptake, and lactate and pyruvate production for 3 h incubation. No correlation was found between a sevenfold range of glucose uptake or pyruvate production rates and alanine or glutamine release. Basal alanine release (20.1±1.1 mμmoles/g per min) was increased by the addition of 10 mM concentrations of aspartate (53.2±6.4), cysteine (43.5±4.5), glycine (27.4±2.8), histadine (25.4±1.8), isoleucine (27.5±2.1), leucine (29.7±1.5), methionine (28.0±2.3), serine (31.2±2.0), threonine (28.2±3.0), and valine (29.4±2.5), but not lysine, phenylalanine, or tyrosine. In contrast, the latter three amino acids increased basal glutamine release (30.1±1.2) by 30–50%. Glutamine release was also increased by cysteine (40.1±1.5), isoleucine (39.4±1.8), leucine (44.3±2.8), methionine (39.4±1.8), and valine (43.6±3.0). Aminoxyacetate (2 mM), a potent transaminase inhibitor, decreased basal alanine release 70% (5.29±1.0) and increased aspartate release from 0.88±0.10 to 6.40±1.02 mμmoles/g per min, but did not alter glutamine release. These data indicate that: (*a*) amino acids serve as the carbon source as well as the nitrogen precursor for alanine formation, (*b*) alanine but not glutamine formation depends primarily upon transamination pathways, and (*c*) alanine formation in muscle represents a mechanism by which both "gluconeogenic" as well as "ketogenic" amino acids can contribute to hepatic gluconeogenesis. (Research supported by NIH grants AM 1921 and 1F03 AM 54124.)

112. The Rate and Quantity of Radiolabeled Antihuman Lymphocyte Globulin Which Binds to Cultured Human Lymphoblasts or Thymus Cells Predicts Immunosuppressive Activity in Monkeys. RAUL GARCIA-RINALDI,* ROGER D. ROSSEN,* KENNETH W. SELL,* EVAN M. HERSH,* AND WILLIAM T. BUTLER, Houston, Tex., and Bethesda, Md.

We developed a highly sensitive and reproducible in vitro model to measure the rate and amount of binding of radiolabeled antibodies to target cells. Quantitative target cell recovery is achieved using a specially designed cup that simultaneously allows washing and collection of the cells. Using this model, we measured the rates and the quantity of antibody which binds to cultured human lymphoblasts, thymus and, in some cases, Hela cells in 28 antihuman and 2 antibaboon lymphocyte sera and in 9 normal sera submitted to the National Naval Medical Center for immunosuppressive testing in primates. The quantity of ^{125}I-labeled antihuman lymphocyte globulin (ALG) which binds to 1×10^6 lymphoblasts during 60 min incubation at 37°C correlates well ($r = 0.648$) with the degree of immunosuppression produced by these same ALG's in rhesus monkeys. 21 of the 22 immunosuppressive sera bound $\geq 6\times10^{-13}$ moles IgG per 10^6 lymphoblasts under conditions of the test, whereas none of the 9 normal sera and only 3 of the 8 nonimmunosuppressive sera bound this amount. Further studies of the nonimmunosuppressive ALG's which contained lymphoblast-binding antibodies revealed that they either lacked antibodies able to bind to human thymus cells, or they contained both much less thymus cell-specific antibody and much less antibody cross reactive with other human cells. The chief feature of highly immunosuppressive ALG's was their large quantities of avid antibodies reactive with human thymus cells. Thus this in vitro test not only rapidly identifies ALG's which are immunosuppressive, but more importantly, measures directly the relative avidity, specificity, and quantity of their antibodies reactive with lymphoid target cells. (Research supported by grants from ACS CI 22, Bureau of Medicine and Surgery, Navy Dept., Research Task No. MF 51 524.013 1002AA2C, NIH AM 015494, HE 05435, GM 1965, RR00350, and the Methodist and VA Hospitals, Houston, Tex.)

113. Regulation of Membrane Cation Transport by Catecholamines: Role of Adenylate Cyclase-Cyclic AMP System. JERRY D. GARDNER,* HAYDEN L. KLAEVEMAN,* JOHN P. BILEZIKIAN,* AND G. D. AURBACH, Bethesda, Md.

We have found that β-adrenergic catecholamines rapidly stimulate sodium transport across the plasma membrane of intact turkey erythrocytes. Sodium influx and outflux increase 2- to 4-fold in parallel with cellular accumulation of cyclic AMP. The potency of catecholamines for stimulation of sodium fluxes and cellular cyclic AMP correlate directly with their potency for activation of adenylate cyclase in the turkey erythrocyte membrane. Stimulation of sodium fluxes by β-adrenergic catecholamines was blocked by low concentrations of propranolol and could be reproduced by adding cyclic AMP to the medium. The catecholamine-stimulated rise in cellular cyclic AMP continued unimpaired for at least 2 h. In contrast, stimulation of sodium transport began to diminish 30 min after addition of catecholamines, and after 3 h of incubation, sodium fluxes were only slightly greater than control. This phenomenon presumably reflects accumulation of an inhibitor or depletion of a necessary factor for the transport system activated by cyclic AMP. Addition of ouabain does not alter catecholamine-stimulated cellular cyclic AMP accumulation but potentiates and prolongs the effect of catecholamines on sodium transport. This potentiating effect of ouabain on catecholamine-stimulated sodium transport suggests that the mechanism activated by cyclic AMP shares some common biochemical pathway with the Na,K-dependent ATPase system. The effects of catecholamines on sodium transport, as well as results with other systems controlled by hormones through cyclic AMP, suggest that endocrine regulation of sodium transport is of general biological significance.

114. Importance of Fe As a Regulator in Thrombocytosis of Blood Loss. SUDERSHAN K. GARG,* MARK WEINER,* AND SIMON KARPATKIN, N. Y.

Blood loss-induced thrombocytosis was studied by measuring thrombocyte and megathrombocyte (young platelet) kinetics in 60 guinea pigs which were either acutely bled for 5 days, chronically bled for 40–64 days, or on an Fe-deficient diet for 92 days. Appropriate controls were sham manipulated. Median platelet volume, hematocrit, hemoglobin, mean corpuscular volume, and serum Fe were also measured. Acute or chronic blood loss raised the platelet count 1.2- and 1.4-fold, respectively. Simultaneous replacement of Fe loss raised the platelet count 2.1- and 2.5-fold, respectively. Paradoxically, an Fe-deficient diet also raised the platelet count 1.4-fold. Megathrombocyte number increased 1.4-fold *greater* than the increase in platelet count for both acute and chronic

blood loss. In chronic blood loss the rise was biphasic, peaking on days 9 and 30 and falling below basal levels on day 16. Simultaneous replacement of Fe loss abolished the biphasic response and increased megathrombocyte number 1.8- and 1.6-fold *greater* than the increase in platelet count for acute and chronic blood loss, respectively. With an Fe-deficient diet the megathrombocyte number did not rise above the rise in platelet count. Median platelet volume declined during chronic blood loss or on an Fe-deficient diet. The decline in the former was temporary and paralleled the megathrombocyte fall on day 16. A two-compartment system is postulated for the regulator effect of Fe on thrombocytosis. (*a*) Fe inhibits the rise in platelet count above steady-state levels. (*b*) Fe is also required for maximum platelet production, via the production of megathrombocytes. The increment in megathrombocyte production after blood loss could account for 1/4 of thrombocyte production if one megathrombocyte gives rise to one thrombocyte. Alternatively, the increment could account for total thrombocyte production if one megathrombocyte gives rise to four thrombocytes in the peripheral circulation. This latter mechanism may be important physiologically.

115. Direct Lymphocyte-Mediated Cytotoxicity. An Assay of Sensitization Pretransplantation. M. R. GAROVOY,* V. FRANCO,* D. ZSCHAECK,* C. B. CARPENTER, T. B. STROM,* AND J. P. MERRILL,** Boston, Mass.

