ABSTRACTS

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

1. Inotropic and Chronotropic Responses to Conversion from Ventricular Fibrillation in Intact Dogs and Isolated Hearts. FRANCOIS M. ABBOUD, DONALD G. PANSEGRAU,* AND HOWARD E. MAYER,* Iowa City, Iowa.

Ventricular fibrillation was induced electrically in anesthetized ventilated dogs and terminated with a 400 watt-second capacitor discharge. Conversion was associated with transient sinus node depression and increased ventricular irritability; cardiac output and arterial pressure increased. Atropine and bilateral vagotomy prevented the bradycardia and reduced the incidence of spontaneous recurrence of fibrillation; increases in cardiac output and arterial pressure were greater, and there was a tachycardia. Blood levels of catecholamines increased after defibrillation. The administration of propranolol prevented the positive inotropic and chronotropic responses. Pretreatment with reserpine depleted myocardial catecholamines and prevented successful conversion. To evaluate the contribution of peripheral circulatory arrest to the cardiac responses, a balloon catheter was inserted into the ascending aorta and inflated while cardiac output was diverted through a carotid-jugular shunt. Inflation of the balloon caused peripheral circulatory arrest without cardiac arrest or ventricular fibrillation. Restoration of normal circulation was not associated with cardiac stimulation. Studies were also carried out in the isolated perfused rabbit heart. Ventricular fibrillation was induced electrically. Coronary flow was maintained. Conversion was associated with transient bradycardia and increased myocardial contractile force (MCF). In six rabbit hearts MCF increased by an average of 75% after conversion, 52% after the administration of norepinephrine (100 ng), and 95% after the administration of calcium gluconate (2.25 mg). Corresponding values after propranolol were 5%, 7%, and 95%. Atropine reduced the negative chronotropic response. We conclude that conversion from ventricular fibrillation is associated with cholinergic and adrenergic cardiac stimulation. Cholinergic activity increases the incidence of spontaneous recurrence of fibrillation. The adrenergic stimulus does not appear to result from arrest of the peripheral or coronary circulation and does not require an intact cardiac innervation. Myocardial catecholamines, however, appear to mediate this inotropic response and are essential for successful conversion. (Supported by NIH grants HE-09835 and HE-02644.)

2. Alpha Cell Function in Man. EUGENIO AGUILAR-PARADA,* ANNA M. EISENTRAUT,* AND ROGER H. UNGER,** Dallas, Texas.

Specific radioimmunoassay of pancreatic glucagon in human plasma has heretofore been prevented by cross-reactivity of the previously available antisera with circulating enterogenous glucagon-like immunoreactivity. The chance finding of a sensitive antiserum, which cross-reacts weakly with gastrointestinal extracts and gives "0" values for plasma from depancreatized dogs, made possible these first studies of α-cell function in man. The fasting glucagon in 16 normal subjects averaged 136 $\pm$20 $\mu\mu$g/ml (SEM). Arginine, infused at 700 mg/kg per min for 40 min to stimulate glucagon secretion, induced in all 16 a significant rise within 5 min to a peak at 40 min. The mean maximal increment of glucagon was 270 $\pm$30 $\mu\mu$g/ml, of immunoreactive insulin 48 $\pm$56 μU/ml, and of plasma glucose 16 $\pm$10 mg/100 ml. In 13 presumably genetic diabetics (9 juveniles, 4 adult type), fasting glucagon averaged 150 $\pm$26 $\mu\mu$g/ml. In all 13, arginine induced a significant glucagon rise to a peak at 30 min; in 3 the rise was extreme. The mean maximal increment of glucagon was 340 $\pm$60 $\mu\mu$g/ml, of insulin 24 μU/ml (4 adult types only), and of glucose 48 $\pm$60 mg/100 ml. In 3 diabetics with fasting normoglycemia, arginine induced a 46 mg/100 ml mean glucose rise. In 4 pancreatic diabetics, fasting glucagon averaged 103 $\mu\mu$g/ml (0–150); the arginine-induced mean maximal rise of glucagon was 185 $\mu\mu$g/ml (8–340), of insulin 16 μU/ml (0–36), and of glucose 16 mg/100 ml (1–29). One patient with severe calcific pancreatitis had "0" glucagon and insulin throughout the infusion; in another, glucagon rose only 30 $\mu\mu$g/ml. The findings indicate that (1) arginine uniformly stimulated pancreatic glucagon secretion in normals and genetic diabetics (the absence of α-cell hypofunction in genetic diabetes suggests that the β-cell hypofunction may represent an isolated defect rather than part of diffuse islet involvement); (2) in severe pancreatitis, hypoglucagonemia and hypoinsulinemia may coexist; (3) arginine-induced hyperglycemia is greater in genetic diabetics than in normals or pancreatitic diabetics, suggesting that aminogenic hyperglucagonemia, when unaccompanied by appropriate hyperinsulinemia, may cause hyperglycemia. (Supported by a grant from the NIH.)

3. A Novel Mechanism for Hypocalcemia. S. AKGUN* AND D. RUDMAN, Atlanta, Ga.

Natelson et al. showed that mobilization of FFA in rabbits by hormones causes acute hypocalcemia. Present experiments revealed that injection of 0.5 mg ACTH in rabbits causes, 2 hr later, 260–680% increase in serum FFA concentration, 27% reduction in serum calcium concentration, and 500–1100% increase in adipose tissue calcium concentration. The latter level rises from 8.1 to 50 μg per g tissue; assuming that adipose organ equals 30% and extracellular fluid 20% of body weight, the increment of calcium in adipose organ accounts for 80% of the decrement of calcium in the extracellular fluid. Incubation of rabbit, rat, or hamster adipose tissue slices in the homologous serum containing 10 μg/ml ACTH or epinephrine causes, in association with lipolysis, a 40–49% decrease in medium calcium concentration (average reduction from 10.7 to 5.7 mg/100 ml) and a 150–460% in-

crease in tissue calcium concentration (average increase from 24.7 to 94.2 μg/g). The in vitro fall in extracellular calcium concentration and increase in adipose tissue calcium concentration occur only in association with lipolysis and are similar in magnitude to those observed in vivo. When the incubation medium is Krebs-Ringer phosphate with or without albumin, the lipolytic response of hamster or rabbit adipose tissue slices to ACTH is similar to that in serum, but no detectable redistribution of calcium from medium to tissue occurs. *Conclusions:* Calcium moves in vivo or in vitro from extracellular fluid into adipose tissue during lipolysis of stored triglyceride, with resultant hypocalcemia; this movement (but not lipolysis) requires the presence of an unidentified serum factor. (Research supported by grants FR-39, AM-13129, and AM-13122 from the USPHS.)

4. Cardiovascular Effects of Weight Reduction. JAMES K. ALEXANDER AND KIRK L. PETERSON,* Houston, Texas.

Nine very obese subjects (seven women, two men) in the age range 22 to 59 yr were studied by the method of cardiac catheterization before and after weight losses of 85 to 184 lb. over periods of 4 to 36 months. In each case body oxygen uptake, plasma and blood volume, cardiac output, and arteriovenous oxygen difference were reduced after weight loss. Systemic arterial pressure, elevated in four subjects, fell in all but one of the nine. With these substantial reductions in adipose tissue mass, blood flow per kilogram body weight increased. There were variable effects upon heart rate and pulmonary artery pressure at rest, with no mean change after weight reduction. Left ventricular diastolic and/or pulmonary wedge pressures rose to abnormal levels during exercise both before and after weight loss. These results have been interpreted as indicating that the circulatory effects of gross obesity are largely reversible with weight reduction. However, left ventricular dysfunction secondary to myocardial hypertrophy and reduced compliance persisted, a fact which suggests that hypertrophy did not regress significantly for periods up to 3 yr after weight loss. (Research supported by NIH grants HE-3006 and HE-5435.)

5. Environmental Factors Affecting Lymphocyte Transformation. ROBERT ALFORD* AND RICHARD BRYANT,* Nashville, Tenn. (introduced by Roger Des Prez).

Several factors affecting lymphocyte transformation were investigated because of the increasing importance of this phenomenon in clinical and basic immunology. Human lymphocytes were stimulated with PHA in medium 199 and autologous plasma. ^{3}H-thymidine uptake indicated transformation. Plasma was required. Transformation increased linearly between 0 and 5% plasma, was maximum between 5% and 25%, and did not increase above this concentration. Heating plasma to 56°C for 30 min, zymosan adsorption at 37°C, or a combination of the two did not impair transformation. Increasing osmolality with NaCl to a level of 450–500 mOsm/kg completely inhibited transformation; essentially no effect was noted at lower osmolalities. Addition of urea to the medium inhibited transformation abruptly at approximately 600 mOsm/kg. In medium 199 and 25% plasma which contained 1.6 mM Ca^{++} and 0.8 mM Mg^{++}, sodium EDTA was completely inhibitory at greater than 1.2 mM. This effect was only partially reversed by addition of excess Ca^{++}, and was not affected by added Mg^{++}. Increasing plasma concentrations partially protected against EDTA inhibition. Lymphocyte transformation may occur (1) under conditions of low plasma concentration, (2) in the absence of complement (surface-bound components have not been excluded), and (3) in markedly increased osmolality due to NaCl or urea. Inhibition by EDTA indicates that divalent cations are required. However, failure of Ca^{++} and Mg^{++} to correct fully EDTA inhibition suggests that this effect is not exclusively due to chelation of these ions.

6. A Hyposynthetic Genetic Variant of C'3F. CHESTER A. ALPER* AND FRED S. ROSEN, Boston, Mass.

Previous work has established genetic polymorphism of human C'3 detectable by electrophoresis. In Caucasians, there are two common alleles, *C'3F* and *C'3S*, which, in heterozygotes, are approximately equally expressed. Seven individuals in a kindred described earlier had one-half of the normal serum concentration of C'3 owing to autosomal dominant inheritance of a nonexpressed C'3 allele. We have observed an individual whose C'3 appeared to be indistinguishable from the common C'3FS except that the faster band contained visibly less protein than the slower band. Similar patterns were found in the serum C'3 of his son and his mother, but in none of 62 other C'3FS patterns. The C'3 concentration in these three sera was normal (133–157 ml/100 ml), as was total hemolytic complement. By elution from stained electrophoretic patterns it was determined that whereas the common C'3FS contained 50.1% F and 49.9% S, in the variant pattern there was 40.4% F and 59.6% S. C'3FF and C'3SS were separately isolated from plasma of C'3 homozygotes and labeled with radioactive iodine. Metabolism studies were carried out in the propositus, a C'3FF individual, and a C'3SS individual. Each subject received ^{125}I-C'3FF and ^{131}I-C'3SS in a single intravenous injection. The fractional catabolic rates of the two forms of C'3 were similar in each subject and were within the previously determined normal range. The synthesis rate of C'3F in the individual with the variant pattern was 0.35 mg/kg per hr, whereas that of C'3S was 0.61 mg/kg per hr. In the C'3FF and C'3SS subjects, "synthesis rates" of C'3F and C'3S were similar. These findings describe a hyposynthetic genetic variant of C'3F inherited as an autosomal dominant trait. (Supported by USPHS grants HD-02723 and AI-05877.)

7. Altered Biosynthesis of Lactic Dehydrogenase (LDH) Subunits in Myocardium and Skeletal Muscle of Congenitally Cyanotic Patients. MICHAEL ALTMAN,* JAMES SCHEUER,* AND EUGENE D. ROBIN,** Pittsburgh, Pa.

Human LDH isoenzymes consist of tetramers of two polypeptide subunits, M-LDH and H-LDH. Tissues with high degrees of O_2-requiring metabolism generally contain predominantly H-LDH, whereas tissues with high glycolytic capacity contain predominantly M-LDH. Since enzyme activity of the H type is more inhibited by rising pyruvate concentrations than is that of the M type, it has been suggested that the type of LDH plays an important role in reg-

ulating anaerobic glycolysis versus oxidative phosphorylation. In these studies the electrophoretic patterns of LDH from myocardium and skeletal muscle of congenitally cyanotic patients were compared with the patterns from noncyanotic subjects. All in the cyanotic group represented specimens obtained during corrective surgery for cyanotic tetralogy of Fallot. In the acyanotic group, one was obtained during surgery for acyanotic tetralogy of Fallot; the remainder were obtained at autopsy from patients who were not cyanotic during life. The relative amounts of H and M subunits were calculated from the patterns, and the results are expressed as the ratio of M to H. In the six control patients, M/H ratios averaged 0.56 $\pm$0.04 (SE). In the five patients with cyanotic heart disease, M/H ratios averaged 0.77 $\pm$0.07 (SE). These ratios were significantly different ($P < 0.05$). Only two of the five patients showed any overlap. M/H ratios in skeletal muscle averaged 1.48 in the two acyanotic patients and 2.14 in the two cyanotic patients, with no overlap. Since biosynthesis is affected in both cardiac and skeletal muscle, it appears that oxygen plays an important role in regulating LDH production. The increased synthesis during hypoxemia of M subunits suggests that LDH type plays an important role in modifying the balance between aerobic and anaerobic energy metabolism.

8. Enzyme Activity of the Intestine. JAMES W. ANDERSON* AND DAVID ZAKIM,* San Francisco, Calif. (introduced by Marvin H. Sleisenger).

The metabolic basis for specialized functions of different segments of the intestine is not established completely. Extension of our studies on the activity of glycolytic enzymes in the intestine indicates that the activity of enzymes regulating intermediary metabolism varies in different segments of intestine. Enzyme activity was measured on 105,000 *g* supernatants of intestinal mucosa obtained from 1–8, 15–22, or 48–55 cm from the pylorus and from a 7 cm long segment of terminal ileum. The total hexokinase activity of intestine of fed rats was the same in the first three segments, but was increased 3-fold in the terminal ileum ($P < 0.001$). When rats were fasted for 72 hr, the hexokinase activity fell in all portions ($P < 0.001$); but the activity was still highest in the terminal ileum. In contrast to these findings, the activity of pyruvate kinase was lower in the terminal ileum than in the proximal three segments. Pyruvate kinase activity fell slightly in all segments in fasted rats. The distribution of enzyme activity within the cell was studied. The hexokinase activity (per mg protein) of the 105,000 *g* pellet was greater in terminal ileum than in segment 2. However, the fraction of whole cell homogenate activity which was present in the particulate portion was the same in these two segments. Variations in enzyme activity in different portions of the intestine were noted for phosphofructokinase, glucose-6-phosphate dehydrogenase, and fructose-1,6-phosphate aldolase. Variations from animal to animal in the activities of pyruvate kinase, G-6-P dehydrogenase, and phosphofructokinase were seen in similar intestinal segments. These findings help to explain differences in the physiological functions of different portions of intestine and may account for the different clinical responses of patients who have had similar anatomic portions of intestine removed.

9. Water, Acidosis, and Pyelonephritis. VINCENT T. ANDRIOLE,* New Haven, Conn. (introduced by P. K. Bondy**).

Susceptibility of the nonobstructed renal medulla to infection has been ascribed to its chemical composition, particularly its hypertonicity and content of ammonia producing enzymes that inhibit complement activity. Of these, only complement inactivation (induced by ammonium chloride) has been well established as a significant factor in promoting Gram-negative bacillary pyelonephritis. Therefore, the relative roles of medullary hypertonicity (controlled by water intake) and NH_4Cl acidosis in renal medullary susceptibility to coliform pyelonephritis were investigated. To alter the chemical composition of the medulla, water intake was restricted in rats (tube fed only 15 ml water daily) and urinary osmolality increased to 2124 $\pm$494 as compared with 1019 $\pm$254 mOsm/kg in control rats tube fed 15 ml daily and allowed water ad lib. Serum Na^+, K^+, CO_2^-, Cl^-, and BUN concentrations were not significantly different in the two groups. Water restriction increased the incidence of pyelonephritis. An inoculum of *E. coli* induced gross infection in 19 of 30 water-restricted rats and in only 5 of 38 rats allowed liberal water intake. To study the effects of medullary hypertonicity and NH_4Cl acidosis on susceptibility to infection, rats were tube fed 10 ml of a 300 mM solution of NH_4Cl daily and some were allowed water ad lib. Urinary osmolality was lower in NH_4Cl-fed rats allowed tap water (Uosm 1533 $\pm$235) as compared with rats fed NH_4Cl only (Uosm 2501 $\pm$522). Concentrations of serum electrolytes and BUN were not significantly different. *E. coli* pyelonephritis was observed in 29 of 37 NH_4Cl-treated, water-restricted rats, and 7 of 21 supplemented rats, respectively. Furthermore, renal infection was observed in only 2 of 16 rats tube fed twice as much NH_4Cl (6 mM) daily and allowed water ad lib. Water intake did not significantly alter the kidney's ability to excrete an acid load, as evidenced by determinations of titratable acidity and ammonium in urine. These data, while supporting the role of NH_4Cl-induced acidosis in renal medullary susceptibility to infection, confirm that the hypertonic environment of the renal medulla is a major determinant of this tissue's unique susceptibility to infection and provides further experimental evidence for "forcing fluids" in patients with pyelonephritis.

10. A Spectrum of Cytomegalovirus Infections during Renal Transplantation. DONALD ARMSTRONG,* SAIDAPET L. BALAKRISHNAN,* LYNN STEGER,* BESSIE YU,* KURT H. STENZEL,* AND ALBERT L. RUBIN,* New York, N. Y. (introduced by Martin Sonenberg**).

A series of six patients followed for 4 to 8 months after renal transplantation were studied in an attempt to evaluate (1) whether they would develop a cytomegalovirus (CMV) infection and how this would be clinically manifest, (2) whether the infection was from an intrinsic or extrinsic source, and (3) whether there was an adequate quantitative circulating antibody response as indicated by semiquantitative immunoglobulin measurements along with specific anti-CMV complement-fixing (CF) antibody. Five of the six patients showed serological evidence of infection with CMV. Two of these appeared to be new, the others reactivation of old in-

fections. Asymptomatic infection occurred in four of the five. One patient developed "cytomegalovirus mononucleosis" along with a significant rise in antibody titer (<1:8 to 1:256), suggesting that this was the initial infection. This patient also developed a viremia lasting over a month, and during about one-half of this period she was asymptomatic. Another patient developed a viremia (despite CF antibody levels of 1:256) lasting at least a month while he was asymptomatic. In both these patients, virus isolation was achieved from the carefully separated erythrocyte layer of heparinized blood both with and without isolations from the plasma and leukocyte layer. High-titer CF antibody responses (up to 1:512) were seen in the immunosuppressed patients along with rises in IgM and IgG levels. Precipitating antibodies were also detectable by Ouchterlony tests showing up to three precipitin bands with a standard AD169 strain of CMV. In vitro studies demonstrated that CMV can persist for up to 3 days in a cell culture consisting of erythrocytes or lymphocytes and tissue culture medium at a temperature of 37°C. Atypical lymphocytosis was not seen, and replication of virus was not measurable. In established lymphocyte cell lines from patients with leukemia, CMV did not alter cell morphology, but virus could be recovered up to 65 days after infection of the cells. (Research supported by grant CA-08748 from the National Cancer Institute.)

11. Effect of Severe Hypoxia on Turnover and Synthesis of Mitochondria from Rat Heart. VACLAV ASCHENBRENNER,* MURRAY RABINOWITZ, RADOVAN ZAK,* AND K. G. NAIR,* Chicago, Ill.

Using a nonreutilizable precursor, ^{14}C–guanido-arginine, the apparent half-life of total mitochondrial protein in rat heart was found to be only 4 days. The effect of severe hypoxia on mitochondrial turnover and synthesis was studied by exposing groups of Sprague-Dawley rats to atmospheres of 4% or 5% oxygen for a period of 6 hr. Survival was over 75%. Mitochondrial proteins were labeled with ^{14}C–guanido-arginine 3 days before exposure to hypoxia. Rats were killed immediately after hypoxia, and at daily intervals thereafter. Mitochondria were isolated using Nagase and purified by multiple differential centrifugations. Electron micrographs confirmed the purity of the preparations. The specific activity (cpm/mg mitochondrial protein) of mitochondria from hypoxic rats was 10–20% lower than that of controls, reflecting dilution by a period of increased synthesis of unlabeled mitochondrial proteins. Dilution was apparent 18 hr after return to room air. The subsequent rate of decrement of specific activity of mitochondrial protein was the same as in control animals. 1 hr pulses of ^{3}H-leucine were given at various times during the 18 hr after hypoxia and at daily intervals thereafter. Incorporation into mitochondrial proteins was about 2-fold greater than controls during the first 8 hr after hypoxia, with little difference thereafter. The data indicate that there is a brisk stimulation of cardiac mitochondrial protein synthesis immediately after a period of severe hypoxia. Experiments are in progress to decide whether the increment in synthesis is a result of repair or replacement, during the posthypoxic period, of mitochondria selectively destroyed by hypoxia, or is a direct effect of hypoxia. (This research was supported by a MIRU contract and grants from the NIH and the Chicago Heart Association, Argonne Cancer Research Hospital, AEC.)

12. The Nature of the Defect in Intestinal Calcium Absorption in Chronic Renal Disease. LOUIS V. AVIOLI, SOOK WON LEE,* STANLEY J. BIRGE,* EDUARDO SLATOPOLSKY,* AND HECTOR F. DELUCA,* St. Louis, Mo., and Madison, Wis.

An intestinal defect in calcium absorption is a hallmark of disordered calcium metabolism in patients with chronic renal disease. A defect in vitamin D_3 metabolism was previously documented in the chronic uremic state and presumed to account for the associated defect in calcium absorption. To test this hypothesis, chronic uremia was experimentally induced in rats and dogs by segmental infarction of one kidney and contralateral nephrectomy, and the metabolic fate of intravenously administered ^{3}H-vitamin D_3 (^{3}H-D_3) was evaluated. The uremic animals all displayed decreased intestinal absorption of calcium as measured by ^{45}Ca transport in everted gut sacs, as well as striking derangements in ^{3}H-D_3 metabolism. The latter were similar to those previously defined in uremic subjects and characterized primarily by an increased fractional turnover of ^{3}H-D_3 and decreased plasma concentration of the biologically active 25-hydroxycholecalciferol (25-OHC) vitamin D_3 metabolite. Abnormalities in calcium absorption and vitamin D_3 metabolism were also associated with decreased 25-OHC levels in the intestinal mucosa of uremic animals. In addition, a calcium-binding protein (CaBP) routinely extracted from the intestinal mucosa of control animals was notably lacking in animals with chronic uremia. Whereas vitamin D_3–treated uremic animals demonstrated insignificant changes both in the mucosal CaBP and in intestinal ^{45}Ca transport, uremic animals treated with 25-OHC responded with significant increments in both parameters. The results collectively suggest that a biologically active vitamin D_3 metabolite normally controls the synthesis of an intestinal calcium-binding protein. They also imply that the decrease in calcium absorption characteristic of the chronic uremic state stems from an acquired defect in vitamin D_3 metabolism. This derangement presumably results in a decreased concentration of the vitamin D_3 metabolite in the intestinal mucosa, defective synthesis of a specific mucosal calcium-binding protein, and decreased calcium transport.

13. The In Vitro Induction of Lysosomal Enzymes by Phagocytosis. STANTON G. AXLINE* AND ZANVIL A. COHN, New York, N. Y.

Prior studies indicated that the uptake of soluble macromolecules by pinocytosis stimulated the formation of lysosomal enzymes in cultivated peritoneal macrophages. Since interiorization of both plasma membrane and substrate occurred simultaneously and continuously, it was not possible to separate their roles in enzyme induction. This problem has now been approached by pulsing macrophages with a variety of ingestible particles. Initial experiments compared the enzymatic response after the uptake of equal numbers of erythrocytes (RBC) and nondigestible particles of the same diameter

($\pm 0.5\ \mu$). Phagocytosis of RBC produced a marked increase in the levels of acid phosphatase, β-glucuronidase, and cathepsin D. The 2- to 3-fold increase in acid phosphatase was associated with a marked shift in its isozyme pattern. Puromycin inhibited the enzyme response. In contrast, phagocytosis of polyvinyl toluene, polystyrene, and insoluble starch particles produced no increase in macrophage lysosomal enzymes, although fusion of phagosome with preexisting lysosomes occurred normally. The endocytic stimulus to synthesis of inducible lysosomal enzymes, therefore, occurred at or beyond the stage of digestion. The substrate requirements are currently being investigated with purified protein aggregates and amino acid homopolymer coacervates. Natural proteins and homopolymers containing as few as two L-amino acids are effective inducers of lysosomal acid phosphatase, β-glucuronidase, and cathepsin D. Protein substrates thus induce enzymes not directly involved in their hydrolysis. Both the quantity of phagocytized substrate and its rate of enzymatic hydrolysis appear to control the level and persistence of lysosomal hydrolases. (Supported by grant PF-418 from the American Cancer Society and by USPHS, NIH grant AI-07012-03.)

14. Correction of Oxidative Deficiencies of Leukocytes in Chronic Granulomatous Disease (CGD). ROBERT L. BAEHNER,* MANFRED L. KARNOVSKY,* AND DAVID G. NATHAN, Boston, Mass. (introduced by Charles A. Janeway**).

Phagocytizing leukocytes in CGD fail to develop the normal increase in oxygen consumption, H_2O_2 production (indicated by $^{14}CO_2$ from $H^{14}COOH$), and hexose monophosphate shunt (HMPS) activity. The first two defects are due to a lack of NADH oxidase, an enzyme that produces H_2O_2. We attempted replacement of peroxide-generating activity in CGD leukocytes. Glucose oxidase was bound to latex spherules at low ionic strength. Normal and CGD leukocytes were incubated with enzyme-coated spherules and $H^{14}COOH$. The rate of catalatic formation of $^{14}CO_2$ was measured. Formate oxidation by CGD leukocytes increased 4-fold when these cells ingested the spherule-bound oxidase. Addition of glucose oxidase (not spherule bound) did not increase $H^{14}COOH$ oxidation. Formate oxidation and H_2O_2 production apparently occurred within the cells; media in which normal leukocytes had previously been incubated with uncoated spherules did not oxidize $H^{14}COOH$ in the presence of glucose oxidase unless cell lysate containing glucose and catalase was added. Production of $^{14}CO_2$ from 1-^{14}C-glucose by normal and CGD leukocytes at rest and during phagocytosis of regular and glucose oxidase–coated spherules was determined. Stimulation of $^{14}CO_2$ production was about 10-fold. In CGD cells this occurred only when glucose oxidase–coated spherules were phagocytized. Thus the oxidative defect in CGD leukocytes was corrected when peroxide was generated intracellularly. A link between HMPS activity and H_2O_2 production (normally via NADH oxidase) was demonstrated during phagocytosis. (Research supported by grant AI-08173 from the NIH and by the John A. Hartford Foundation.)

15. The Biosynthesis of Lecithins in Lung, Liver, and Heart in Dogs. JOHN BALINT, STUART BONDURANT, SAMUEL POWERS,* AND EMILIOS KYRIAKIDES,* Albany, N. Y.

To compare the metabolism of lecithins in surfactant with those in lung, liver, and heart, 12 dogs were injected with isotope mixtures containing $^{32}PO_4$ to measure de novo synthesis, ^{3}H-choline-methyl to follow the CDP-choline pathway, and either ^{14}C–methionine-methyl to follow methylation of phosphatidyl ethanolamine (PE) or 1-^{14}C-palmitic acid. Specific activities (SA, dpm/μmole P) of lecithin and PE were determined for each isotope after extraction with chloroform-methanol and by silicic acid column and thin-layer chromatography. ^{32}P SA of hepatic PE exceeded that of lung PE by a factor of 15.5 and that of heart PE by 76.2. Corresponding ratios for lecithin were 1.7 and 31.2, respectively. The ratio of ^{3}H to ^{32}P in liver lecithin was 5.8, in lung lecithin 13.5, and in heart lecithin 69.8. SA of ^{3}H in surfactant lecithin was about 10% of that in whole-lung lecithin. Methionine-methyl incorporation into lung and heart lecithin was minimal (^{14}C-methyl:^{32}P <1), but it was significant in liver (ratio $= 3.6$). Palmitate ^{14}C to ^{32}P ratios in liver and lung lecithin were 3.4 and 3.2, while in heart lecithin the ratio was 27.8 and in surfactant lecithin 8.4. These findings confirm that the methylation pathway of lecithin synthesis is insignificant in heart and lung. Based on ^{32}P incorporation as a measure of de novo synthesis, the data indicate, firstly, that choline enters myocardial lecithin primarily by exchange, and, secondly, that the turnover of palmitate in heart and surfactant lecithin, especially, is faster than that of the rest of the molecule. Further, the data indicate that surfactant lecithin is renewed much more slowly than whole-lung lecithin. (Supported by grants from the NIH and the American Medical Association, Education and Research Foundation.)