Decreased renal allograft survival occurs in recipients sensitized to histocompatibility antigens before transplantation. Patients with lymphocytotoxic antibodies (Ab) against $\geq 10\%$ of a random panel of cell donors have a higher failure rate despite a negative donor-specific crossmatch. Of 31 consecutive cadaver kidney recipients, 12/14 without Ab and 10/17 with Ab are functioning at 1 yr. Since cellular presensitization may play a role in early rejection, we have evaluated the direct lymphocyte-mediated cytotoxicity (LMC) procedure, a 4 h assay of cellular sensitization to major and minor histocompatibility antigens in which blood lymphocytes from potential recipients are mixed with ^{51}Cr-labeled donor lymphocytes. In comparison with the complement-mediated Ab crossmatch, the LMC release of ^{51}Cr was more sensitive in detecting presensitization in potential recipients. 20 hemodialysis patients with varying incidences of Ab presensitization were studied. Every patient with cytotoxic Ab against a panel member had direct LMC activity against the same cells (range of specific ^{51}Cr released: 6–30%). However, in 11/20 combinations the patients had positive direct LMC assays with cells against which no cytotoxic Ab was detected. One patient had a positive LMC with a sibling who was compatible at the major histocompatibility locus. Subsequently, the patient severely rejected this transplant. Of three other patients with donor-specific LMC reactions two had severe rejections with loss of the kidneys. Thus, assessing the presence of circulating effector cells of cellular immunity (thymic dependent) is a promising adjunct to measurements of humoral Ab (bone marrow dependent) in evaluating the total immunologic status of transplant recipients. (NIH grants AM-05700 and AI-18516.)

116. Effect of Sex Hormones on Arterial Subendothelial Connective Tissue. EVELYN GAYNOR,* Bronx, N. Y. (introduced by Theodore H. Spaet**).

Profound hormonally related sex differences in the incidence and severity of atherosclerosis and its thromboembolic complications have been well established. Although biochemical effects of sex hormones on vascular connective tissue (CT) have been reported, to date these have not been structurally identified. However, the thrombogenicity of subendothelial CT has recently been shown to vary with structural differences. Therefore, an effect of sex hormones on subendothelial CT in rabbit arteries was sought, and striking morphological alterations were noted after prolonged treatment. Depot preparations of testosterone cypionate, 100 mg/wk, or estradiol cypionate, 200 μg/wk, were injected intramuscularly into intact adult New Zealand male rabbits for 8–16 wk. The iliac arteries were prepared for electron microscopic examination from five rabbits in each treatment group and in four normal male rabbits. Thin sections were prepared from three randomly selected cross-sectional tissue blocks for each animal. A minimum of 7 μ of vessel circumference was studied in each block. Selection of area for electron microscope examination was random, except that those sites in which the internal elastic lamina showed fragmentation were discarded. Electron micrographs at a single final magnification were evaluated "blind" by several observers for qualitative differences in subendothelial constituents. In the control group, the subendothelium contained moderate numbers of elastin-associated microfibrils (MF), but all the rabbits given testosterone consistently showed significantly fewer MF than the controls. In contrast, rabbits given estradiol all had significantly greater numbers of MF. Statistical analysis of variance showed these differences to be significant at $P = < 0.001$. Variations in platelet affinity for different subendothelial connective tissue elements has recently been reported. Sex-dependent differences in thrombogenicity of exposed subendothelium may be reflected in the hormonally induced morphologic changes described in this report. (Research supported by grants from NIH.)

117. Demonstration of Gliadin Toxicity in Vitro: Evidence for an Endogenous Effector Mechanism in Gluten-Sensitive Enteropathy (GSE). R. L. GEBHARD,* Z. M. FALCHUK,* C. SESSOMS,* AND W. STROBER,* Bethesda, Md. (introduced by R. Gordon**).

In vitro culture of jejunal biopsy tissue was utilized to study the pathogenesis of GSE. During 48 h culture, alkaline phosphatase activity of normal tissue increases by 42%. Tissue from GSE patients has lower initial activity but shows proportionally greater enzyme increase (230%). Sucrase and trehalase show qualitatively similar changes. This increase is probably due to maturation of crypt epithelial cells. If tissue is cultured with a peptic-tryptic digest of gliadin (GLI), increased enzyme activity is still observed in tissue from normals, patients with other diseases, and GSE patients in remission. In contrast, under these conditions, increase in enzyme activity is greatly inhibited in tissue from GSE patients in relapse. Purified α-gliadin also causes inhibition, but not peptic-tryptic digests of casein. This interference with epithelial cell maturation mediated specifically by gliadin represents an in vitro model of GSE. The fact that culture with GLI affects tissue of GSE patients only after gluten challenge suggests that the toxic effect of GLI requires activation of an endogenous mechanism. To investigate this possibility, jejunal tissue from GSE patients in remission was cultured alone and with tissue from patients in exacerbation. In tissue cultured alone, GLI did not inhibit alkaline phosphatase increase. However, in four out of five instances, increase in enzyme activity was inhibited when the same tissue was cultured with tissue from a patient with active disease. These mixed cultures confirm the presence of an endogenous effector mechanism which mediates mucosal injury in GSE; furthermore they demonstrate that this

mechanism can be "transferred," making the immune system a likely candidate.

118. Antiketotic Effect of Alanine in Diabetic Man. SAUL GENUTH,* Cleveland, Ohio. (introduced by Bernard Landau).

L-Alanine has been previously shown to suppress ketosis in man under two circumstances when plasma glucose and gluconeogenesis are reduced: in prolonged starvation and in ketotic hypoglycemia of childhood. The present study determined the influence of alanine on ketosis when plasma glucose (and presumably gluconeogenesis) are elevated—in diabetes mellitus. Plasma beta-hydroxybutyrate (mM/liter), free fatty acids (FFA) (mM/liter), insulin (μU/ml), and glucose (mg/100 ml) were measured after administration of *l*-alanine, 0.5 g/kg, orally in previously untreated adult diabetics (AD) and insulin-dependent diabetics (IDD) temporarily withdrawn from treatment. In AD, mean beta-hydroxybutyrate (0.48) decreased 0.09±0.026 (mean±SE of differences) within 15 min ($P < 0.01$) and 0.39±0.11 by 150 min ($P < 0.01$) returning to base line at 240 min. Mean insulin (10) increased 11±3 at 15 min ($P < 0.01$), 17±3.5 at 60 min ($P < 0.001$), and 6±0.7 ($P < 0.001$) at 180 min. Mean FFA (1.08) was unchanged for 60 min, then decreased 0.49±0.13 at 90 min ($P < 0.005$), this reduction persisting till 180 min. Mean glucose (279) decreased only 21±7.7 at 180 min ($P < 0.025$). In IDD, mean beta-hydroxybutyrate (2.12) was much higher but promptly decreased 0.22±0.06 within 15 min ($P < 0.025$) and 1.08±0.32 by 90 min ($P < 0.025$). Return to base line was evident by 180 min. Mean FFA (1.42) *never fell* significantly but actually increased 0.84±0.23 by 120 min ($P < 0.025$). Mean glucose (364) increased 28±8 by 45 min ($P < 0.025$) and 84±20 by 150 min ($P < 0.01$). We conclude that alanine, although insulinogenic, can suppress ketosis independently of lipolysis and without releasing insulin (IDD). Its potent antiketotic effect in diabetes suggests that hepatic ketogenesis could in part be regulated by alanine availability.

119. Quantitative Effects of Glucose on Glucagon and Insulin Responses to Arginine in the Isolated Perfused Rat Pancreas. JOHN E. GERICH,* M. ARTHUR CHARLES,* BARBARA FRANKEL,* RUDY FANSKA,* AND GEROLD M. GRODSKY,* San Francisco, Calif. (introduced by R. J. Havel**).

Glucose stimulates insulin (IRI) secretion and inhibits glucagon (IRG) release. The role of IRI in glucose's effect on IRG release is unclear. To further characterize this interrelationship, responses to arginine (2.1–19.2 mM)±glucose (0–150 mg/100 ml) were studied in the isolated perfused rat pancreas. Arginine without glucose evoked biphasic IRG secretion. 4.6 mM arginine caused half-maximal stimulation for both phases. IRI responses were monophasic (no first phase) with half maximum at 18 mM arginine. IRI and IRG secretion correlated directly ($r = 0.6$; $P < 0.005$). Glucose, over 75 mg/100 ml, progressively inhibited both phases of IRG responses to arginine (3.2 mM). Half-maximal inhibition occurred at 110 mg/100 ml glucose. IRI secretion was biphasic. The glucose concentration yielding half-maximal IRI release differed for first phase (80 mg/100 ml) and second phase (130 mg/100 ml). IRI and IRG release were inversely related ($r = -0.7$; $P < 0.005$). Varying arginine (3.2–19.2 mM)+constant glucose (100 mg/100 ml) caused biphasic release of both IRI and IRG. Arginine + glucose enhanced IRI and diminished IRG responses compared to arginine alone without altering arginine half maximums. Total IRI and IRG release correlated directly ($r = 0.36$; $P < 0.05$). Despite a 3-fold increase in IRI secretion, no further inhibition of IRG release occurred. We conclude that pancreatic α-cells have greater sensitivity to arginine than β-cells. Arginine alone causes biphasic IRG secretion, but glucose is necessary for arginine to evoke biphasic IRI release. IRI may be required for suppression of IRG release, but glucose concentration is the major factor determining the magnitude of inhibition.