16. The Effect of Acute Hypertension on Sodium Transport by the Distal Nephron. NORMAN BANK, HAGOP S. AYNEDJIAN,* VINOD K. BANSAL,* AND DAVID M. GOLDMAN,* New York, N. Y.

In order to determine whether renal perfusion pressure might influence net transport of sodium in distal segments of the nephron, solute-free water reabsorption ($T^c_{H_2O}$), free water clearance (C_{H_2O}), and urinary sodium-potassium ratios were measured in rats during acute hypertension induced by epinephrine infusion. It was found that $T^c_{H_2O}$ was markedly suppressed when hypertension was induced during hypertonic saline loading. During hypotonic mannitol or hypotonic saline loading, free water clearance per calculated amount of sodium delivered to the loop of Henle fell abruptly when hypertension was induced. The amount of potassium in the urine, relative to sodium, also decreased during the periods of hypertension. These observations suggest that acute elevation of renal perfusion pressure inhibits net sodium transport in the medullary portions of the ascending limb of Henle's loop, perhaps in the cortical diluting segment, and in the distal convoluted tubule. Since filtration fraction rose in every experiment during the infusion of epinephrine, implying that the protein concentration in the peritubular capillaries increased, it seems unlikely that colloid oncotic pressure changes could account for the suppression of sodium transport in these

experiments. Hydrostatic pressure, on the other hand, was found to rise significantly in the capillaries adjacent to distal convoluted tubules on the surface of the kidney. We suggest that the rise in hydrostatic pressure in the peritubular capillaries was the initiating event in inhibiting sodium transport in the loops of Henle and distal convolution. Peritubular Starling forces may be important in regulating the countercurrent mechanism and the rate of sodium excretion in the urine. (Research supported by grants from the NIH and the New York Heart Association.)

17. Contractile-Element Power as a Measure of Ventricular Performance in Man. W. A. BAXLEY,* C. L. GARRARD,* C. E. RACKLEY,* M. FRIMER,* AND H. T. DODGE, Birmingham, Ala.

Left ventricular (LV) wall contractile-element power (Pce) was calculated as the product of wall stress (S) and contractile-element velocity (Vce) at peak S in 48 patients with heart disease. LV mid-wall equatorial S was 176 to 760 g/cm^2 as calculated from LV dimensions determined from biplane angiocardiograms (12 or 6/sec) and LV pressure recorded during filming. Vce was determined at peak S as the velocity of mid-wall circumference shortening per cm of circumference. Vce values were 0.16 to 2.04 cm/sec per cm. LV volume and volume curves and mass were also determined from the angiocardiograms. LV pressure and volume were related to determine stroke work (SW), which ranged from 37 to 393 g-m. Pce values were 63 to 767 g cm/sec per cm. These values for Pce were correlated with other indices of LV performance: ejection fraction (range 0.12–0.85), $r = 0.62$ ($P < 0.01$); SW/EDV, $r = 0.59$ ($P < 0.01$); and SW per g LV mass, $r = 0.81$ ($P < 0.01$). From Pce, Vce can be normalized for a common stress value by the hyperbolic expression $\text{Vce} \times \text{S} = \text{Pce}$. Such normalized Vce when compared with these other indices of LV performance gives the same results as above. This study demonstrates the use of Pce to evaluate LV performance and a method for normalizing Vce, and indicates that SW related to LV mass may be a better index of LV performance than stroke volume normalized for end-diastolic volume (ejection fraction) in man with chronic heart disease.

18. Autosomal Recessive Inheritance of Intestinal Lactase Deficiency: Evidence from Ethnic Differences. THEODORE M. BAYLESS,* NICHOLAS L. CHRISTOPHER,* AND SAMUEL H. BOYER, Baltimore, Md.

Hereditary control of intestinal lactase deficiency, and the potential for milk-induced cramps or diarrhea, has been suggested, but proof of simple inheritance has not been provided. We have examined a proposition of autosomal recessive inheritance based on ethnic differences in (*a*) prevalence of lactase deficiency and (*b*) levels of enzyme activity. In North American whites, the frequency of lactase deficiency is 10/120 (8.3%). Assuming that these individuals are homozygotes (*lac/lac*), the (*lac*) gene frequency is 0.29 and the gene frequency for nondeficiency (*Lac*) is 0.71. In the current study 4 of 38 white adults were lactase deficient. Mean enzyme activity, 0.82 units (range 0.5–1.0); mean lactase activity of the unaffected whites, 7.7 U (range 2.2–22.6). By hypothesis, these nondeficients include *Lac/Lac* and *Lac/lac* individuals. If (*Lac*) gene contribution is fixed, the mean enzyme activity contribution of each (*Lac*) can be calculated to be 9.83 U. In Baltimore Negroes, the proportion of unaffected individuals as well as their level of enzyme activity could be predicted from (1) white gene frequencies and (2) estimates of 30% western European and 70% African origin gene admixture. All immigrant African Negroes were assumed to be lactase deficient. It was predicted, without reference to measurement, that 62% of Baltimore Negroes would be lactase deficient (*lac/lac*). Deficiency (activity < 2 U) was observed in 70% (14 of 20) randomly selected Negro adults. The predicted lactase activity level in unaffected Negroes (largely *Lac/lac*) is 5.67 U, a value in close agreement with the observed mean of 4.74 U (range 2.2–8.5) in 13 unaffected Negroes. Alternative propositions of multiple loci and of dominant inheritance did not agree with the observations. The consistent agreement between predicted and observed values suggests that lactase deficiency is inherited as a recessive autosomal trait and that the gene is common in all Americans. (Supported by NIH grants TI-AM-5095, IRO1-10581, and 5-K3-AM-6308-07.)

19. Effects of Immunization of Baboons with Human Thyroid Tissue. G. N. BEALL,* P. M. DANIEL,* O. E. PRATT,* AND D. H. SOLOMON, Torrance, Calif.

Because thyroid-stimulating activity which resembled the long-acting thyroid stimulator (LATS) was found in the serum of rabbits after immunization with thyroid microsomes, but the rabbits showed no evidence of thyroid hyperfunction, we elected to do a comparative study in baboons. Five adolescent baboons of both sexes were immunized with the microsomal fraction of human thyroid. Thyroid-stimulating activity was measured by a modification of the McKenzie bioassay. Three of the baboons developed large amounts of thyroid-stimulating activity in their serum (response indices greater than 500), two did not. All developed thyroglobulin antibodies. Thyroid function and ocular position remained unchanged in all. These findings were similar to those previously seen in rabbits. Serum thyroxine (T_4) binding by gamma globulin was found in the rabbits, but was not prominent in the baboons. The thyroid-stimulating activity and thyroid function were suppressed by the administration of triiodothyronine (T_3) to the baboons. In contrast, equal numbers (4 out of 6) of both T_4- and saline-treated rabbits developed thyroid-stimulating activity after immunization. The serum thyroid stimulator had all the characteristics of TSH: 2 hr/8 hr response ratio well over 1.0; a pattern of delayed elution from DEAE-cellulose and Sephadex G-200; extractability with NaCl-ethanol; complete neutralization by anti-human TSH antiserum and lack of neutralization by anti-human gamma G. These findings were in marked contrast to the evidence that the thyroid stimulator in rabbits was a gamma globulin. Biopsies of the baboon thyroid demonstrated a severe thyroiditis, the severity of which correlated with the presence of the thyroid stimulator in the serum. Histological changes in rabbit thyroid have been negligible. These major differences in the effects of heteroimmunization in rabbits and baboons suggest that human response to autoimmunization

may also differ and such differences might determine whether thyroiditis or Graves' disease resulted.

20. Contractile Protein of Platelets and Endothelial Cells. CARL G. BECKER* AND RALPH L. NACHMAN,* New York, N. Y. (introduced by Paul Reznikoff**).

Human platelets and megakaryocytes contain a contractile protein, thrombosthenin, which has biochemical properties resembling those of smooth-muscle actomyosin. Endothelial cells have recently been shown to contain an intracellular component antigenically similar to uterine smooth-muscle actomyosin. In order to define further the potential biochemical and functional interrelations of platelets and endothelial cells, we have examined the antigenic relations of the contractile protein present in these separate cell systems. Thrombosthenin was extracted from washed human platelets. Antisera were made in rabbits and conjugated with fluorescein isothiocyanate. Fluoresceinated antithrombosthenin absorbed with lyophilized platelet-free plasma brilliantly stained human endothelial cells of arteries, veins, capillaries, sinusoids, and heart and also stained arterial and uterine smooth muscle. Immunofluorescence was completely inhibited by absorption with purified thrombosthenin and blocked by unfluoresceinated antithrombosthenin serum. This antiserum did not stain striated cardiac or skeletal muscle. Fluoresceinated anti-uterine actomyosin brilliantly stained endothelial cells, platelets, megakaryocytes, and vascular and uterine smooth muscle. Antiserum to purified uterine actomyosin precipitated thrombosthenin on immunodiffusion in 0.6 M KCl agar. Antithrombosthenin markedly inhibited thrombosthenin ATPase activity and slightly inhibited purified uterine actomyosin-ATPase activity. Striated cardiac and skeletal muscle actomyosin ATPase activities were not inhibited by prior incubation with the thrombosthenin antiserum. These studies indicate that platelets and smooth muscle cells contain structurally similar actomyosin proteins. The results further suggest that similar contractile proteins are present in endothelial cells. The presence of a similar or possibly identical contractile system in platelets and endothelial cells may be essential for the normal development of the hemostatic process. (Supported by grants from the NIH and the American Cancer Society.)

21. Demonstration of Defective Hepatic Bilirubin Clearance (C_{BR}) in Gilbert's Syndrome (GS) Using Unconjugated Bilirubin (^{14}C-UCBR). PAUL D. BERK,* JOSEPH R. BLOOMER,* ROBERT B. HOWE,* AND NATHANIEL I. BERLIN,** Bethesda, Md.

Recent studies indicate that some patients with GS have low-grade hemolysis. Nevertheless, the plasma UCBR concentration ($\overline{BR}$) in such cases, as well as in some patients with overt hemolysis (OH), is elevated out of proportion to the rate of bilirubin production (BRP). In order to investigate the relation between hepatic function, $\overline{BR}$, and RBC survival, plasma clearance studies with ^{14}C-UCBR were performed in 3 nonhemolyzing patients with GS, 10 patients with OH, and 19 normals. The data were used to calculate C_{BR}, BRP, and the mean red cell life span (RBCLS). In normals, $\overline{BR} = 0.4 \pm 0.1$ mg/100 ml, ^{14}C-UCBR retained in plasma at 4 hr (R_{BR}) $= 5.0 \pm 1.9\%$, $C_{BR} = 47 \pm 10$ ml/min, and RBCLS $= 101 \pm 12$ days. In GS, abnormal bilirubin kinetics ($R_{BR} = 18$–30%, $C_{BR} = 15$–21 ml/min) resulted in increases in $\overline{BR}$ up to 1.9 mg/100 ml in the absence of hemolysis (RBCLS $= 97$–119 days). Five of the patients with OH had normal bilirubin kinetics ($R_{BR} = 4.3$–5.8%, $C_{BR} = 23$–53 ml/min), and in only one of these (RBCLS $= 8$ days) was $\overline{BR}$ greater than 1.0 mg/100 ml. The other four maintained $\overline{BR}$ below 1.0 mg/100 ml, despite RBCLS $= 37$–59 days. The last five patients had similar rates of BRP and hemolysis (RBCLS $= 11$–63 days). More marked elevations of $\overline{BR}$ (2.3–7.7 mg/100 ml) resulted from abnormalities in hepatic function ($R_{BR} = 16.2$–40.6%, $C_{BR} = 5$–21 ml/min) similar to those of GS. Standard liver function tests (LFT) and biopsy (done in 4 of 5) were also compatible with GS. In all 32 subjects, there was good agreement between the $T_{\frac{1}{2}}$ of ^{51}Cr-labeled RBC and RBCLS calculated from ^{14}C-UCBR kinetics ($r = 0.86$). These studies indicate that (1) the diagnosis of GS should be based on demonstration of a reduced C_{BR}, irrespective of the presence of hemolysis, provided that standard LFT are normal; and (2) clearance studies with ^{14}C-UCBR are useful for the clinical evaluation of hepatic function and RBCLS.

22. The Correction of Glutathione Reductase Deficiency by Riboflavin Administration. ERNEST BEUTLER, Duarte, Calif.

Most erythrocyte enzyme deficiencies have been associated with relatively uniform clinical findings. Glutathione reductase (GR) deficiency is unique in this respect: low levels of GR have been found in patients with a variety of hematologic and nonhematologic disorders and even in clinically well subjects. Recently, the finding that erythrocyte GR is a flavine enzyme has suggested a possible relation between riboflavin metabolism and GR activity. Subjects with one-half or less the normal red cell GR activity were identified by screening hospital personnel and patients. The addition of 1 μM flavine adenine dinucleotide (FAD) to the assay system increased by 2- or 3-fold the GR activity of hemolysates prepared from GR-deficient erythrocytes from several otherwise normal subjects without signs of nutritional deficiency. The addition of FAD to normal hemolysates produced only 0–75% stimulation. Both normal and GR-deficient erythrocyte FAD levels were between 0.25 and 0.5 μM, as estimated fluorometrically. The GR of normal and deficient hemolysates was "stripped" of FAD with acid. The K_m for FAD was found to be approximately 0.02 μM both for normal and for deficient enzyme. Administration of only 5 mg of riboflavin daily for 5 days to a deficient subject resulted in a doubling of GR activity with a modest rise of erythrocyte FAD levels. Erythrocyte GR in normal subjects increased only slightly with a similar change in FAD levels. The GR activity of the erythrocytes of all GR-deficient subjects was not equally sensitive to FAD; it seems probable that GR deficiency is a heterogenous disorder. The riboflavin-responsive type is unique among erythrocyte enzyme deficiencies in that the activity of the deficient enzyme from nutritionally normal subjects can rapidly be restored to normal by incubation with coenzyme in vitro or by vitamin administration in vivo. (This work was supported in part by a grant from the NIH, HE-07449.)

23. Selective Regulation of Deoxycorticosterone (DOC), Corticosterone (B), and Aldosterone. E. G. BIGLIERI, M. SCHAMBELAN,* AND P. E. SLATON, JR.,* San Francisco, Calif.

Urinary excretion of tetrahydro-DOC (THDOC), tetrahydro-B (THB), and the acid-labile conjugate of aldosterone, measured by double-isotope dilution derivative techniques, was used to assess adrenal production of DOC, B, and aldosterone in six normal subjects during specific maneuvers. A single infusion of ACTH (25 units i.v. for 8 hr) effected a 3- to 10-fold increase in all three steroids, but continued daily infusions for 3–17 days sustained the increase of only THDOC and THB. After cessation of ACTH, aldosterone production was reduced and refractory to stimulation by sodium restriction (20 mEq/day). 2 days of dexamethasone suppression of ACTH had little effect on aldosterone but reduced THDOC and THB by more than 50%. Sodium restriction increased production of aldosterone but not of THDOC and THB. Administration of B (200 mg/day), DOC acetate (DOCA, 20 mg/day), or 9α-fluorocortisol (9FF, 600 μg/day) during sodium restriction did not affect excretion of THDOC and THB, but after "escape" from DOCA or 9FF on sodium intakes of 120 mEq/day, excretion of aldosterone was virtually abolished but levels of THDOC and THB remained unchanged. The level of aldosterone production may condition the maximum response of DOC and B to ACTH, since the daily infusion of ACTH for 3 days during sodium restriction produced higher levels of THDOC and THB than when ACTH was similarly administered after "escape" from 9FF. In summary, within a group of biologically similar adrenal steroids the principal mineralocorticoid secreted is determined by differing regulatory influences. DOC and B are primarily ACTH dependent and are influenced little by conditions that increase or decrease aldosterone secretion.

24. Leukokinetic Studies in Neutropenic Patients. C. R. BISHOP* AND J. W. ATHENS, Salt Lake City, Utah.

To assess neutrophil kinetics in neutropenia, 31 patients with stable neutrophil counts $<1800/mm^3$ were studied by the in vitro $DF^{32}P$ method. The total blood granulocyte pool was small in all subjects. On the basis of half disappearance time ($T\frac{1}{2}$) and granulocyte turnover rate (GTR), three neutropenic groups were identified. *Group I:* In seven patients a short $T\frac{1}{2}$ (<3.5 hr) and GTR greater than twice normal indicated accelerated destruction neutropenia. These subjects had cirrhosis or Felty's syndrome. Splenomegaly was present in six. *Group II:* In nine subjects a short $T\frac{1}{2}$ and GTR less than twice normal suggested the combined effect of accelerated destruction and inadequate production. A variety of clinical disorders were represented in this group; splenomegaly was present in five. *Group III:* In 15 subjects a normal $T\frac{1}{2}$ and a GTR less than normal indicated decreased production as the only mechanism. This group included drug toxicity, hematologic malignancy, and idiopathic neutropenias without splenomegaly. Measurements of (*a*) marrow granulocyte reserve; (*b*) marrow cellularity on aspiration and biopsy; (*c*) in vitro marrow labeling index with ^{3}H-TdR; and (*d*) serum lysozyme values did not correlate with the kinetic studies. In vivo $DF^{32}P$ kinetic studies in four patients from group I were compared with similar studies in five patients from group III. No deviation from normal myelocyte to blood transit time (phase I + II) was present in either group. However, in two group I subjects, myelocyte generation time appeared to be short ($T\frac{1}{2}$ phase III = 1 and 1.7 days). (Research supported by grants from the NIH.)

25. Hypercatabolism of Several Serum Proteins in the Wiskott Aldrich Syndrome. R. MICHAEL BLAESE,* WARREN STROBER,* AND THOMAS A. WALDMANN, Bethesda, Md.

The Wiskott Aldrich syndrome is a sex-linked recessive disease with thrombocytopenia, eczema, and increased numbers of infections. The patients have depressed antibody responses and are deficient in many natural antibodies in spite of normal amounts of total serum gamma globulins. The metabolism of IgG, IgA, IgM, and albumin was studied in five patients using purified radioiodinated proteins. The serum level of IgM in the patients was moderately but significantly decreased owing to depressed synthesis. IgM survival was normal. Serum concentrations of IgG (9.6 $\pm$2.7 mg/ml versus normal 8.9 $\pm$2.8), IgA (4.8 $\pm$2.2 versus 1.6 $\pm$1.1), and albumin (43 versus 43 $\pm$5) were all normal or elevated in the patients as compared with age-matched controls. The survival of each of these proteins, however, was significantly shortened, with half times of 7.5 days for IgG (normal 22 $\pm$2), 2.9 days for IgA (normal 6 $\pm$1), and 9.3 days for albumin (normal 17 $\pm$2). The fractional catabolic rates were significantly increased to 19.8% of the intravenous pool per day for IgG (normal 6.7 $\pm$2), 48% for IgA (normal 25 $\pm$4), and 16% for albumin (normal 10 $\pm$2). Thus, for each of these proteins increased rates of synthesis are clinically masking significant hypercatabolism. In the case of IgA, the synthetic rate averaged almost 5 times normal. Gastrointestinal albumin clearance studies using ^{51}Cr-albumin showed a slight degree of gastrointestinal protein loss, but this could account for only a small fraction of the hypercatabolism observed. There was no proteinuria or abnormality of thyroid, adrenal, renal, or liver function. Therefore, since the previously recognized causes for increased serum protein catabolism were not operative, the hypercatabolism of IgG, IgA, and albumin in these patients should be added to the primary abnormalities of this sex-linked disorder.

26. Defective Oxidation of Pyruvic Acid in a Child with an Intermittent Movement Disorder. JOHN P. BLASS,* JOEL AVIGAN,* AND WILLIAM UHLENDORF,* Bethesda, Md. (introduced by James H. Baxter).

An inherited defect in the oxidative decarboxylation of pyruvic acid has been demonstrated in a patient with intermittent ataxia recently studied in our laboratory. The patient is an 8 yr old boy who since infancy has had episodes of a combined cerebellar and choreoathetoid movement disorder occurring with stress. He has hyperpyruvicemia, less marked hyperalanemia and hyperlacticemia, and hyperalanuria. We developed methods for studying his metabolic abnormality in dilute suspensions of white blood cells and cultured skin fibroblasts. Oxidation of 1-^{14}C–pyruvic acid to $^{14}CO_2$ was low

both in the patient's white cells (patient, 1.4 cpm/10^4 cells; controls, 10.0–46.4 cpm/10^4 cells) and in his fibroblasts (patient, <0.4 cpm/10^8 cells; controls, 4.0–11.0 cpm/10^8 cells). The defect has also been demonstrated in preliminary experiments with cell-free sonicates of fibroblasts. Oxidation of U-^{14}C–glutamic acid and 1-^{14}C–palmitic acid by the patient's cells was normal, as was incorporation of U-^{14}C–alanine into protein. The concentration of pyruvic acid in his father's blood and the rate of oxidation of pyruvic acid by his father's fibroblasts (2.0 cpm/10^8 cells) were intermediate between those of the patient and the normal ranges. Available information on pyruvic acid metabolism is consistent with the hypothesis that the patient's symptoms could accompany altered demands on an already reduced cerebral pyruvic dehydrogenase. Another patient with the combination of hyperpyruvicemia and an intermittent movement disorder has recently been described. This combination may characterize a distinct syndrome in which the metabolic error is etiologically related to the symptoms.

27. Hypoglycemia Associated with Intrathoracic Mesothelioma: Studies and a Proposed Mechanism. SHELDON J. BLEICHER* AND FAIZUR CHOWDHURY,* Brooklyn, N. Y. (introduced by Stanley Wallach).

The association of fasting hypoglycemia with a large extrapancreatic tumor was observed in a 72 yr old man with a 2300 g mesothelioma. Preoperatively, fasting plasma glucose concentrations were 24 to 52 mg/100 ml; plasma immunoreactive insulin (P-IRI) was barely detectable and responded poorly to oral glucose or i.v. tolbutamide. Significantly, parenteral glucagon evoked a prompt hyperglycemic response despite the presence of hypoglycemia. After removal of the tumor, fasting plasma glucose concentrations were normal, and P-IRI rose briskly after oral glucose or i.v. tolbutamide. Assays of homogenized tumor for IRI or for insulin-like activity by three bioassays (rat fat pad, rat hemidiaphragm, rat intraperitoneal assay of Rafaelson) revealed less than 2 units of insulin in the entire tumor. Tumor slices incubated with glucose, ^{14}C-glucose, and free fatty acids (FFA) took up 5 mg glucose per g tumor per hr, did not utilize FFA, and were unresponsive to added insulin. When projected for 24 hr, as much as 276 g glucose would be taken up by the tumor, approximating this patient's daily hepatic glucose output. Although the tumor contained 8.3 g/100 g of glycogen, it was devoid of glucose-6-phosphatase activity, indicating that the rise of plasma glucose after glucagon was of hepatic origin. The patient's central nervous system apparently did not perceive his hypoglycemic state: he was asymptomatic, and plasma cortisol, growth hormone, and FFA concentrations were not higher preoperatively, during hypoglycemia, than postoperatively during normoglycemia. We propose that fasting hypoglycemia associated with these tumors is the consequence of two interrelated factors: loss of glucose from the circulation (to body tissues and the tumor) at a rate exceeding hepatic glucose output, and failure of hypothalamic centers to activate the mobilization of available hepatic glycogen, owing to the gradual evolution of hypoglycemia.

28. Hepatitis Virus and Australia Antigen. BARUCH S. BLUMBERG, BETTY JANE S. GERSTLEY,* W. THOMAS LONDON,* IRVING MILLMAN,* ALTON I. SUTNICK,* AND VERONICA ZAVATONE,* Philadelphia, Pa.

Our impression that Australia antigen, Au(1), may be a hepatitis virus has been strengthened by recent findings. Au(1) is identified in human sera by immunodiffusion using human, rabbit, and mouse anti-Au(1) antisera. (1) Au(1) occurs early in acute viral hepatitis (post transfusion, 21 of 51, 41.1%; infectious, 28 of 129, 21.7%). (2) It is common in institutionalized Down's syndrome (DS) patients but rare in other mentally retarded patients, and absent in DS outpatients. DS patients with Au(1) have chronic anicteric hepatitis. (3) Au(1) is common in certain chronic diseases (leukemia, Hodgkin's, chronic renal diseases, lepromatous leprosy). These patients show immunologic defects and evidence suggesting chronic anicteric hepatitis. (4) Isolated Au(1) is a virus-like particle 200 A in diameter. (5) Liver cells of patients with acute and chronic viral hepatitis who have Au(1) in their blood have fluorescent granules in their nuclei when stained with fluorescein-tagged anti-Au(1). Granules are not seen in cells of patients without viral hepatitis. (6) Patients transfused with donor blood subsequently shown to contain Au(1) may develop hepatitis with Au(1) in their blood, or may develop anti-Au(1) antibodies. (7) Millions of apparently normal people living (primarily) in the tropics have persistent Au(1). In these populations susceptibility to chronic infection with Au(1) appears to be controlled by genes segregating at a single autosomal locus. Individuals homozygous for the gene (Au^1/Au^1) are the susceptibles. We interpret these data as follows: (*a*) Australia antigen is an antigen of a hepatitis virus, Au(1) virus; (*b*) Au(1) virus can cause acute and chronic hepatitis or can persist in asymptomatic carriers. Differences in clinical manifestations are related to differences in genetic and immunologic factors in the host. (Supported by NIH grants CA-08069 and CA-06551, the World Health Organization, and the Commonwealth of Pennsylvania.)

29. Suppression of Pyrogen Release from Human Leukocytes by Estradiol. P. BODEL,* G. M. DILLARD,* S. KAPLAN,* AND S. E. MALAWISTA,* New Haven, Conn. (introduced by Elisha Atkins).

A number of naturally occurring steroids affect temperature regulation in man. The pyrogenic action of etiocholanolone, as well as its suppression by cortisone, can be demonstrated in vitro with human blood leukocytes. Estrogens are thought to lower body temperature in women, and have been reported to suppress both natural and induced fevers in man. In order to investigate the mechanism for these antipyretic actions, the effects of estradiol on leukocytes were examined. Human blood leukocytes were incubated in a 22% serum-buffer medium, with and without added steroids, and stimulated to release pyrogen by addition of heat-killed staphylococci. After 18 hr incubation, supernatant fluids were injected into rabbits for pyrogen assay. Human leukocytes, preincubated with 17β-estradiol, 25 μg/ml, released one-third as much pyrogen as did normal cells, although phagocytosis was

not impaired. The normal increase in oxygen consumption and glucose C-1 oxidation with phagocytosis was also reduced, and there was significantly less granule lysis. When progesterone was substituted for estradiol, however, pyrogen release was normal, even though oxygen consumption was comparably suppressed. These and other studies of hormonal actions on cell respiration, granule lysis, and pyrogen production revealed a clear dissociation of these normally associated aspects of cell function. Pyrogenic steroids do not cause fever in rabbits. Studies of the effects of estrogen on production of pyrogen in rabbits failed to demonstrate any inhibitory action of the hormone, either in vivo or in vitro. Estradiol, like actinomycin D and puromycin, only suppresses formation of human endogenous pyrogen during the early induction phase of production of this protein. Further studies of the action of steroid hormones on production of endogenous pyrogen may elucidate certain aspects of temperature regulation in man as well as some mechanisms of hormonal control of cell function.

30. Granuloma Induction by a Soluble Factor from Schistosome Eggs. Dov L. Boros* and Kenneth S. Warren, Cleveland, Ohio.

Granuloma formation around schistosome eggs in mice is accelerated and augmented by previous exposure to eggs. This reactivity is transferred by cells rather than serum and responds to various immunosuppressive measures, suggesting that schistosome egg granuloma is a form of delayed hypersensitivity. A soluble factor has now been obtained from schistosome eggs which sensitizes mice to granuloma formation around intact eggs. Eggs, or materials prepared from them, were injected intraperitoneally. 1 wk later, intact eggs were injected intravenously. After 8 days resultant pulmonary granulomas were measured. Mean granuloma volume in unsensitized animals averaged 16 ± 0.8 and in sensitized animals $38 \pm 2.1 \times 10^{-4}$ mm^3. Intact heat-killed eggs sensitized mice; eggs ruptured by grinding in phosphate-buffered saline (pH 7.2) at room temperature did not. Grinding at 0°C provided material which sensitized mice. After centrifugation of this material at 100,000 *g* for 2 hr, 0.1 ml (equivalent to 1000 eggs, protein content < 1 μg) of the clear supernatant also sensitized mice. The sensitizing soluble factor (SF) in this supernatant was stable at 23° and 37°C for 2 hr and for months at — 30°C. SF from immature unembryonated eggs was inactive, that from hatched embryos (without eggshells) was active. Intact or ruptured eggs implanted intraperitoneally within diffusion chambers and fluid from eggs cultured in vitro sensitized mice. SF activity, although unimpaired by DNase, was abolished by trypsin and RNase. The active factor was neutral electrophoretically. Immunoelectrophoresis of a rabbit antiserum to SF and an egg homogenate revealed two faint precipitin lines. A soluble factor has been isolated from schistosome eggs. In apparently minute amounts and without adjuvant it sensitizes mice to a delayed hypersensitivity-type response. This substance may also be involved in the initiation of the granulomatous reaction. (Research supported by NIH and Rockefeller Foundation grants.)