120. Heme Synthesis by Cultured Human Marrow Cells in Response to Cyanate-Treated Erythropoietin. ANTHONY S. GIDARI,* MARK H. COHEN,* AND RICHARD D. LEVERE, Brooklyn, N. Y.

Cyanate inhibits sickling *in vitro* and prolongs the survival of sickle-cell erythrocytes *in vivo*. This action appears to be mediated, in part, by the carbamylation of the amino-terminal valine residues of hemoglobin S. Although plasma levels of erythropoietin appear to be elevated in sickle-cell anemia, the effect of cyanate on the biological integrity of erythropoietin has not been examined. In these studies a single preparation of erythropoietin (step III, sheep plasma) was used. Freshly prepared 0.2 M KOCN was diluted to 50 mM with the erythropoietin (24 IU/ml), neutralized, incubated 1–1.5 h at 37°C, and then dialyzed. In controls 0.9% NaCl replaced the KOCN solution. In five replicate experiments ($n = 18$), the cyanate-treated erythropoietin or the saline-treated erythropoietin was assayed for activity at a concentration of 0.4 IU/0.8 ml media in normal human marrow cultures. After 66 h of incubation 0.5 μCi ^{59}Fe was added to each culture and at 72 h the radioheme was extracted into 2-butanone and quantitated. [^{59}Fe] heme radioactivity in treated cultures is expressed as a percentage of that in untreated controls. Cyanate did not inhibit the effectiveness of erythropoietin (208±10%) in stimulating heme synthesis when compared with the saline-treated erythropoietin (214±9.9%). NaO^{14}CN-treated erythropoietin exhibited the same banding pattern in polyacrylamide gel as saline-treated erythropoietin. Moreover, the ^{14}C label was associated with the bands suggesting that carbamylation of protein occurred. These studies show that the amount of cyanate employed, which exceeds clinically useful concentrations, while apparently carbamylating erythropoietin does not alter its biological effectiveness in human marrow cultures. (Supported by NIH grants AM-09838 and HL-15170.)

121. Abstract withdrawn.

122. Effects of Hyperoxia upon Physiologic Dead Space in Normal Adults. JOHN N. GLOVER,* DAN H. KEREM,* JOHANNES A. KYLSTRA,* AND HERBERT A. SALTZMAN, Durham, N. C.

Systematic capillary blood flow is known to decrease on exposure to abnormally high partial pressures of oxygen. A similar response of the pulmonary capillary bed might cause an increase in the physiological dead space. This, in turn, might cause alveolar hypoventilation in patients with a decreased ventilatory reserve. The physiological dead space (V_D) was measured in 22 normal adults with inspired pressures of oxygen ($P_{I_{O_2}}$) ranging from 0.4 to 1.53 atmospheres absolute (Ata), at fractional concentrations of oxygen ($F_{I_{O_2}}$) ranging from 0.21 to 0.81. Gas volumes were determined by spirometry, fractional concentrations of oxygen and carbon dioxide in respired gas were determined by gas chromatography, and partial pressures of oxygen and carbon dioxide in arterial blood were determined electrochemically. When compared to results obtained at 0.21 Ata of oxygen, values for the physiological dead space (V_D) increased from 137.3±44.7 (mean±standard deviation in milliliters BTPS) to 158±44.2 at 0.4 Ata of oxygen, from

110.6±27.4 to 140.6±44.8 at 0.5 Ata of O_2, from 143.1±49.0 to 166.4±55.7 at 0.8 Ata of oxygen, and from 110.4±23.7 to 140.0±27.0 ml BTPS at 1.53 of oxygen. Responses were variable, however, and approached statistical significance only at the highest P_{IO_2} administered ($P < 0.08$ at 1.53 Ata P_{IO_2}). These findings of an increased V_D, although not conclusive, suggest a change in the balance between ventilation and perfusion caused by hyperoxia. (Research supported by NIH grant HL07896, ONR contract N00014-67-A-0251-0007, and by the North Carolina Heart Association.)

123. Digestion and Absorption of Synthetic [^{3}H] Pteroylhepta-γ-L-glutamate ([^{3}H] PteGlu$_7$) in Dogs. HERMAN A. GODWIN* AND PHILLIP L. ROBERTS,* Houston, Tex., and Boston, Mass. (introduced by Harold Brown**).

We have investigated digestion and absorption of conjugated folate in anesthetized mongrel dogs by direct placement of synthetic [^{3}H] PteGlu$_7$ (1972. *J. Biol. Chem.* **247**: 2266) into isolated jejunal segments and by continuous perfusion of segments of jejunum. When graded amounts of [^{3}H] PteGlu$_7$ (equivalent to 70–250 μg pteroylmonoglutamate) are introduced into the lumen of isolated jejunal segments, progressive hydrolysis of [^{3}H] PteGlu$_7$ to [^{3}H] pteroylmonoglutamate through intermediate folate compounds is detectable in samples of intraluminal contents. Increases within mesenteric blood of microbiologically active folate and radioactivity begin as early as 5 min after [^{3}H] PteGlu$_7$ placement. Maximum rises are attained by 20–50 min. Increments of *L. casei*-active folate in mesenteric blood are 2–5 times greater than corresponding rises in *S. faecalis*-active folate. Increases of folate within systemic venous blood are detected at approximately 15 min with peaks occurring around 60 min; these increments support only the growth of *L. casei*. Cumulative absorption (60 min) ranges from 66% of small test doses to 36% of larger doses. Ligation of the common bile duct 4 days before study does not alter results. Continuous perfusion (9–10 ml/min) of jejunal segments with physiologic saline-glucose solution containing polyethylene glycol and graded quantities of [^{3}H] PteGlu$_7$ (equivalent to 100–500 mμg/ml pteroylmonoglutamate) demonstrates that between proximal and distal sampling sites partial hydrolysis and absorption of [^{3}H] PteGlu$_7$ occurs. Calculated folate absorption approximates 60% for smaller [^{3}H] PteGlu$_7$ concentrations and 40% for greater ones. These studies suggest that (*a*) partial hydrolysis of [^{3}H] PteGlu$_7$ occurs within the jejunal lumen; (*b*) [^{3}H] PteGlu$_7$ absorption is dose related; and (*c*) partial methylation of folate occurs during absorption. (Research supported by NIH grants AM-00795 and AM-05391.)

124. Human Preleukemia: Identification by a Maturation Defect In Vitro. DAVID W. GOLDE* AND MARTIN J. CLINE, San Francisco, Calif.

Preleukemia is an ill-defined condition of bone marrow dysfunction with associated morphological abnormalities that precedes the onset of diagnosable acute leukemia. No reliable means currently exists for identifying the preleukemic state in man. We studied the proliferative and maturational characteristics of human marrow cells in vitro using a recently developed liquid culture technique. Bone marrow was obtained from four patients with preleukemia, from four patients with refractory anemias, four acute and three chronic granulocytic leukemias, and from eight normal subjects. Cultures were harvested at intervals up to 30 days for viable and differential cell counts, [^{3}H] thymidine labeling indices, histochemistry, electron microscopy, and tests of leukocyte function. Although total cell counts and labeling indices in culture were similar in preleukemic and normal marrows, a striking maturation defect was observed in preleukemia. Only acute leukemia cultures had a comparable maturation arrest. Since development of mature cells proceeded normally in refractory anemia, this condition was readily distinguished from preleukemia. The defect in preleukemia resulted in the appearance of a morphologically homogeneous population of immature cells early in culture. Chronic granulocytic leukemia cells had a proliferative capacity 2–3 times normal; however, they maintained a normal maturational pattern. These studies demonstrate a defect in cellular differentiation in preleukemia, supporting a concept of acute granulocytic leukemia as a primary maturation disturbance. Identification of the preleukemia state in man permits consideration of therapy at a time when the leukemic cell population is small or when the leukemic maturational block is incomplete.

125. Factor VII: A Biological Probe for Initiation Sites of Blood Coagulation. PAUL B. GOLDENFARB,* FRANCES A. PITLICK,* AND YALE NEMERSON, New Haven, Conn.

To identify potential sites of initiation of blood coagulation, we previously used an immunohistochemical technique to define the anatomic localization of tissue factor (thromboplastin). This approach showed that blood vessels, particularly intima and endothelial cells, were intensely stained for tissue factor antigen. Biologically active tissue factor binds factor VII, a plasma coagulation factor, and the product of this reaction, the tissue factor-factor VII complex, initiates the extrinsic system of coagulation. We therefore labeled factor VII and used it as a probe for biologically active binding sites. Bovine factor VII was purified by a previously published technique and was either radiolabeled with ^{125}I or coupled to glucose oxidase with glutaraldehyde. The labeled protein was incubated with unfixed frozen sections of bovine or human tissues; bound factor VII was visualized by autoradiography or by tetrazolium reduction in the presence of glucose. Enzyme-conjugated factor VII bound specifically to plasma membranes of vascular endothelial cells. In addition, membranes of other cells as well as some medial and adventitial staining of vessels was noted. Specificity of the binding was established by demonstrating a requirement for Ca^{2+} and by inhibition of the binding by native factor VII. Endothelial binding was confirmed by autoradiography. Thus, good correspondence between tissue factor antigen and factor VII binding was established. From these experiments we conclude that labeled factor VII is a specific probe for tissue factor *in situ*. Further, we conclude that endothelial cells bind factor VII and are therefore potentially thrombogenic. Whether membrane binding leads to the appropriate changes in factor VII such that further coagulation is catalyzed is yet to be demonstrated. (Supported by grants from the NIH and AHA.)

126. A New Role for Complement: Lysosomal Fusion and Enzyme Secretion. IRA M. GOLDSTEIN,* MELCHIORRE BRAI,* ABRAHAM G. OSLER,* AND GERALD WEISSMANN, New York.

Lysosomal enzymes and inflammatory substances are released from surface-stimulated polymorphonuclear leukocytes (PMN's) after lysosomal and plasma membranes fuse. We now report that a complement reaction product of low molecular weight generated via the alternate pathway interacts with PMN's and induces selective release of lysosomal enzymes in the absence of phagocytosis. Treatment of fresh human serum with zymosan (1.0 mg/ml), inulin (1.0 mg/ml), bacterial lipopolysaccharide (1 μg/ml), or cobra venom factor (100 U/ml) yields a fluid phase component which in-

duces cytochalasin B (5 μg/ml)-treated PMN's to release β-glucuronidase (e.g., 17.9±1.5% of total enzyme vs. 6.5± 0.5% from controls) without leakage of cytoplasmic lactate dehydrogenase. Involvement of the alternate pathway of complement activation is evidenced by failure of serum to yield enzyme-releasing activity after pretreatment with rabbit antihuman C3 proactivator, 0.01 M EDTA but not 0.01 M EGTA, 0.02 M salicyclaldoxime, 0.02 M hydrazine, or by heating to 56°C for 30 min. Levels of C3 proactivator in treated sera correlated inversely with enzyme-releasing activity. Chromatography on Sephadex G-75 of zymosan-treated serum and of trypsinized (10 min) purified human C5 yielded similar low molecular weight fractions containing enzyme-releasing activity, implicating C5a. Ultrastructural histochemistry of PMN's exposed to treated serum revealed degranulation, fusion of lysosomal with plasma membranes, and release of endogenous myeloperoxidase. Agents which regulate secretion of other inflammatory mediators (Kaliner et al. 1972. *J. Exp. Med.* **136:** 556 and Weissmann et al. 1971. *Nature New Biol.* **231:** 131) influenced complement-dependent release; cyclic AMP (0.001 M) and theophylline (0.001 M), or prostaglandin E_1 (0.1 mg/ml) inhibited enzyme release, whereas cyclic GMP (10^{-6} M) mimicked the effect of treated serum. Data suggest that a low molecular weight complement component generated from C5 promotes release of lysosomal enzymes via a secretory process: "reverse endocytosis."

127. Genetic and Medical Significance of Neonatal Hyperlipidemia. JOSEPH L. GOLDSTEIN,* JOHN J. ALBERS,* WILLIAM R. HAZZARD,* HELMUT R. SCHROTT,* EDWIN L. BIERMAN, AND ARNO G. MOTULSKY,** Seattle, Wash., and Dallas, Tex.

To assess the genetic and medical significance of neonatal hyperlipidemia, cholesterol and triglyceride levels were determined on 2000 consecutively collected umbilical cord blood (CBL) samples. Fasting blood lipid levels and documentation of coronary heart disease (CHD) were then obtained on parents and grandparents (n = 645) of 134 newborns with normal CBL and compared to those of parents and grandparents (n = 614) of 125 newborns whose CBL exceeded the 95th percentile of the 2000 CBL samples. 55 newborns had hypercholesterolemia, 54 had hypertriglyceridemia, and 16 had elevations in both lipids. As a group, the mean levels of cholesterol and triglyceride of parents and grandparents of infants with hypercholesterolemia and/or hypertriglyceridemia were not significantly different from those of controls. No difference was observed in frequency of deaths attributable to CHD (documented by death certificates) in 70 grandparents of newborns with hyperlipidemia as compared with 90 control grandparents (31.4% vs. 30.2%). However, 26.4% of newborns with hyperlipidemia had one hyperlipidemic parent in contrast to 14.9% of newborns with normal CBL ($X^2 = 13.2$, $P < 0.005$). Using the criterion of three-generation transmission (i.e., from grandparent to parent to newborn), a pattern compatible with autosomal dominant inheritance was observed in 9 of 125 newborns with elevated CBL: five had familial hypercholesterolemia (FHC) and four had familial combined hyperlipidemia (FCHL). Therefore, a *minimal* estimate of the heterozygote frequency of the familial hyperlipidemias in the general population is approximately 0.45% (9 of 2000). These studies demonstrate that most newborns with elevated CBL do not have a genetically determined lipid disorder. However, both FHC and FCHL may sometimes express at birth and can *only* be diagnosed after extensive family studies. (Supported by grants from NIH and AHA.)

128. Cyclic AMP Levels in Young and Senescent Fibroblasts: Effects of Epinephrine and Prostaglandin E_1. SAMUEL GOLDSTEIN* AND RICHARD J. HASLAM,* Hamilton, Ontario, Canada (introduced by J. Fraser Mustard**).

Recent studies suggest that cyclic AMP levels control growth of cultured mammalian cells. To explore possible mechanisms of senescent decline in diploid human cells, cyclic AMP levels were determined by a protein-binding method in cultured skin fibroblasts from a normal adult at early and late passage. In young cells cyclic AMP levels were 10.7±0.5 and 10.9±1.9 (mean ± SE) pmoles/mg protein in growing and contact-inhibited cultures, respectively. Cyclic AMP levels in senescent cells ranged from 100 to 150% of these values. However, the molar cyclic AMP concentration in senescent cells was lower than in young cells because the ratios of mean cell volumes in old vs. young cells (4–6) were considerably greater than the ratios of cellular protein contents (1.7–2.2). Prostaglandin E_1 (PGE_1, 1 μM) increased cyclic AMP levels in young cells to 2000–4000 pmoles/mg protein under both growth conditions, while with senescent cells, values in the range 1000–1600 pmoles/mg protein were obtained. Values of cyclic AMP were maximal 10 min after the addition of PGE_1. Epinephrine (10 μM) increased cyclic AMP levels in young cells to a maximum of 90 pmoles/mg protein at 2 min and to about 200 pmoles/mg protein in senescent cells after the same interval. The ratio of the peak cyclic AMP level with PGE_1 to that seen with epinephrine was 50.4 in young and 3.7 in old confluent cultures and similar differences in ratio were seen during growth. The results suggest that senescence of cultured human fibroblasts is associated with a change in the ratio of specific hormone receptors. However, no evidence was found that an increase in the molar concentration of cyclic AMP occurs during senescent decline or during contact inhibition of growth in the cell strain studied. (Supported by the Medical Research Council of Canada and the Canadian Diabetic Association Foundation Fund.)

128a. Mechanical and Metabolic Correlations During Regional Myocardial Ischemia in the Pig. SIDNEY GOLDSTEIN* AND JAN WILLEM DE JONG,* Rochester, N. Y., and Rotterdam, The Netherlands (introduced by David D. Thompson**).

Correlations between local mechanical and metabolic events were studied during 20 min of controlled decrease in coronary blood flow (CBF) in six open-chest baby pigs. A fixed decrease in CBF was produced using a screw slamp and flow probe around the left anterior coronary artery. Regional venous blood was obtained from a local anterior coronary vein simultaneous with arterial samples. Changes in ventricular wall thickness (VMT) were measured using a harpoon-type mercury strain gauge placed through the anterior left ventricular wall. Left ventricular pressure, peak dP/dt, and peak Vce were also measured. Blood samples for potassium (K) and lactate (L) were obtained serially. After decrease in CBF to 25% of control, VWT decreased to 47±28% (P 0.02) and remained at that level during the ischemic period, returning to 76±25% (P 0.05) with reinstitution of CBF. There was no significant change in LVEDP, peak dP/dt, or Vce during ischemia. Local venous K increased within 2 min of ischemia at which time K_{ven} exceeded K_{art} ($K_{ven} = 4.20 \pm 0.27$ mM; $K_{art} = 3.76 \pm 0.08$ mM: (P 0.05). K_{ven} fell to control level at 6 min and equaled K_{art} for the remainder of ischemia. Decreased lactate extraction was demonstrated by an increase in venous L concentration from control (L = 0.79±0.38 mM) to 1.49±0.40 mM at 2 min of ischemia (P 0.02). L_{ven} remained sig-

nificantly elevated during the remainder of ischemia and returned to control levels 10 min after reinstitution of CBF. Arterial L and K did not change significantly during the study. Thus, decrease of VWT, K release, and decreased lactate extraction demonstrate that controlled myocardial ischemia in this model causes major mechanical and metabolic abnormality without significant change in ventricular function when measured as a whole.