31. Heterogeneity of Red Blood Cell Membrane As Revealed by Phospholipase C. M. H. Bowman,* C. E. Mengel,* A. C. Ottolenghi,* and S. P. Balcerzak,* Columbus, Ohio (introduced by Wallace N. Jensen).

The effect of phospholipase C from *Bacillus cereus* on erythrocytes was studied as an approach to understanding the structure of the cell membrane. Washed red cells were incubated at 37°C with the enzyme, and the reaction was stopped at intervals with *o*-phenanthroline. After as little as 30 sec of incubation, multiple, punctate depressions were apparent on RBC surfaces examined by the scanning beam electron microscope. These defects were located principally in the central concavity of the cell and not on the edge. Cells so injured often tended to be more spherical than biconcave. After 20 min of incubation, almost all cells exhibited similar surface defects and became spherical. At this time cell osmotic fragility measurements showed two distinct populations. Membrane lipid phosphorus was lost throughout the incubation and reached 26% by 20 min in association with a decreased MCV. Hemolysis began shortly thereafter and was complete by 50 min, at which time 65% of the original lipid phosphorus had been lost and the MCV had diminished to 50% of initial values. Ghosts formed during this reaction were observed by phase and transmission electron microscopy to have isolated, uniform-sized sudanophilic electron-opaque spots on the membrane. These data suggest that hemolysis by this enzyme occurs in the following sequence: hydrolysis of exposed phospholipid with loss of lipid phosphorus, production of visible surface defects, osmotic swelling with formation of spherocytes, and lysis by osmotic force. The uneven distribution of the ghosts indicates a heterogeneous surface distribution of phospholipid available to the action of phospholipase C. (Research supported by NIH training grant 5-TO1-CA-05192.)

32. Cardiovascular Effects of Elevated Cerebrospinal Fluid (CSF) Pressure. Richard E. Brashear,* Indianapolis, Ind. (introduced by Joseph C. Ross).

A variety of incompletely defined factors involving the central nervous system have been associated with pulmonary hypertension and edema. This is a study of the relations between acute elevations of CSF pressure and pulmonary hemodynamics. Heart rate, cardiac output, central blood volume (CBV), stroke volume, systemic and pulmonary vascular resistances, arterial blood gases, and pressures in CSF, aorta, pulmonary artery (PA), left ventricle (end diastolic, LVEDP), and right atrium (RA) were measured in 10 anesthetized, paralyzed dogs (24 ± 3 kg) at a control CSF pressure and 2, 6, 10, 20, and 30 min after elevation of CSF pressure. Pressure was increased by injecting 100 ml of saline (38°C) into the cerebellomedullary cistern over a 4 min period. CSF and aortic pressures were 8 ± 1 and 145 ± 8 before and 24 ± 10 and 181 ± 31 mm Hg 2 min after increasing CSF pressure, and remained elevated ($P < 0.05$) for 6 min. PA pressure increased from a control 15 ± 4 to 28 ± 9 mm Hg at 2 min and remained elevated ($P < 0.05$) for 10 min. LVEDP increased from 4 ± 2 to 18 ± 6 mm Hg at 2 min and remained elevated for 30 min ($P < 0.05$). RA pressure increased from 1 ± 1 to 4 ± 3 mm Hg at 2 min (P

< 0.05). Heart rate was unchanged. Cardiac output and stroke volume increased from 199.3 ±44.2 and 1.10 ±0.21 to 294.9 ±119.3 ml/min per kg and 1.61 ±0.66 ml/beat per kg after 2 min and remained elevated ($P < 0.05$) for 10 min. Pulmonary vascular resistance was increased ($P < 0.05$) for 30 min, while systemic resistance was unchanged. CBV increased from 26.5 ±4.1 to 38.2 ±9.0 ml/kg after 2 min and remained elevated ($P < 0.05$) for 20 min. These findings suggest a shift of blood volume from peripheral vessels to the lungs during CSF pressure elevations.

33. Relation between Peritubular Capillary Protein Concentration and Fluid Reabsorption by the Rat Proximal Tubule. B. M. BRENNER,* K. H. FALCHUK,* R. I. KEIMOWITZ,* AND R. W. BERLINER,** Bethesda, Md.

It has been proposed that proximal sodium reabsorption is regulated in part by the rate of capillary removal of reabsorbate and that this removal is dependent upon the balance of Starling forces acting across the capillary wall. We determined the effect of graded changes in capillary colloid osmotic pressure (COP) on proximal reabsorption, using recollection micropuncture techniques. Tubule fluid to plasma inulin ratios, nephron filtration rates, and capillary protein concentrations were measured before, during, and after rapid (15–25 sec) intra-aortic injections (at level of renal artery) of 2 ml of colloid-free, isoncotic, and hyperoncotic solutions. Fractional reabsorption declined in 26 of 26 tubules in the 15–25 sec during colloid-free infusions (mean change, −36.2 ±3.8% [SE]), increased in every tubule during 9–10 g/100 ml albumin (+26.6 ±7.6%, n = 10) and 15 g/100 ml albumin infusions (+41.5 ±6.6%, n = 6), and remained unchanged during isoncotic infusions (−0.2 ±3.3%, n = 16). Fractional as well as absolute reabsorption varied directly with the change in capillary protein concentration (mean change during colloid-free infusion, −21.6 ±4.1%; isoncotic, −0.5 ±2.8%; hyperoncotic, +16.2 ±3.1%). The roles of GFR and hematocrit were excluded as factors mediating these changes in reabsorption. Furthermore, the rapidity with which these divergent adjustments in reabsorption were elicited excludes an extrarenal humoral factor and makes unlikely one of intrarenal origin. 20 min after fluid injection, capillary protein concentration approximated control values. Although reabsorption after colloid-free solutions also returned to control, after colloid infusions (which led to expansion of ECF volume) reabsorption declined progressively. These results indicate that capillary COP is one important determinant of proximal sodium reabsorption. Nevertheless this mechanism is inadequate to explain the delayed inhibition of reabsorption that accompanies expansion of ECF volume by colloid solutions.

34. The Role of Erythrocyte Intermediates in "Programing" for Oxygen Release in the Normal Human and in Hypoxic Diseases. GEORGE J. BREWER AND JOHN W. EATON,* Ann Arbor, Mich.

Recent work of others has demonstrated that physiological concentrations of 2,3-diphosphoglycerate (DPG) and ATP greatly decrease hemoglobin oxygen affinity. We have previously shown (1) a negative correlation between DPG levels and blood hemoglobin levels, and (2) significant elevation of levels of DPG in altitude-acclimatized people (known to have decreased oxygen affinity). These findings suggest that variation in DPG levels significantly influences hemoglobin function. We hypothesize that this occurs through an effect on rate of oxygen release. Under physiological conditions, erythrocytes never come to complete equilibrium with tissue oxygen tensions. Therefore the rate of oxygen release is important in determining oxygen delivery. A technique has been developed which allows measurement of the rate at which freshly drawn erythrocytes release oxygen. There is variation among individuals in this rate, and a significant negative correlation exists between desaturation rate and whole blood hemoglobin levels (e.g., 20 women: $r = -0.55$; $P < 0.02$). Women have a significantly greater mean desaturation rate than men, a fact which suggests one reason for the females' ability to attain adequate tissue oxygenation with lower hemoglobin levels. Levels of DPG plus ATP in erythrocytes are significantly and positively correlated with desaturation rates in normal individuals of both sexes (e.g., 20 women: $r = +0.46$; $P < 0.05$), accounting for part of the programing for oxygen release. The mean desaturation rate is significantly increased in hypoxic conditions, such as anemia or pulmonary disease. The mean level of DPG plus ATP is also markedly and significantly elevated in these diseases, a fact which probably explains the increase in rate of oxygen release. These findings indicate that variation in the metabolism and in the levels of metabolic intermediates in the red cell, by affecting desaturation rates, plays a vital role in the maintenance of normal tissue oxygen tensions in health and disease.

35. Demonstration of Cholesterol Feedback Deletion in Intact Hepatoma-Bearing Rats. LEE A. BRICKER* AND MARVIN D. SIPERSTEIN, Dallas, Texas.

Though we have previously demonstrated deletion of hepatic cholesterol feedback regulation of mevalonate synthesis in isolated animal and human hepatomas, it is not known (1) whether such uncontrolled synthesis of sterols can be detected in tumor-bearing animals or (2) whether the sterols synthesized by hepatomas are released into the bloodstream. The influence of exogenous cholesterol on the ability of hepatomas to synthesize and release sterols into plasma was therefore examined by determining plasma desmosterol in triparanol (MER-29)–fed rats bearing minimal deviation hepatoma 7787 in which hepatic steroidogenesis was blocked with a high-cholesterol diet (HCD). MER-29–fed normal rats showed marked HCD suppression of plasma desmosterol (28 ±7 vs. < 1.0 mg/100 ml), while in hepatoma-bearing rats desmosterol was 54 ±2 mg/100 ml on a low-cholesterol diet (LCD) and 56 ±11 on the HCD. A similar loss of feedback control was demonstrated in rats implanted with a different minimal deviation hepatoma, 9121; i.e., blood desmosterol concentrations on MER-29 + HCD in normal rats was < 10 mg/100 ml, and in hepatoma 9121–bearing rats, 93±18 mg/100 ml. Finally, the concentration of blood desmosterol in hepatoma-bearing rats treated with MER-29 and a HCD could be directly correlated ($r = 0.77$) with tumor size. These experiments therefore demonstrated that (1) a functional cholesterol feedback system is operative in intact nor-

mal rats; (2) by contrast, in rats bearing either of two types of minimal deviation hepatoma, dietary cholesterol causes little or no suppression of steroidogenesis; (3) finally, hepatomas can clearly release newly synthesized sterols into the circulation. Hence, these studies represent the first in vivo evidence of deletion of a feedback mechanism in tumor-bearing animals. (Research supported by NIH grants CA-08501 and CA-05200, and DRG-747.)

36. Regulation of Gene Expression in Reticulocytes by Changes in Ribonuclease Activity. EDWARD R. BURKA,* Philadelphia, Pa. (introduced by Franklin R. Miller**).

The increase in the rate of protein synthesis caused by addition of RNA to the reticulocyte cell-free system (RCFS) has been ascribed to the template properties of the added material. Direct proof of such template activity is lacking. The present studies indicate that the increase in protein synthesis induced in the RCFS by RNA cannot be wholly attributed to direct genetic influences, but that alteration of a nongenetic factor, RNase activity, is instrumental in the effect. Supplementation of the RCFS with reticulocyte RNA stimulated ^{14}C-labeled amino acid incorporation into protein as much as 2-fold over control values. Simultaneously, the inherent RNase activity in the RCFS, assayed by degradation of minute amounts of added native ^{32}P–reticulocyte RNA, was decreased. RCFS RNase activity degraded both tRNA and rRNA (rate = 10%/hr at 37°C) and did not require energy. Experimental factors common to both stimulation of protein synthesis and inhibition of RNase activity were temperature dependence, concentration dependence, maximal effect at a concentration of added RNA of 3 mg/ml (94% stimulation of ^{14}C incorporation and 50% inhibition of RNase activity), and occurrence of both effects with addition of whole RNA, rRNA, or tRNA. Hemin and Co^{++}, which also stimulate the rate of protein synthesis in the RCFS, cause a similar concentration-dependent inhibition of RNase activity. The similarity between conditions under which exogenous RNA or hemin simultaneously stimulates protein synthesis and inhibits RNase activity in the RCFS indicates a close relation between the two effects. Thus, the action of RNA in stimulating protein synthesis in the RCFS cannot be ascribed totally, if at all, to inherent template activity. The results indicate that the expression of genetic information in the reticulocyte can be moderated, at least in part, by alteration of RNase activity. (Supported by grants from the USPHS.)

37. The Cell Membrane: A Common Site of Action of Thyrotropin and Long-Acting Thyroid Stimulator. GERALD BURKE,* Chicago, Ill. (introduced by Eric Reiss).

We have recently shown that the initial interaction of both thyrotropin (TSH) and long-acting thyroid stimulator (LATS) with the thyroid is rapid, firm binding to a superficial cell site, possibly the cell membrane. In order to evaluate those structural features of the cell membrane requisite for hormonal action in the present study, sheep thyroid slices were treated with phospholipase C, which is known to affect membrane phospholipids, and the subsequent response to TSH or LATS as measured by ^{32}P incorporation into phospholipids was examined. Phospholipase C (5–25 μg/ml) abolished this response although basal and acetylcholine-stimulated phospholipogenesis were unaffected, demonstrating the specificity of hormonal inhibition. Thus, a membrane phospholipid appears to be important for the action of both TSH and LATS on the thyroid cell. Since phospholipid may play an important role in membrane transport of ions, the effects of cations and ouabain on TSH and LATS stimulation of ^{32}P incorporation into sheep thyroid slice phospholipid were studied. Omission of Na^+ from the buffer markedly decreased base-line incorporation and abolished the response to both stimulators. Addition of 12 mM Na^+ restored TSH and LATS action without increasing base-line incorporation. Ca^{++} and Mg^{++} were not essential for TSH or LATS stimulation of ^{32}P incorporation. Ouabain (10^{-4} M) inhibited the effects of both stimulators on phospholipogenesis. These findings indicate that both TSH and LATS stimulation of ^{32}P incorporation into phospholipids have an absolute dependence on Na^+ and are similarly influenced by the ionic composition of the medium. It would thus appear that despite their marked physicochemical and immunologic differences, the in vitro effects of the two stimulators are mediated in similar fashion, presumably via interaction with the thyroid cell membrane. (Research supported by grant AM-11136 from the NIH.)

38. IgE Deficiency Associated with Chronic Sinopulmonary Infection. WILLIAM A. CAIN,* ARTHUR J. AMMANN,* RICHARD HONG,* KIMISHIGE ISHIZAKA,* AND ROBERT A. GOOD,** Minneapolis, Minn.

This study was designed to investigate the role of IgE using patients with immunological deficiency diseases. Tissue levels of IgE were determined by evaluation of the response to intradermal injections of guinea pig anti–human IgE in 27 patients with various immunological deficiencies, and 50 normal individuals. All the normal subjects produced a typical wheal and flare reaction. No reactions to normal guinea pig serum were observed. 3 patients with isolated IgA deficiency and 5 with ataxia-telangiectasia had normal skin levels of IgE. 17 patients did not respond to anti-IgE. These included 11 with ataxia-telangiectasia, 5 with hypogammaglobulinemia, and 1 with isolated IgE deficiency. There was no correlation between detectability of skin IgE and serum IgD. Passive transfer of IgE was accomplished by intravenous infusions of plasma, but not by intramuscular injection of Cohn fraction II. Passive transfer was also accomplished locally by intradermal injection of serum from a ragweed-sensitive donor. Injected sites reacted, upon challenge after 24 hr, with anti-IgE or ragweed allergen, indicating normal binding to skin and normal histamine-releasing mechanisms. The patient with isolated IgE deficiency had chronic sinopulmonary disease with bronchiectasis. Serum IgM, IgG, and IgA levels, salivary IgA, humoral antibody responses, delayed hypersensitivity, and leukocyte functions were normal. Other patients with IgE deficiency either currently have chronic sinopulmonary infections or have a syndrome which includes a high probability of sinopulmonary disease. The three patients with deficiency of IgA only were free of sinopulmonary infection. It is postulated that a normal function and the selective value of IgE is protection of the sinopulmo-

nary mucosa from infection. (Supported by the NIH and the NF.)

39. Phospholipid Synthesis in Human Platelets. FRANK L. CALL, II,* CHARLES T. LUCAS,* AND WILLIAM J. WILLIAMS, Philadelphia, Pa.

Previous studies in this laboratory have demonstrated that inorganic $^{32}PO_4$ is rapidly incorporated into phosphatidic acid and phosphatidylinositol by human platelets in vitro. Since phosphatidic acid is an early intermediate in the biosynthesis of phospholipids, studies were undertaken to determine the metabolic fate of this compound. Phosphatidic acid was prepared from purified egg phosphatidylcholine by the action of cabbage phospholipase D and was further purified by column chromatography. Human platelets were collected by centrifugation, washed, and then disrupted by freezing and thawing or by homogenization. Such preparations were found to contain phosphatidic acid phosphatase as well as the enzymes necessary for two steps in phospholipid synthesis: the formation of cytidine diphosphate diglyceride (CDP-diglyceride) from phosphatidic acid and ^{3}H–cytidine triphosphate, and the formation of phosphatidylinositol from CDP-diglyceride and ^{3}H-myoinositol. The products of the synthetic reactions were identified by thin-layer chromatography. Mg was required for maximal rate of formation of CDP-diglyceride. Mn was less effective and Ca was inhibitory. The pH optimum was 7.5 in Tris-HCl buffer. The specific activity of the enzyme was greatest in the microsomal fraction of homogenized platelets, but the yield was poor. Mn was required for maximal rate of formation of phosphatidylinositol. Mg was less effective. The pH optimum was 8.5–9.0 in Tris-HCl buffer. The rates of formation of CDP-diglyceride in platelet preparations from 10 normal subjects and 12 patients with diseases known to interfere with platelet thromboplastic function were similar (0.5 ± 0.1 mμmole/mg protein per min). It is concluded that platelets are able to convert phosphatidic acid to phosphatidylinositol via CDP-diglyceride. The significance of these reactions in human disease remains to be determined. (Supported by training grant AM-5228 from the NIH.)

40. Use of Odd-Numbered Fatty Acid as a Marker in Studies of Turnover of Adipose Tissue. ROBERT G. CAMPBELL* AND SAMI A. HASHIM,* New York, N. Y. (introduced by W. Henry Sebrell**).

The present study utilizes a system whereby adipose tissue is enriched with a fatty acid (undecanoate, C_{11}) which is not synthesized endogenously, yet is incorporated into adipose tissue glycerides after its ingestion as triglyceride. In adipose tissue, C_{11} responds to factors influencing its mobilization and appears as plasma free fatty acid but not in lipoproteins. 60 weanling rats received the following diet: protein 19% of calories, carbohydrate 51%, and fat 30%, a 7:3 mixture of C_{11}-triglyceride and corn oil. At 4 wk C_{11} was discontinued, and animals were divided into two groups. Group 1 received an identical diet except that corn oil was the sole fat source. Group 2 received "fat-free" diet (2% linoleate) wherein carbohydrate constituted 70% of calories. At weekly intervals, adipose tissue samples were analyzed for their fatty acid composition. Growth rate was progessive and comparable in the two groups. At 4 wk there was a striking amount ($31.4 \pm 2.3\%$) of C_{11} in adipose tissue, representing the level reached at discontinuance of C_{11} administration. Thereafter, for 6 wk, based on decreasing content of C_{11} in adipose tissue, an exponential curve of fatty acid disappearance was observed. A semilogarithmic plot of data against time revealed two distinct rates of disappearance of C_{11}. Animals on conventional diet (group 1) displayed a faster turnover rate of C_{11} than animals on high-carbohydrate diet (group 2), with calculated half-life of 12 and 19 days, respectively. The results indicate that C_{11}-enriched adipose tissue in the intact animal provides a model for study of fatty acid turnover in response to physiologic and experimental conditions that may influence the kinetics of adipose tissue. (Supported by a grant from the NIH.)

41. Altered Synthesis Rates of Primary Bile Salts in Portal Cirrhosis and Cholestasis. J. B. CAREY, JR., R. F. HANSON,* AND B. K. MONSON,* Minneapolis, Minn.

Primary bile salts are those synthesized in the liver from cholesterol. Portal cirrhosis patients usually have a different primary bile salt predominating in their blood from patients with cholestasis. The major bile salt in blood was chenodeoxycholate in 81% of 95 portal cirrhosis patients, whereas cholate predominated in 82% of 75 patients with cholestasis. The predominance of chenodeoxycholate has been attributed to suppression of 12-alpha hydroxylase activity necessary for cholate synthesis. In order to determine whether these differences were related to changes in primary bile salt synthesis rates, the concentration ratios of the fecal bile salt metabolites (secondary bile salts) were measured by GLC and compared with those of their precursors in blood and bile on the same day. Comparison of the primary bile salt concentration ratio in blood and bile in 14 patients yielded a correlation coefficient of 0.86 ($P < 0.01$), demonstrating that the primary bile salt concentration ratio in serum is an accurate reflection of that in bile. When chenodeoxycholate predominated in blood, its metabolites (lithocholate + others) predominated in feces, and when cholate predominated in blood, its metabolites (deoxycholate + others) predominated in feces in all patients. Therefore the primary bile salt having the highest concentration in blood also predominates in bile, and its metabolites account for the major quantity of bile salts excreted in the feces. Since excretion rates must closely approximate or equal synthesis rates for each primary bile salt, it may be concluded that (*a*) the bile salt predominating in blood and bile has the greater synthesis rate, and (*b*) patients with portal cirrhosis synthesize mainly chenodeoxycholate when it is the major serum bile salt, whereas those with cholestasis synthesize mainly cholate when it predominates in serum. (Supported by a grant from the NIH.)

42. Studies in a Denervated Human Heart. RICHARD A. CARLETON, STANLEY J. HELLER,* HASSAN NAJAFI,* AND JAMES G. CLARK,* Chicago, Ill.

Cardiac catheterization of a 50 yr old man was performed 3 wk after cardiac transplantation to quantify bedside observations suggesting hyperresponse of the denervated heart to isoproterenol and to study the cardiac response to heart

rate changes, increased venous return, and exercise. During isoproterenol infusions of 0, 0.4, and 0.8 μg/min, the recipient's sinus nodal rates were 73, 77, and 83/min, while the donor sinus rates were 88, 100, and 112/min. A-V conduction time, estimated from P-R intervals during multiple-rate atrial pacing, shortened by 60 msec/μg per min of isoproterenol at any paced rate, contrasted with only 4–14 msec/μg per min in five normal subjects. Stroke volume and left ventricular end-diastolic pressure were, respectively, 40 ml/beat per m^2 and 7 mm Hg (paced rate 130/min) at rest, 47 and 10 (paced rate 96/min) at rest, and 52 and 13 (paced rate 98/min) with passive leg elevation. At 3 min of supine bicycling exercise, stroke volume was 57 with an end-diastolic pressure of 18 (rate 103/min), suggesting that the Starling effect was the dominant mechanism regulating cardiac output. Endogenous adrenergic stimulation during exercise was estimated by comparing exercise donor sinus rate and left ventricular diastolic filling times with those attained during isoproterenol infusion. Filling time, measured at multiple paced rates, decreased linearly as rate was increased; 0.4 and 0.8 μg/min of isoproterenol increased filling time by 20 and 40 msec at any rate. Neither donor sinus rate nor filling time showed adrenergic effect at 1.5 min of exercise, but both demonstrated changes equivalent to 0.4 μg/min of isoproterenol at 3 min of exercise. We conclude that the denervated human heart has (1) abnormal sensitivity to circulating catecholamines; (2) major dependence upon ventricular filling to alter performance; and (3) a belated, minor catecholamine effect during exercise.

43. Mechanisms of Pyrogenic Tolerance to Endotoxin. FRANK A. CAROZZA, JR.,* EDWARD J. YOUNG,* AND SHELDON E. GREISMAN, Baltimore, Md.

Previous studies employing continuous intravenous endotoxin infusions indicated that pyrogenic tolerance involves two temporally distinct mechanisms. The initial or early phase appeared best explicable by cellular desensitization. It was postulated that when endotoxin injections are closely spaced, optimal conditions are provided for cellular desensitization; when endotoxin challenge intervals are lengthened, desensitization wanes and tolerance becomes increasingly dependent upon antibodies which enhance reticuloendothelial clearance and destruction of toxin. The present studies test this concept. Employing a single initial intravenous injection of *Escherichia coli* endotoxin in acclimatized rabbits, pyrogenic tolerance could be readily separated into two distinct phases, early (<48 hr) and late (>48 hr). Early tolerance (*a*) was directly proportional to initial pyrogenic response, (*b*) exhibited no interendotoxin specificity, (*c*) was unassociated with increments in anti-"O" antibody. Early tolerance, however, was not a generalized refractory state; full responsiveness persisted to another pyrogen, staphylococcal enterotoxin B. In contrast, late tolerance (*a*) bore no direct relation to initial pyrogenic response, (*b*) exhibited significant interendotoxin specificity, and (*c*) coincided with increments in anti-"O" antibody. Late, but not early, tolerance was drastically reduced if initial immunizing endotoxin preparations had poor "O" antigenicity, or if endotoxins employed for testing tolerance lacked "O"-specific polysaccharide side chains. Subsequent studies employing daily endotoxin injections indicated that the protective function of anti-"O" antibody during late pyrogenic tolerance was usurped by additional humoral factors, which, unlike anti-"O" antibody, could not be suppressed by splenectomy or 6-mercaptopurine. The following hypothesis is proposed: Endotoxins are common core (toxophore)-haptene ("O"-specific polysaccharide) complexes. Cellular hypersensitivity exists to core. A single endotoxin injection evokes transient cellular desensitization, and subsequently two classes of protective antibodies, anticore and antihaptene, the latter predominating functionally. Repeated endotoxin injections evoke sustained cellular desensitization and increasing anticore and antihaptene antibodies. Protective anticore antibodies, however, are less readily suppressed by splenectomy and 6-mercaptopurine than antihaptene antibodies. This concept is currently under further investigation. (Research supported by grants from the USPHS.)

44. Complement Metabolism in Patients with Hereditary Angioedema. C. B. CARPENTER,* S. RUDDY,* I. H. SHEHADEH,* D. J. STECHSCHULTE,* H. J. MÜLLER-EBERHARD, J. P. MERRILL,** AND K. F. AUSTEN, Boston, Mass., and La Jolla, Calif.

Patients with hereditary angioedema (HAE) either lack the normal α2-globulin inhibitor of C'1 esterase, or have the inhibitor in a nonfunctional form. It has been postulated that the persistently low serum C'4 levels, measured functionally or immunochemically, in these patients are a result of accelerated utilization of C'4 as a substrate of the uninhibited C'1 enzyme. Formation of an active 4, 2a complex (C'3 convertase) has been in doubt, however, since serum C'3 levels are generally normal. Highly purified and radioiodinated human ^{131}I-C'4 (β1E-globulin) and ^{125}I-C'3 (β1C-globulin) were administered intravenously to four patients with asymptomatic HAE in order to determine their catabolic and synthetic rates. Catabolic rates for C'4 were 3.7, 5.8, 7.0, and 8.8% of the plasma pool per hour in HAE (normal, 1.8± 0.9%/hr; 2 SD), and serum C'4 levels were 0.14, 0.10, 0.04, and <0.03 μg/ml, respectively (normal 0.42 ±0.22; 2 SD). Synthetic rates of C'4 were 0.30, 0.23, 0.10, and <0.11 mg/kg per hr in the same patients (normal, 0.35–1.34). Although serum C'3 levels were normal, moderate hypercatabolism of C'3 was present: 2.6, 2.8, 2.8, and 3.2% of the plasma pool per hr (normal, 1.4 ±0.6%/hr; 2 SD). C'3 synthesis rates were normal. ^{125}I-C'4i, the in vitro product of esterase-treated ^{125}I-C'4, was rapidly removed from the plasma space of a control subject, only 25% of the injected dose remaining 10 min after injection, and less than 5% at 24 hr. The marked hypercatabolism of C'4 in HAE constitutes the first direct evidence for the in vivo destruction by uninhibited C'1 esterase of its natural substrate C'4. Moderate hypercatabolism of C'3 is also present, indicating that C'3 convertase is formed. (Research supported by grants from the NIH.)

45. Differential Effects of Porcine Proinsulin on Rat Fat Pieces and Fat Cells. DAVID R. CHALLONER,* Indianapolis, Ind. (introduced by John B. Hickam**).