129. New Concepts in Assessing Critical Coronary Stenosis: Instantaneous Flow Response and Regional Distribution During Peak Coronary Hyperemia. L. GOULD,* K. LIPSCOMB,* AND G. HAMILTON,* Seattle, Wash. (introduced by R. Evans**).

Quantitative physiologic assessment of critical coronary stenosis has not been reported. Resting coronary flow and regional distribution are insensitive for determining critical stenosis, but flow response to hyperemic stimulus, i.e., exercise equivalent, permits quantitation of restrictions on maximum flow due to coronary lesions. Coronary flow responses to temporary occlusion and selective injection of Hypaque 75M were studied in 12 dogs with surgically implanted electromagnetic flowmeter and separate micrometer constrictor on left circumflex coronary artery. Selective Hypaque injection adequate for coronary cineangiography increased coronary flow to 3 times resting base line, peaking at 7 s and lasting 3 min, a response equivalent to hyperemia following 10 s circumflex occlusion. With progressive micrometer constriction, resting flow did not fall until 95% stenosis by micrometer scale and angiography; flow disappeared with minimal further narrowing. Hyperemia after Hypaque began to decrease with 60% stenosis and disappeared at 85% stenosis, before resting flow was affected. In intact dogs, flow response to Hypaque may be followed during arteriography with a velocity sensing, Doppler tip, coronary catheter. Myocardial images by gamma camera after left atrial injection of ^{131}I macroaggregated albumin demonstrated uniform regional distribution at resting flow in spite of 85% circumflex stenosis. However, 7 s after selective Hypaque injection, left atrial injection of Tc^{99m} macroaggregates demonstrated distinct perfusion abnormalities in region of circumflex constriction. Thus, flow distribution was normal at rest but showed marked differences due to restricted circumflex vs. normal anterior descending response after Hypaque injection. Flow response and regional distribution during coronary hyperemia caused by Hypaque are sensitive quantitative methods for assessing critical coronary stenosis with direct applicability to patients.

130. Human Uremic Serum Stimulates Net Fluid Secretion in Proximal Straight Renal Tubules In Vitro. JARED GRANTHAM,* RICHARD IRWIN,* PATTI QUALIZZA,* DONALD TUCKER,* AND FREDERICK WHITTIER,* Kansas City, Kans. (introduced by Norton Greenberger).

Renal transport of sodium and organic acids is inhibited by uremic serum. To define this relationship better, we studied the effect of serum from 11 patients with acute or chronic renal failure on fluid transport in isolated rabbit tubules. Proximal convoluted (PCT) and straight (PST) tubules were perfused at constant pressure with isotonic fluid in a bath of normal rabbit serum. The distal end of the tubule was occluded. Transtubular fluid transport rate was determined optically from the movement of an oil column in the perfusion pipet. Undiluted normal human serum in the bath decreased fluid absorption 40 and 65% in PCT's and PST's, respectively. Undiluted human uremic serum decreased absorption in PCT's 63%. More importantly, uremic serum caused PST's to actually secrete fluid. Secretory rate of PST's with uremic serum was 0.05 nl/min·mm and was reversible. Fluid secretion was also demonstrated indirectly by observing in nonperfused PST's but not PCT's that the normally collapsed lumens opened widely in uremic serum. Serum from acutely uremic rabbits possessed secretory activity but normal rabbit serum did not. Lumen expansion was detected in some uremic sera after 256-fold dilution with normal rabbit serum. Uremic serum stimulated secretion of fluid in PST's was inhibited by cooling, ouabain, and probenecid. Secretory activity of serum was removed by dialysis but not by boiling. Paraminohippurate and benzoate caused fluid secretion in PST's but urea, creatinine, guanidinosuccinate, and urate did not. We suggest that relatively high concentrations of a secretory factor in the serum of uremic patients may significantly influence the transport of salt and water in residual nephrons.

131. Degradation of Thyroid Hormones by Thyroid Tissue In Vitro. WILLIAM L. GREEN,* Seattle, Wash. (introduced by C. J. Goodner).

Several similarities between phagocytosing leukocytes (PL) and active thyroid tissue have been reported, including the ability to organify iodine by peroxidase-catalyzed reactions. The demonstration that iodothyronines (ITh) are rapidly degraded by PL (Klebanoff and Green. 1973. *J. Clin. Invest.* **52**: 60) and that myeloperoxidase may be involved in this process, prompted a further examination of ITh metabolism by the thyroid. Thyroid minces and slices from iodine-deficient rats were incubated in KRP with ^{125}I-labeled thyroxine (T4) or triiodothyronine (T3); [^{131}I] Na was also added, and its metabolism determined as an index of iodide peroxidase activity. Labeled substrates and products were separated by paper chromatography. In confirmation of Haibach's report (1971. *Endocrinology* **88**: 918), this system can degrade [^{125}I] T4, and T3 comprises 20–30% of the labeled products. Significant degradation of T3 was also seen. ITh metabolism was inhibited by 1 mM propylthiouracil, as it is in PL. However, 1 mM azide, 1 mM cyanide, and hypoxia, which inhibit ITh metabolism in leukocytes and iodide metabolism in both tissues, had much less effect on thyroidal ITh breakdown. Also, the enhancement of ITh deiodination produced by 1 mM methimazole in PL did not occur in the thyroid preparations, although the usual inhibition of iodide metabolism by methimazole was observed. It is concluded that ITh degradation in the thyroid is mediated by a system which differs in several respects from the peroxidative systems responsible for ITh breakdown by PL and for iodide organification in the thyroid. The existence of discrete systems in the thyroid for metabolism of iodide and of ITh makes it possible that ITh breakdown is a separate control point for the regulation of thyroid secretion. (Supported by NIH grants AM 15810 and AM 05331.)

132. Cutaneous Vasoconstriction Due to Prostaglandin B_2—a Potential Model for Raynaud's Phenomenon. STANLEY GREENBERG,* JAMES A. ENGELBRECHT,* AND WILLIAM R. WILSON,** Iowa City, Iowa.

Raynaud's phenomenon is characterized by digital vasospasm and enhanced reactivity of digital vessels to cold. This study was done to evaluate the effects of prostaglandin B_2 (PGB_2) on cutaneous vascular resistances and responses of the perfused canine paw to intra-arterial norepinephrine, tyramine, and nitroglycerin, and exposure of the paw to cold (4°C, 90 s) and to heat (45°C, 30 s). Responses were obtained before and during intra-arterial infusions of PGB_2 (0.14–2.24 nmoles/kg per min—rates without effect on ar-

terial pressure). Data were expressed as change in perfusion pressure (ΔPP) for constrictor stimuli or percent change (%ΔPP) for dilator stimuli. In five dogs, ΔPP averaged 56±SE 25 mm Hg after PGB_2 (0.14 nmoles) and 228±41 mm Hg after 2.24 nmoles. The pressor responses to norepinephrine (0.1–1.0 μg) were not altered during PGB_2 (all doses) compared to those before PGB_2. Pressor responses to tyramine (50 μg) were enhanced from 61 to 96 mm Hg during PGB_2 (0.14 nmoles). The pressor response to cold increased from 34 to 53 mm Hg with PGB_2 (0.14 nmoles). The dilator response to nitroglycerin was unaffected but that to heat was reduced by 33 and 54% after low and high doses of PGB_2, respectively. The data suggest that PGB_2 enhances the release of norepinephrine from adrenergic nerves since it enhances the vasoconstrictor responses to tyramine and cold but not vasoconstrictor responses to norepinephrine. Its abilities to produce intense cutaneous vasoconstriction, enhance the response to cold, and antagonize that to heat make this preparation a potentially useful model for Raynaud's phenomenon and suggest that a prostaglandin, perhaps PGB_2, may play a role in cutaneous vasospastic disorders. (Research supported by VA-TR-105 and grants from NHLI.)

133. Abstract withdrawn.

134. Metabolism of Plasma Very Low Density Lipoproteins (VLDL) by Isolated Adipocytes. RICHARD C. GROSS,* La Jolla, Calif. (introduced by Henry O. Wheeler **).

Impaired removal of triglyceride (TG) from plasma VLDL may be important in the genesis of hyperlipoproteinemia. To study regulation of this process, rat epididymal adipocytes isolated by Rodbell's method were incubated with human VLDL labeled with [1-^{14}C] glyceryl trioleate. After 15 min incubation, fat cells contained 5–8% of medium radioactivity per 10^6 cells, and the rate of net uptake was decreasing. Radioactivity appeared in all cell lipid classes, with > 80% in TG at 15 min. Cell lipid radioactivity increased most rapidly in diglycerides, rising to 15% of total at 15 min, while the proportion of label in TG fell slightly. Medium TG decreased 30–50% in 15 min. Free fatty acids increased to 25–25% of medium radioactivity during the first 5 min, then fell gradually to 30% of peak levels. The role of lipoprotein lipase (LPL) was assessed using diethyl *p*-nitrophenylphosphate, diisopropylfluorophosphate, and apolipoprotein Ala. These inhibitors of lipase activity depressed hydrolysis of medium TG by 3–80% and incorporation of radioactivity into cells by 25–60%. Though hydrolysis of medium TG was inversely proportional to concentration of apolipoprotein Ala, cell uptake was inhibited by only 25% at all concentrations used. At ratios of VLDL TG to cell number ranging from 40 to 270 nmoles TG per 10^6 cells, 15-min values for hydrolysis plus uptake of medium TG never exceeded 50% of VLDL TG initially added. These studies demonstrate that rat adipocytes avidly hydrolyze and incorporate human VLDL TG and that this uptake is dependent at least partially on hydrolysis by LPL. They also suggest that not all TG initially present in VLDL can be hydrolyzed by these cells. (Research supported by NIH grant HL-13119 and AHA grant 70-1023.)

135. Induction of Intravascular Platelet Aggregation in the Heart by Stress. JACOB I. HAFT* AND KAZEM FANI,* New York (introduced by L. J. Soffer **).

In vitro, catecholamines will cause intravascular platelet aggregation in vessels of the dog heart. To determine if catecholamines produced endogenously during stress are sufficient to cause similar intravascular aggregation of platelets in heart vessels, 16 rats were stressed by immersion in ice water until fatigue (25–45 min) (group A); 8 rats were similarly immersed in hot water (50°C, group B); 15 rats were placed in a chamber with an electrified grid floor and stressed by the delivery to their paws of 150 V (250 ohms) 80-ms shocks 12 times per min for 3–5 h (group C); 14 were not stressed and served as controls (group D). The hearts of the 53 rats were studied with the electron microscope. All rats in group A, 7 of the 8 rats in group B, and 13 of the 15 rats in group C were found on electron microscopic study to have platelet aggregates in the small vessels of the heart varying from a few platelets adherent to each other and to the vessel wall to complete occlusion of the vascular lumina by aggregated platelets. 13 of the 14 control hearts (group D) were free of any evidence of intravascular platelet aggregation. It is concluded that stress can cause intravascular aggregation of platelets in the heart. In patients who suffered myocardial infarction or sudden death during stress, one mechanism may be via intravascular platelet aggregates that form at or travel to sites of atherosclerotic narrowing in the coronary tree and cause acute coronary occlusion. If the thrombogenic theory of atherosclerosis is valid, these findings may help to explain the high incidence of coronary disease among aggressive, driving, frequently stressed individuals.

136. Unusual Features of Human Factor VIII Antibody Induced in Hemophilic Canines. JAMES W. HAMPTON* AND RALPH G. BUCKNER,* Oklahoma City, Okla. (introduced by Stewart Wolf **).

A colony of beagles with no antihemophilic factor (factor VIII) activity in an X-linked genetic pattern have been used as subjects to investigate the site of origin for the plasma procoagulant. To develop an experimental situation which would provide an immunological approach for study, human factor VIII was injected into hemophilic canines and the serum tested at 4, 6, and 12 wk. 1 ml (8 U) factor VIII plus 1 ml Freund's adjuvant were injected subcutaneously into each shoulder and both hips of the hemophilic beagles. Two later injections (20 U) were made at 1 and 3 wk. A human antibody to factor VIII was used for comparative purposes. At 4 wk the canine serum cross-reacted with the human VIII in two bands on immunodiffusion and neutralized the human activity by 60%. At 6 wk the human VIII was completely neutralized and the canine antihuman VIII was studied. Adsorption of the canine serum with fibrinogen did not alter the neutralization of VIII. Immunoelectrophoresis of normal human plasma and normal canine plasma against the canine anti-VIII showed that the antiserum did not react with the latter and reacted with the former in a single sharp arc. The canine anti-VIII resembled the human anti-VIII and did not react with normal canine plasma. The human anti-VIII migrated as an IGG. No cross-reaction resembling fibrinogen appeared between normal human plasma and the canine anti-VIII. Characterization of the canine antihuman VIII and the use of the fluorescent-labeled antiserum offers an opportunity to explore the cellular sites of localization of human antihemophilic factor. (Research supported by NIH grants HE-12316 and RR00412.)

137. Modification of the Primary Structure of Plasma α_2-Macroglobulin Associated with Protease Inhibition. PETER HARPEL,* New York (introduced by M. Mosesson).

Although α_2-macroglobin (α_2M) is a major circulating inhibitor of plasmin, thrombin, kallikrein, and trypsin, the biochemical nature of its interaction with these enzymes is not well defined. This investigation has examined the primary

structure of purified human plasma α_2M. A characteristic alteration in subunit structure has been identified in association with binding and inhibition of these proteases. Incubation mixtures of α_2M and each enzyme were sampled periodically and the subunit structure of α_2M was assessed by SDS-polyacrylamide-gel electrophoresis. Upon reduction with dithiothreitol, α_2M yielded a single major band whose apparent mol wt was 185,000. An additional band, mol wt 85,000, was present in the reduced α_2M-enzyme mixture. This alteration in the structure of α_2M could also be produced in a plasma environment through the urokinase-induced generation of plasmin. Formation of the hydrolytic derivative chain was dependent upon time and enzyme concentration and was accompanied by depletion of the precursor subunit chain. The hydrolytic derivative remained with the core α_2M molecule in the unreduced α_2M-enzyme mixture, indicating that the cleavage product was covalently linked by disulfide bridges. Furthermore, the electrophoretic migration of unreduced α_2M was consistent with the concept that α_2M has a urea dissociable dimeric structure with each monomeric unit consisting of two similar sized chains linked by disulfide bonds. Since only a single derivative band is observed after the α_2M-enzyme interaction, the hydrolytic cleavage occurs at or near the center of the intact precursor chain. This enzymic modification of α_2M structure may prove essential for its protease inhibition.

138. Composition of Glomerular Filtrate: Variations in Phosphate Handling in the Rat. CAROL A. HARRIS,* P. G. BAER,* AND J. H. DIRKS, Montreal, Canada.

The composition of mammalian glomerular filtrate is not accurately known due to inaccessibility of glomeruli to micropuncture in most species. Samples of glomerular filtrate (GF) were collected by micropuncture of 70 surface glomeruli in 37 hypropenic rats (Wistar-Munich strain). Tubule fluid (TF) was collected from 94 random or late proximal tubules for comparison with GF. Glomerular or tubular fluid concentrations were also related to corresponding plasma water (P) values. Mean GF/P inulin was 1.00±0.01 (SE) and was unchanged over a wide range of plasma inulin concentrations. Mean GF/P Na was 0.96±0.02 and TF/GF Na was 0.98±0.01 (not significantly different from 1). Mean GF/P Ca was 0.63±0.02 and TF/GF Ca was significantly greater than 1 at 1.08±0.03. Ultramicro phosphorus (PO_4) was analyzed and two distinct groups of rats were observed with respect to PO_4 handling. (I) Mean TF/P PO_4 was 1.02±0.01 with 100% ultrafilterable PO_4 at the glomerulus and a fractional PO_4 excretion rate of 25%: all rats were female. (II) Mean TF/P PO_4 was 0.60±0.02 with 80% ultrafilterable at the glomerulus and fractional PO_4 excretion rate of 16% and with one exception all rats were male. These data indicate that (*a*) inulin and Na are freely ultrafilterable across the glomerular membrane, (*b*) about 65% plasma Ca is ultrafiterable, and (*c*) there appear to be two identifiable groups of Munich-Wistar rats with respect to PO_4 handling defined by characteristics of glomerular ultrafiltration and reabsorption, presumably dependent on the sex of the rat. (Research supported by grant from MRC[C].)