Porcine proinsulin and insulin were incubated with rat epididymal fat pieces or isolated cells (Rodbell) in Krebs-bicarbonate-albumin buffer at 37°C. Dose-response curves

were determined for the effects of proinsulin and insulin on 1-^{14}C-glucose conversion to $^{14}CO_2$ and inhibition of glycerol release stimulated by 10^{-4} M theophylline. In fat cells, insulin antilipolysis appeared at 35×10^{-12} M with an apparent K value (½ maximum) of 50×10^{-12} M, and the corresponding values for proinsulin were 600 and (K) 1000×10^{-12} M. In contrast, antilipolysis in fat pieces appeared at 70 with a K of 100×10^{-12} M insulin, and the corresponding values for proinsulin were 50–180 and (K) 900×10^{-12} M. $^{14}CO_2$ production by fat cells was stimulated at 7 with a K of 50×10^{-12} M insulin; the corresponding values for proinsulin were 600 and (K) 1500×10^{-12} M. In fat pieces an effect was found at 140 $(K = 250) \times 10^{-12}$ M insulin and 600 $(K = 1000) \times 10^{-12}$ M proinsulin. In three paired 1 hr fat cell incubations with added insulin or proinsulin, immunoassayable insulin decreased by 30–50%, whereas immunoreactivity was unchanged (88–102%) with proinsulin (the anti-insulin was known to cross-react significantly with proinsulin). These results demonstrate that the biologic activity of porcine proinsulin on rat adipose tissue is significantly less than that of insulin. Contamination of the proinsulin by small amounts of insulin is unlikely but cannot be ruled out. The dose-response curves are closer with fat pieces than with isolated cells, consistently with the hypothesis that proinsulin may exert some of its effect after conversion to insulin by enzymes which would therefore seem to be located in the extracellular compartments digested away by collagenase. The fat cell membrane binding sites necessary for the disappearance of insulin from the medium (and possibly for its biologic effect) do not appear to bind or degrade proinsulin as actively as insulin. (This research was supported by grants from the NIH.)

46. Electrolyte Movements across the Gastric Mucosa: The Effects of Bile on the Permeability of Antrum and Fundus. M. L. CHAPMAN,* J. RUDICK,* W. D. DYCK,* J. L. WERTHER,* AND H. D. JANOWITZ,** New York, N. Y.

The permeability characteristics of the gastric mucosa are different in the fundus and antrum, and this may influence both the release of gastrin and the localization of ulcers in the antrum. Since increased reflux of bile into the stomach has been demonstrated in patients with active gastric ulcers, we investigated the effects of hepatic bile on the movement of H^+ and other ions across the gastric mucosa of both antrum and fundus. HCl solutions containing a nonabsorbable dilution indicator were instilled into canine antral and fundic pouches before and after the topical application of bile obtained from the T tubes of patients after cholecystectomy and choledochostomy. Bile (approximately 15 mM conjugated bile salts per 60 min) increased the loss of H^+ in the antrum from 500 ±320 μeq (SD) to 1150 ±520 μeq per 30 min, and from 390 ±220 μeq to 1650 ±560 μeq per 30 min in the fundus. Net gain of Na^+ rose from 530 ±180 μeq to 850 ±250 μeq per 30 min in the antrum, and from 340 ±170 μeq to 1460 ±620 μeq per 30 min in the fundus. Net fluid gain increased from 1.9 ±1.3 ml to 2.3 ±1.1 ml in the antrum, and from 1.6 ±1.0 ml to 4.6 ±3.2 ml in the fundus. Antral mucosa was relatively more permeable than fundic mucosa. The presence of bile reversibly increased the permeability of both, but the effects on the fundus were more pronounced, so that the fundus tended to resemble the antrum. These findings may account for the hyposecretion of acid by gastric ulcer stomachs and their increased permeability to an instilled acid load. (Supported by NIH grants AM-11580, AM-02290, and AM-05126.)

47. A Kinetic Model of Hematopoietic Stem Cell Repopulation and Differentiation. PAUL A. CHERVENICK* AND DANE R. BOGGS, New Brunswick, N. J.

Erythrocytes, neutrophils, and megakaryocytes share a common pluripotential stem cell. After hematopoietic damage by such agents as irradiation, proliferation of stem cells repopulates the stem cell pool. Differentiation of these cells restores the various mature cell lines to normal. The rate of repopulation and differentiation and the effect of an increased demand for differentiation upon the rate of repopulation were studied in irradiated mice. Stem cell repopulation rate was determined from the rate of increase in endogenous spleen colonies after irradiation. Two exposures to X-irradiation were given, the second 0–5 days after the first. 10 days later the number of colonies in the spleen was counted. Colonies per spleen increased from 0.1 to 0.7, 2.2, 5.3, and 8.8 at days 1, 2, 3, and 4, respectively. Mean doubling time for stem cells was 22 hr. The effect of an increased demand for differentiated cells upon the rate of stem cell repopulation was determined by bleeding mice after the first exposure to irradiation. No significant difference was observed in the rate of stem cell repopulation or doubling time after bleeding. Stem cell differentiation rate (erythropoietic recovery) was studied by determining iron-59 uptake by the spleen and bone marrow at daily intervals after irradiation. Iron-59 uptake increased within 2 days after 100–300 R, but after 400, 500, 600, and 700 R there was no evidence for differentiation until day 4, 6, 7, and 9, respectively. Once erythropoiesis began, the rate of increase in iron-59 uptake was similar at all doses of irradiation. These results suggest that (1) stem cell repopulation begins almost immediately after the pool size is reduced, (2) rate of repopulation is not increased by an increased demand for differentiated cells, (3) differentiation of stem cells is delayed until the stem cell compartment is repopulated to some critical size. (Research supported by grant AM-12295 from the NIH.)

48. Antibody Synthesis by Human Circulating Lymphocytes. LAWRENCE N. CHESSIN,* CAROLYN J. RINEHART,* DONALD S. SCHALCH,* AND MICHAEL W. BRANDRISS,* Rochester, N. Y. (introduced by John H. Vaughan**).

In recent studies from this laboratory, the role of the circulating lymphocyte in man has been identified in a variety of cellular immune reactions. Lymphocytes in the circulating pool are heterogeneous at both the fine-structural and functional levels. Circulating lymphocytes synthesize immunoglobulins and, in various lymphoproliferative states, have the potential for long-term proliferation in vitro. In the present study, we have examined the biosynthetic capabilities and proliferative potential of human circulating lymphocytes during the immune response to keyhole limpet hemocyanin (KLH). After injection of KLH, antibody-synthesizing cells appeared in the circulating pool. Employing isotopic labeling with ^{14}C amino acids, newly synthesized antibody molecules

were detected by radioimmunoelectrophoresis on polyacrylamide gels. Though antibody synthesis by peripheral lymphocytes could be maintained in vitro for as long as 36 days, these cells did not have the potential for long-term proliferation in vitro, in contrast to lymphocytes from patients with infectious mononucleosis and other lymphoproliferative states. (Research supported by grants from the NIH, AI-09030-01 and AI-07352-03.)

49. The Growth Fraction and Proliferative Rate of Leukemic Cells in Patients with Slowly Progressive Acute Leukemia. BAYARD CLARKSON,* MAKOTO OGAWA,* AKIO TODO,* AND JERROLD FRIED,* New York, N. Y. (introduced by David A. Karnofsky**).

Previous studies in patients with acute leukemia employing continuous infusions of ^{3}H-thymidine (^{3}H-T) and radioautographic analysis have shown that significant fractions of the leukemic populations (usually about 10%) may remain dormant (i.e. unlabeled) for at least 10 days. Three patients were given 20 day i.v. ^{3}H-T infusions to determine the proliferative rates and dormant fractions of their leukemic populations; although the patients were not receiving chemotherapy, serial marrows showed only slowly progressive leukemic infiltration. The ^{3}H-T labeling indexes (LI) of the marrow leukemic cells initially (in vitro) were: G. S. (myeloblastic), 6%; J. T. (lymphoblastic), 8%; and W. J. (myelomonocytic), 14%. Some of W. J.'s leukemic cells matured from primitive (type I) to partially differentiated (type III) cells which could no longer divide; their initial LI were: type I, 60%; II, 18%; and III, 0%. During the ^{3}H-T infusions in vivo, all leukemic mitoses were labeled after a few days and the LI of the interphase cells (marrow and blood) increased rapidly for about a week and then more slowly; after 20 days the LI of the marrow interphase leukemic cells were: G. S., 99%; J. T., 92%; and W. J., 98% (type I, 100%; II, 99%; and III, 98%). The mean generation times (T_G) of the labeled mitotic and interphase marrow leukemic cells were determined separately and were, respectively (hours): G. S., 96 and 133; J. T., 99 and 237; and W. J., mitotic (I and II) 53, and interphase (I) 52, (II) 71. Within each population, T_G was variable; the longer interphase T_G indicate that some cells entered a resting state after their parents had divided. Since previous studies in patients with rapidly expanding leukemic populations have shown similar T_G and ^{3}H-T labeling patterns, the slower progression of leukemia in these patients (especially W. J.) is more probably attributable to a higher death rate of their leukemic cells than to their slower proliferation. Chemotherapy subsequently caused remissions in all patients and immediately prolonged the T_G. The proliferative kinetics will be discussed in relation to chemotherapeutic schedules and the problem of eradicating long-term dormant cells. (Research supported by grants P-494 from the American Cancer Society, and CA-08748, CA-05826 from the NIH.)

50. Demonstration of the Polyol Pathway in Rabbit and Human Aorta. REX S. CLEMENTS, JR.,* ANTHONY D. MORRISON,* AND ALBERT I. WINEGRAD, Philadelphia, Pa.

Increased activity of the polyol pathway of glucose metabolism has been implicated in the pathogenesis of diabetic cataract and neuropathy. The polyol pathway has now been demonstrated in the intima and media of rabbit and human aorta. The sorbitol content of whole rabbit aorta (13.7 ±0.8 mμmoles/g) is significantly greater than that of blood. After incubation, the mean sorbitol concentration in paired segments of rabbit aortic intima and media rose from 24.5 to 47.9 mμmoles/g (mean Δ 23.4 ±7.0) when the medium glucose was increased from 5 to 50 mM. Thus, ambient glucose concentration appears to regulate the activity of this pathway in the aorta as previously demonstrated in lens. Two NADP-polyol dehydrogenases which catalyze the reduction of glucose to sorbitol have been isolated and partially purified from rabbit and human aorta by ammonium sulfate fractionation and DEAE-cellulose chromatography. One enzyme has the characteristics of aldose reductase and the other those of TPN-L-hexonate dehydrogenase, a component of the uronic acid cycle. The K_m for glucose of rabbit and human aldose reductases are 106 and 180 mM, respectively. Both TPN-L-hexonate dehydrogenases have K_m for glucose which are approximately 2 molar. Rabbit aortic homogenates contain a soluble NAD-polyol dehydrogenase which oxidizes sorbitol to fructose. It has been established that exposure of the lens to increased concentrations of substrate for aldose reductase results in intracellular accumulation of the corresponding polyol. This is associated with abnormalities in water, electrolyte, amino acid, and myoinositol metabolism and eventual cataract development. The demonstration of the polyol pathway in the aorta, in which transport is not rate limiting for glucose metabolism, suggests for the first time a mechanism by which hyperglycemia, itself, may lead to significant derangements in the metabolism of the arterial wall.

51. On the Renal "Threshold" for Chloride. JORDAN J. COHEN,* JOSEPH A. CHAZAN,* SERAFINO GARELLA,* AND YAACOV BAR-KHAYIM,* Providence, R. I. (introduced by Milton W. Hamolsky**).

Cl reabsorption by the renal tubule is commonly assumed, by analogy with HCO_3, to exhibit threshold characteristics. To examine this thesis, 14 dogs were made hypochloremic by ethacrynic acid and a Cl-free diet. After 1–3 days of equilibration, during which filtered Cl was completely reabsorbed, plasma Cl concentration ([Cl]p) was increased over several hours in conjunction with varying degrees of volume expansion in order to examine Cl reabsorption at different levels of fractional Na reabsorption. In five dogs [Cl]p was increased without producing volume expansion by infusing 0.5 N HCl at 1 ml/min. Both fractional Na and fractional Cl reabsorption remained above 0.99 even at [Cl]p > 120 mEq/liter. Consequently, Cl reabsorption rose progressively without exhibiting a threshold. In four dogs [Cl]p was increased by infusing isotonic saline at 0.33 ml/kg per min. The resulting volume expansion reduced fractional Na reabsorption progressively to 0.92–0.95. Only under these circumstances did Cl reabsorption per GFR exhibit a classic threshold, rising with [Cl]p to ~105 mEq/liter and remaining virtually constant thereafter. In five dogs more marked volume expansion was produced before increasing [Cl]p by infusing a solution isometric with each animal's plasma at 1 ml/kg per min for 1–2 hr. As fractional Na reabsorption fell to <0.95, isotonic saline was substituted at the same rate in order to

raise [Cl]p to >120 mEq/liter. When fractional Na reabsorption fell even farther, to <0.80, Cl reabsorption per GFR, which had risen initially to ~104 mEq/liter, fell progressively to levels as low as 80 mEq/liter. *Interpretations:* As [Cl]p is increased, Cl reabsorption does not exhibit threshold characteristics unless, fortuitously, an accompanying decrease in fractional Na reabsorption just counterbalances the increase in filtered Cl load. Cl reabsorption greatly exceeds this "threshold" when Cl is provided faster than cation can be excreted, and falls far below this "threshold," even at high [Cl]p, when fractional Na reabsorption is markedly suppressed.

52. Molecular Nature of Nonchromosomal Drug Resistance Factors (R Factors) in Bacteria. STANLEY N. COHEN,* Stanford, Calif. (introduced by David A. Rytand).