139. The Effects of Chronic Phosphate Depletion on Renal Glucose Transport. H. HARTER,* A. MERCADO,* E. RUTHERFORD,* K. HRUSKA,* E. SLATOPOLSKY,* AND S. KLAHR, St. Louis, Mo.

Previous studies suggesting a relationship between phosphate and glucose transport involved the acute infusion of both sodium and phosphate. Sodium loading is known to depress TmG. No chronic studies in a nonexpanded state have been performed to investigate this suggested coupling between phosphate and glucose transport. We have, therefore, examined the kinetics of glucose reabsorption by the kidney during chronic phosphate depletion. Classic glucose titration studies were performed in eight normal phosphatemic dogs fed a synthetic diet and 1200 mg of supplemental phosphorus. The studies were repeated after several weeks of phosphate depletion at serum phosphate levels of 1 mg/100 ml or less. During phosphate depletion GFR fell from a mean of 73 to 61 ml/min ($P < 0.001$), but the average TmG value rose slightly from a mean of 223 to 236 mg/min. Thus the TmG/GFR ratio increased from a mean of 3.04±0.12 to 3.87±0.14 ($P < 0.001$). Phosphate depletion increased the splay of the glucose titration curves in seven of the eight dogs studied. Administration of PTH during phosphate depletion decreased the TmG/GFR ratios from 3.87 to 2.65 despite a 5-fold decrease in the filtered load of phosphate and the absence of phosphaturia. Also, the increased splay observed during phosphate depletion returned to normal during PTH administration. Fractional Na excretion was less than 1% even during maximal glycosuria. These studies clearly demonstrate a relationship between phosphate and glucose transport. The data also suggest that PTH may depress renal glucose reabsorption through a mechanism which is independent of phosphate transport. The changes observed in the splay of the glucose titration curve during phosphate depletion and PTH administration provide new insight into the physiological mechanisms responsible for the splay.

140. Effect of Adrenergic Blockade on Exercise-Induced Hyperglucagonemia. W. HARVEY,* G. FALOONA,* AND R. UNGER,** Dallas, Tex.

Striking hyperglucagonemia occurs during intense exercise in dogs. To gain insight as to the mechanism of this response, studies were designed to determine if adrenergic blockade alters exercise-induced hyperglucagonemia. 15-h fasted male Sprague-Dawley rats received intraperitoneally either 1 ml saline control (Sal), 4 mg phenotolamine (Phe), or 1 mg propranolol (Pro); half the rats were forced to swim for 45 min. Plasma glucose (G), insulin (IRI), and glucagon (IRG) were measured at that time. Results:

	Resting Rats			Swimming Rats		
	G (mg%)	IRI (μU/ml)	IRG (pg/ml)	G (mg%)	IRI (μU/ml)	IRG (pg/ml)
	Means±SEM			Means±SEM		
Sal	133±5	19±1	98±17	74±5	15±6	642±111
Phe	129±6	98±25	81±6	100±4	72±12	84±8
Pro	134±7	16±3	83±4	59±3	4±0.5	803±80

S = $P < 0.01$

In summary (*a*) in fasted rats, forced swimming causes hyperglucagonemia and a falling glucose, and (*b*) hyperglucagonemia is blocked by α- but not β-adrenergic blockade. In contrast to Iversen's and Luyckx and Lefebvre's reports of β-receptor control of IRG secretion, these findings favor suppression of exercise-induced IRG secretion and stimulation of IRI secretion by α-receptor blockade. (Supported by NIH grant AM 02700-15; Hoechst Pharmaceutical Company, Kalamazoo, Mich.; Pfizer Laboratories, New York; Bristol Myers Company, New York; Mead Johnson Research Center, Evansville, Ind.; Lilly Research Laboratories,

Indianapolis, Ind.; Wm. S. Merrell and Company, Cincinnati, Ohio; and Dallas Diabetes Association, Dallas, Tex.)

141. Infectious and Immunological Determinants in Chronic Mycoplasmal Arthritis of the Mouse. H. J. HARWICK,* G. M. KALMANSON,* M. A. FOX,* AND L. B. GUZE, Los Angeles, Calif.

Mice inoculated intravenously with *Mycoplasma pulmonis* develop lifelong migratory arthritis. Early, acute intra-articular and periarticular inflammation is seen histologically. Later, proliferative synovitis with bone and cartilage erosion predominates. In 140 mice sacrificed from 1 to 46 wk after infection, 93% developed arthritis; *M. pulmonis* was recovered from 30% of inflammed joints. No correlation between duration of infection and recovery of mycoplasmas was found, but among culture-positive joints, level of infection correlated significantly with intensity of clinical arthritis. Anti-*M. pulmonis* antibody peaked at 18 wk. Using both indirect hemagglutination and agar gel diffusion, distinct cross-reactivity was observed between rabbit antimouse synovium serum and *M. pulmonis;* rabbit anti-*M. pulmonis* serum also cross-reacted with mouse synovium. Study of peritoneal macrophage migration revealed significant inhibition of cells from infected but not from age-matched normal mice in the presence of normal mouse synovium antigen ($P < 0.001$). *M. pulmonis* membrane or cell content antigens did not significantly inhibit cell migration. Treatment of half of 200 mice with rolitetracycline, 50 mg/kg twice daily starting at day 21 (peak of arthritic response), significantly reduced clinical arthritis after 6 wk ($P < 0.001$), as did twice weekly treatment of a smaller group with cyclophosphamide 30 mg/kg ($P < 0.05$). The relatively low recovery of mycoplasmas from inflamed joints, presence of an antigenic determinant common to both *M. pulmonis* and mouse synovium, inhibition of macrophage migration by normal synovium in infected animals, and clinical response to both antibiotic and immunosuppressive therapy all taken together suggest that both infection and an immunological response participate in the pathophysiology of this disease. (Research support from VA.)

142. Marginated Blood Neutrophils in Patients with Neutropenia. UTE HASIBA,* CARL SRODES,* AND DANE BOGGS, Pittsburgh, Pa.

Epinephrine-induced neutrophilia is due to a shift of cells from the marginal (MGP) to the circulating granulocyte pool (CGP). The present studies further define this effect of epinephrine in normal volunteers as well as MGP size in neutropenic patients. Epinephrine was infused intravenously and neutrophil response curves to doses ranging from 0.025 to 0.3 mg were sigmoid with no further augmentation of response by doses greater than 0.1 mg. Maximal neutrophilia occurred within 5 min of completing the infusion. In 11 normals, absolute neutrophils increased from 1100 to 3700/dl (mean base line of 3300/dl) and the percentage increase ranged from 36 to 132. The percentage increase tended to be greater with low normal CGP than with high normal CGP although this relationship was not present when increase was determined in absolute values. In 26 studies of 24 patients with neutropenia due to various causes, some increment in CGP was observed in all except for the one without a demonstrable CGP. The mean percentage increase was larger than normal, 167% vs. 57%. There was a significant inverse correlation between the size of the base line CGP and the percentage increment after epinephrine; for example, of seven patients with less than 200 neutrophils per dl mean increase was 362%, and of five with 1000–1400 neutrophils per dl the mean increase was 80%. These results indicate that CGP size may be misleading as respects total blood neutrophils and in a sense confirm the concept of "pseudoneutropenia," a decreased CGP with normal total blood neutrophils. However, the inverse relationship between the size of the CGP and MGP as neutropenia becomes more profound suggests that "pseudoneutropenia" may be the result of a normal physiologic mechanism rather than representing a distinct neutropenic syndrome. (Supported by NIH grant AM 14352.)

143. Use of Fluoride to Prevent Erroneously High Measurements of Human Serum Unsaturated B_{12}-Binding Capacity (UBBC); Evidence That Granulocyte-Derived Binders (Transcobalamin I and III) (TCI and III) Are a Smaller Component of Normal Circulating UBBC Than Previously Believed. V. HERBERT, J. BLOOMFIELD,* R. STEBBINS,* AND J. SCOTT,* Bronx, New York, and Dublin, Ireland.

Different laboratories report widely disparate UBBC. We observed that UBBC varied up to fourfold with time, temperature, and anticoagulant (lowest increment with Na_2-EDTA, highest with Li_2EDTA). Plasma UBBC of 10-ml aliquots of blood collected in 10 mg of Na_2EDTA + 20 mg NaF (Vacutainer #3200XF92) and immediately (T_0) centrifuged was compared with centrifugation after incubation at 23°C for 24 h (T_{24}). The components (TCI, TCII, TCIII) of UBBC were determined by DEAE and Sephadex chromatography. Blood samples collected in NaF-Na_2EDTA had lowest UBBC and contained < 10% combined TCI and TCIII; UBBC at T_{24} was almost identical with T_0. T_0 Na_2-EDTA blood samples, unless kept cold, had higher UBBC than those containing NaF, and had still higher UBBC at T_{24}. Lithium increment was totally blocked by NaF. T_0 UBBC in NaF was slightly lower with lithium present. Incubation of leukocytes rather than whole blood yielded identical increment in UBBC. Increments were all TCIII. Other studies from our laboratory suggest granulocyte granules are the source of TCI and TCIII. These studies suggest that UBBC in vitro measures circulating UBBC without "artefact" increments *only* when blood is collected in high concentration of an agent such as NaF (47 mM) capable of blocking leukocyte degranulation. The same may prove true for measuring serum levels of acid phosphatase, lysozyme, and other enzymes derived from leukocyte granules. In preliminary studies, 1 mM Na arsenate prevented in vitro increments in UBBC as fully as 47 mM NaF, but 1 mM 2-deoxyglucose or NaF only partially prevented increments, and 1 mM 2,4-dinitrophenol, Na azide, KCN, or methotrexate had no effect. (Supported by NIH, VA, and Health Research Council, New York City.)

144. A New Isozyme of α-1,4-Glucosidase Relevant to the Diagnosis of Pompe's Disease and Maltase Deficiency and Showing Genetic Polymorphism. ROCHELLE HIRSCHHORN,* DALLAS SWALLOW,* AND HARRY HARRIS,* New York and London, England (introduced by Blas Frangione).

Two forms of the enzyme α-1,4-glucosidase (maltase) with different electrophoretic mobilities and pH optima have previously been described in tissues from normal individuals. One of these isozymes, with an acid pH optimum, is deficient in both the infantile (Pompe's disease) and adult type of maltase deficiency. We have found a third form of human α-1,4-glucosidase which is present in only about 6% of two populations studied (15 of 239, London; 4 of 99, New York). This new isozyme has an acid pH optimum and is inhibited by turanose, as is the isozyme involved in Pompe's

disease. However, using starch-gel electrophoresis, this new isozyme can clearly be distinguished from the two previously described acid and neutral pH isozymes, as well as from the tissue-specific kidney isozyme. This new isozyme can neither be generated in vitro from acid α-glucosidase by treatment of tissue extracts with neuraminidase, sulphydryl reagents, or "aging," measures known to lead to formation of secondary isozymes, nor can its electrophoretic mobility be altered by these treatments. It is a true maltase, hydrolyzing maltose as well as artificial substrates. The enzyme has a wide tissue distribution, being found in lung, kidney, spleen, and brain of a single individual. Studies of placentae from monozygotic and dizygotic twins suggest that its presence is genetically determined. The activity of this previously undetected isozyme, when present, may interfere with prenatal diagnosis of Pompe's disease and modify the phenotypic expression of the adult and childhood diseases associated with maltase deficiency. (Research supported by grant AI-10343 from NIH.)

145. Efficacy and Mechanism of Action of Chenodeoxycholic Acid in Gallstone Dissolution. ALAN F. HOFMANN, JOHNSON L. THISTLE,* TIMOTHY C. NORTHFIELD,* AND RUDY G. DANZINGER,* Rochester, Minn.

The emphasis by Small that formation of bile which is supersaturated with cholesterol (lithogenic) is necessary for cholesterol gallstone formation and growth, together with the observation of Thistle and Schoenfield that chenodeoxycholic acid (CDC) administration to patients with cholesterol gallstones causes bile to become unsaturated, suggested that CDC treatment should cause gallstone dissolution. To test this, we initiated studies designed to (*a*) compare efficacy of CDC with that of cholic acid or placebo in the treatment of radiolucent gallstones; (*b*) define intestinal absorption of CDC and its effect on the decreased bile acid frequently present in this condition; (*c*) measure effect of CDC on lithogenicity of fasting gallbladder bile; and (*d*) quantify effect of CDC on daily secretion of biliary lipids, using a perfusion technique. In seven patients receiving CDC for 1–3 yr, gallstones dissolved in four and continue to diminish in size in three. In 14 patients receiving CDC for 6 months, gallstones became smaller in eight; in a matched group of patients, no response to cholic acid or placebo occurred during this time interval. CDC was well absorbed and the CDC pool expanded in relation to the amount absorbed. The total bile acid pool also increased and became composed predominantly of CDC (> 90%). Cholic acid synthesis fell, causing a decrease in the cholic acid pool. Fasting gallbladder bile, obtained by duodenal sampling, became unsaturated in cholestrol, indicating that dissolving gallstones did not fully equilibrate with bile, i.e., stone dissolution was rate limiting. CDC significantly decreased the number of hours during which lithogenic bile was secreted, because less cholesterol was secreted for any given rate of bile acid output than before treatment. CDC appears to offer a rational and efficacious treatment of relatively asymptomatic radiolucent gallstones in patients with functioning gallbladders. (Research supported in part by a grant from NIH.)

146. Specific Neutralization of Human Hepatitis A in Marmosets. A. W. HOLMES,* L. WOLFE,* G. G. FROESNER,* B. CASTO,* AND F. DEINHARDT,** Chicago, Ill.

The transmission of human hepatitis to marmosets has been reported previously. Though subsequent confirmatory data were obtained from other laboratories, it has been suggested that the hepatitis observed in inoculated marmosets does *not* result from the transmission of a human virus, but from the activation of a latent marmoset virus. Twenty samples, coded outside the laboratory, have been inoculated into marmosets. Of these, ten were from six individuals with hepatitis A (five aliquots of one specimen), one was from a patient with hepatitis B, and nine were control samples from five individuals (five aliquots of one specimen). All ten unknowns from the hepatitis A patients induced hepatitis A in marmosets. No hepatitis was observed in marmosets inoculated with control materials. As anticipated, the specimen from the patient with hepatitis B did not induce disease. Thus, all hepatitis A unknowns were infectious for marmosets, and inoculation of normal human plasma did not induce or activate hepatitis in marmosets. Neutralization of infectivity by convalescent human serum would offer further proof of the human origin of the infectious agent. In one experiment infectious plasma was incubated with autologous convalescent serum. In another, infectious plasma from one individual was incubated with convalescent serum from another, and in a third study a pool of three infectious plasmas was concentrated on a CsCl gradient and the infectious fraction thus obtained was incubated with convalescent serum. In each case, infectivity was neutralized. Preinfection serum from the donor of the convalescent serum did not impair infectivity. The correct identification of a substantial number of coded specimens and the neutralization of infectivity by convalescent *human* serum prove conclusively that the virus causing hepatitis in marmosets in these studies is of human and not marmoset origin. (Supported by U. S. Army.)

147. Molecular Basis for the Regulation of Purine Biosynthesis De Novo in Man. EDWARD W. HOLMES, JR.,* JAMES B. WYNGAARDEN,** AND WILLIAM N. KELLEY, Durham, N. C.

Previous studies have established a critical role for phosphoribosylpyrophosphate (PRPP) and purine ribonucleotides in controlling purine biosynthesis *de novo* in man. We have recently demonstrated that the first reaction unique to purine biosynthesis, which is catalyzed by glutamine phosphoribosylpyrophosphate amidotransferase (PRPP amidotransferase), is the site at which PRPP and purine ribonucleotides exert their effect in man. The basis for this observation was an analysis of the kinetic and regulatory properties of human PRPP amidotransferase. We found that the apparent K_m for PRPP (4.8×10^{-4} M) was 10–100 times higher than the known intracellular concentration of this substrate, the enzyme demonstrated synergistic inhibition by 6-hydroxy and 6-amino purine ribonucleotides, and the substrate velocity plot for PRPP was changed from a hyperbolic to a sigmoidal function by purine ribonucleotides. However, the molecular basis for the regulation of human PRPP amidotransferase and consequently purine biosynthesis by PRPP and purine nucleotides was not well defined. The following observations now explain the regulation of purine biosynthesis *de novo* at the molecular level. (*a*) Two forms of human PRPP amidotransferase are identified by sucrose ultracentrifugation ($S_{20,w} = 6.5$ and 11.3). (*b*) Purine ribonucleotides convert the small active form of the enzyme (mol wt 140,000) to the large inactive form (mol wt 280,000) by a dimerization reaction. (*c*) PRPP dissociates the large inactive form of the enzyme to the small active form. (*d*) The relative proportion of the active to the inactive form of PRPP amidotransferase depends on the relative concentration of PRPP and purine ribonucleotides, respectively. These physiologically induced changes in the conformation of human PRPP amidotransferase provide a molecular basis for understanding the regulation of purine biosynthesis *de novo* in man.