It is now well established that multiple resistance to a wide variety of antimicrobial agents can be transferred among bacteria as a single unit by nonchromosomal elements of DNA (drug resistance factors or "R factors"); these episomal elements have been shown to play a major role in the development of clinical antibiotic resistance. In the present experiments, the molecular nature of the drug resistance factor R6 (specifying resistance to chloramphenicol, streptomycin, sulfonamide, kanamycin, neomycin, and tetracycline) has been studied by ultracentrifugation, electron microscopy, and DNA-RNA hybridization. R factor DNA was separated from the chromosome of detergent-lysed *Proteus mirabilis* by Hg^{++}-Cs_2SO_4 gradient centrifugation, or from *Escherichia coli* DNA by banding with ethidium bromide in CsCl. These procedures fractionate DNA on the basis of base composition or circularity, respectively. Three distinct R factor subspecies having buoyant densities (ρ) of 1.709, 1.711, and 1.717 g/cm^3 in CsCl were resolved from the episomal band isolated from *Proteus* ($\rho = 1.698$). Ultracentrifugation and electron microscope observation indicate that all three consist of autonomously replicating, closed circular DNA molecules whose relative amounts vary with conditions of bacterial growth. Transfer of only the $\rho = 1.709$ species from *P. mirabilis* to *E. coli* occurs when appropriate media are used to select for the recipient strain in the absence of antibiotics; passage of drug resistance appears to require concomitant transfer of the $\rho = 1.717$ species or transfer of the $\rho = 1.711$ species. Transfer of the 1.717 species alone has not been observed. These findings suggest that the $\rho = 1.709$ species may constitute the RTF (transfer) portion of the R factor, whereas the $\rho = 1.717$ species carries the drug resistance markers. The DNA species banding at $\rho = 1.711$ is associated with both resistance and transfer functions, and may represent the "complete" R factor. These observations suggest a possible mechanism for the emergence of transferable multiple drug resistance in bacteria via the genetic recombination of autonomous R factor units. (Supported by the National Institute of Allergy and Infectious Diseases.)

53. Lack of Correlation between Diuretic Action and Inhibition of Ouabain-Sensitive ATPase. C. H. COLE,* W. B. BLYTHE,* AND L. G. WELT,** Chapel Hill, N. C.

Ethacrynic acid at a concentration of 10^{-3} M has been shown to be a strong inhibitor of ouabain-sensitive ATPase in human red cell membranes. L589-420, an analogue of ethacrynic acid, lacks this characteristic, but, like ethacrynic acid, is a potent diuretic, as shown by studies in five dogs. A dose of 100 mg (less than 3×10^{-4} M) i.v. resulted in an increase in urine flow from 0.27 ml/min to 7.63 ml/min. Sodium excretion was increased 30-fold, potassium excretion 5-fold, calcium excretion 53-fold, and magnesium excretion 5-fold. In vitro studies performed on a microsomal ATPase preparation from dog kidney homogenates revealed that L589-420 increased mean ouabain-sensitive ATPase activity 11% at a concentration of 10^{-3} M; inhibition of ouabain-sensitive ATPase required higher concentrations of the drug. In vivo studies were also performed. The ouabain-sensitive ATPase of a microsomal fraction from renal cortex of 15 control dogs was 28.0 ±2.1 (SEM) μmoles P_i per mg protein per hr. In five dogs given 100 mg L589-420 intravenously, the mean activity was 31.3 ±3.0 (SEM) μmoles P_i per mg protein per hr. The experimental:control ratio in dogs given L589-420 was 1.17. This was not significantly different from the mean ratio of 1.07 in 10 paired controls. Medullary ATPase activity paralleled cortical activity in six dogs in which this was studied. There is no evidence from these studies that the diuretic action of L589-420 is due to an inhibition of ouabain-sensitive ATPase. (Research supported by grants from the NIH: AM-08458, HE-01301, AM-05054.)

54. The Regulation of Myocardial Energy Utilization after Chronic Cardiac Denervation. HENRY NEAL COLEMAN,* PETER J. DEMPSEY,* AND THEODORE COOPER, Rochester, Minn., and Bethesda, Md.

The energy requirements of chronically extrinsically denervated heart muscle, such as would obtain in a cardiac transplant, are unknown. Since myocardial energy utilization (oxygen consumption, $M\dot{V}O_2$) is regulated by the mechanical events during contraction, both the contractile state and $M\dot{V}O_2$ were determined in a polarograph for isolated papillary muscles from five normal and five denervated cat hearts. Extrinsic cardiac denervation was accomplished by mediastinal neural ablation, and studies were done 34–60 days postoperatively. Force-velocity relations, the extent of shortening, external work, and $M\dot{V}O_2$ were determined for afterloaded contractions. The force-velocity relations for normal and denervated muscles were qualitatively similar, and the intrinsic velocity of contraction (V_{max}) was also comparable in the two groups (normal, 1.22 ±0.20; denervated, 1.30 ±0.18 muscle lengths per sec), indicating normal contractility in the denervated muscles. The extent of shortening and thus external work were also unchanged in the denervated muscles. Simultaneous determination of $M\dot{V}O_2$ for these afterloaded contractions revealed no difference in $M\dot{V}O_2$ between the two groups. Isometric contraction at the apex of the length-tension curve resulted in a normal level of tension development (5.0 ±0.25 g/mm^2) in the denervated muscles. The $M\dot{V}O_2$ of isometric contraction per gram of developed tension averaged 0.69 ±0.18 and 0.73 ±0.13 μl/mg per beat $\times 10^{-3}$ for the normal and denervated groups, respectively. Resting $M\dot{V}O_2$ averaged 2.28 ±0.38 μl/mg per hr in the control and 1.90 ±0.12 μl/mg per hr in the denervated muscles. Thus the functional integrity of the contractile apparatus and the regulation of myocardial energy utilization do not

appear to be impaired in the chronic absence of extrinsic cardiac innervation.

55. Folate Uptake by *Lactobacillus casei*. BERNARD A. COOPER, Montreal, Canada.

The growth of *Lactobacillus casei* in folic acid (PtGlu) was observed to parallel the concentration of PtGlu in the medium at concentrations below 300 pg/ml, but at concentrations greater than this, the organism hoarded folate. PtGlu accumulation consisted of a rapid temperature-independent phase and a slower temperature-dependent, parabolic uptake. The concentration of PtGlu at which the velocity of the latter was half maximal was about 10 μg per liter, or 2×10^{-8} M. The primary uptake of cells maintained in PtGlu was higher than that of cells maintained in a medium containing natural folates. During incubation of *L. casei* for 1 hr in medium containing ^{3}H-PtGlu, 75% of the PtGlu in the medium was converted to another ^{3}H-labeled folate which bound weakly to DEAE-cellulose, supported growth of both *L. casei* and *Streptococcus fecalis,* and had an absorption spectrum similar to that of N^{10} formyl PtGlu. Both this material and PGA were released from washed cells incubated in folate-free medium after 1 hr of incubation in ^{3}H-PtGlu. This rate of release was temperature dependent. Methotrexate (1 μg/ml) prevented accumulation of the formyl-folate–like metabolite but did not prevent accumulation of PtGlu by the cells. Puromycin at a concentration which prevented growth of *L. casei* also did not prevent uptake of ^{3}H-PtGlu. It would appear that PtGlu uptake by the cells does not require alteration of the PtGlu molecule. Accumulation in the cells of a metabolic folate which is in equilibrium with the culture medium is prevented by methotrexate. (Supported by Medical Research Council of Canada grant MT-802.)

56. The Role of Lithocholic Acid in the Pathogenesis of Spur Red Cells and Hemolytic Anemia. R. A. COOPER,* W. H. ADMIRAND,* F. GARCIA,* AND C. TREY,* Boston, Mass. (introduced by J. H. Jandl).

Certain patients with severe hepatocellular disease develop marked hemolytic anemia and red cells having projections, or "spurs." Unlike biliary obstruction, in which cholic acid retention predominates (and "target" cells appear), hepatocellular disease leads to the accumulation of chenodeoxycholic acid and its toxic degradation product, lithocholic acid. In three patients with spur cells, the serum concentrations of lithocholic acid were markedly elevated: 0.7, 2.4, and 5.9 μg/ml (normal, $<$0.01 μg/ml). These patients had hemolytic anemia and red cells with an increased content of cholesterol (+50–70%) but a normal content of phospholipid. To explore the possible role of various bile salts in this syndrome, rhesus monkeys were fed diets containing 0.75% lithocholic acid, 2.0% cholic acid, and 2.0% taurocholic acid, alone and in combination. No changes occurred with cholic or taurocholic acid alone. However, in all monkeys receiving lithocholic acid, the red cells developed spurs identical with those seen in patients; spur cells were apparent by day 3 and maximal by day 8. The cholesterol content of these red cells remained normal for 8 days but increased 20% by day 12 and 30% by day 25, with no change in phospholipid content. Normal monkey red cells incubated in serum obtained on day 25 acquired spurs and gained 16–25% cholesterol, but no phospholipid. Affected monkeys developed anemia, reticulocytosis, and hepatocellular injury. Within 30 days after cessation of lithocholic acid, both the cholesterol content and the morphology of red cells returned to normal. Spur cells did not occur in rats, hamsters, or guinea pigs fed the same diet. These studies describe the induction of spur red cells and hemolytic anemia in monkeys fed lithocholic acid and demonstrate that the formation of projections and the accumulation of cholesterol by red cells are distinct and sequential phenomena. Moreover, they support the concept that lithocholic acid is important in the pathogenesis of spur cells in man. (Research supported by grants HE-07652, AM-5391, AM-09115, AM-5413, and FR-00168 from the NIH.)

57. Studies with a Humoral Immunoregulatory Factor Isolated from the Plasma Alpha Globulins (IRA). SIDNEY R. COOPERBAND,* ROBERT C. DAVIS,* KARL SCHMID,* AND JOHN A. MANNICK, Boston, Mass.

In the past few years there have been a number of reports which support the idea of a natural immunosuppressant factor present in the α-globulin fraction of plasma. We have attempted to substantiate these reports and to determine how this factor might act. Alpha globulin–containing fractions have been isolated from human plasma, and Cohn fractions, by a variety of methods (salting out, ion exchange chromatography, gel filtration). 8 of 14 fractionation procedures produced one α-globulin fraction which caused significant prolongation of skin allografts in mice with one intravenous injection of 5–8 mg. The assay was across a H-2 barrier ($C_3H \rightarrow C_{57}Bl$), and gave an average graft duration of 13.1 $\pm$0.7 days (control 10.2 $\pm$1.3 days; $P < 0.01$). Other fractions isolated from the same runs did not produce this effect. 33 of 38 fractionation procedures yielded at least one α-globulin–containing fraction which completely inhibited the stimulatory effect of mitogen (PHA) and antigens (tuberculin, diphtheria toxoid, tetanus toxoid) upon human blood lymphocytes in tissue culture. This inhibition was measured by a decrease in the rate of incorporation of radioactive precursors into DNA and/or protein. The mechanism of action of the IRA has been studied in vitro in the human lymphocyte system and in the antigen-specific guinea pig macrophage immobilization assay. The IRA was loosely bound to lymphocytes and was removed by washing. It did not influence lymphocyte metabolism directly, nor did it influence cells by preincubation. IRA added 24 hr after stimulation with PHA was not suppressive. Lineweaver-Burke type analyses at different doses of stimulus and IRA suggest a noncompetitive inhibition. IRA inhibited antigen responses in the guinea pig macrophage immobilization assays but did not interfere with immobilization induced by passive transfer with "macrophage immobilization factor." These studies suggest that IRA is biologically significant, and may be immunosuppressive by interfering with the earliest events of antigen recognition by lymphoid cells.

58. Uptake of Tritiated Pteroylglutamic Acid (^{3}H-PGA) by Human Bone Marrow Cells In Vitro. JOSE CORCINO,* SAMUEL WAXMAN,* AND VICTOR HERBERT, New York, N. Y.

Izak et al. reported that 1 to 2% of 2 to 10 pg of ^{3}H-PGA was taken up in vitro by 0.16 ml of human erythrocytes (reticulocytes up to 50%). We found similar minimal erythrocyte uptake. In the present study, uptake of PGA by human bone marrow cells was studied. It was hoped to find greater uptake than that by erythrocytes, and thereby to facilitate studies of mechanisms of cellular folate uptake. Uptake was determined after incubation of cell suspensions in Hanks' medium containing ^{3}H-PGA. Uptake increased over a period of 4 hr at 37°C but not at 4°C, and exhibited a pH optimum at 7.4. The percentage uptake decreased as the concentration of ^{3}H-PGA was increased. Uptake by 0.25 ml of marrow (3.38×10^6 nucleated cells) was 7% of 100 pg, 2% of 1000 pg, and 0.5% of 10,000 pg (10 ng) ^{3}H-PGA. Similar uptake of ^{14}C-methyl-tetrahydrofolate was noted. Uptake was not affected by 1 hr preincubation with Dilantin (10 μg/tube) or *p*-chloromercuribenzoate (100 μmoles/tube), but preincubation with equimolar concentrations of unlabeled PGA or methotrexate (MTX) inhibited uptake by 86% and 41%, respectively. These results suggest that MTX shares, at least in part, the uptake mechanism for PGA. This may prove to be involved in the antifolate activity of MTX. Studies correlating in vitro uptake of ^{3}H-PGA by human bone marrow cell suspensions and in vivo folate clearance are in progress. (This work was supported by USPHS grants AM-13358 and AM-11048.)

59. The Role of Messenger RNA and Protein Synthesis in the Stimulation of Enhanced Zinc Uptake by Steroids in Mammalian Cell Cultures. RODY P. COX, New York, N. Y.

Previous studies have shown that the zinc uptake of HeLa S_3 cells is markedly increased after growth in medium containing adrenal hormones with glucocorticoid activity. The present investigation indicates that the synthesis of RNA and protein is required for the steroid-mediated enhancement of zinc uptake. Cycloheximide, an inhibitor of protein synthesis, completely blocks increased zinc uptake even when added as late as 6 hr after the hormone. If cycloheximide is added after enhanced zinc uptake becomes apparent, the inhibitor blocks further increases in zinc accumulation. Similarly, actinomycin D, an inhibitor of RNA synthesis, also completely inhibits the prednisolone-mediated increase in zinc uptake. When protein synthesis is blocked by cycloheximide in HeLa S_3 suspension cultures containing prednisolone for 16 hr and the inhibitor is then removed, there is a marked enhancement of zinc uptake during the subsequent 6 hr. Prednisolone must be present during the 16 hr incubation with cycloheximide in order for this enhancement of zinc uptake to be observed. These findings are consistent with the accumulation of an intermediate, presumably mRNA, which specifies a mechanism for increased zinc uptake. (Research supported by a grant from the NIH.)

60. Effect of Pressure Development on Metabolism of Endogenous Lipids in Rat Heart. M. F. CRASS, III,* AND J. C. SHIPP, Gainesville, Fla.

Tissue lipids of hearts from fed, functionally hepatectomized rats were labeled in vivo with 1-^{14}C-palmitate. All hearts were then perfused (nonrecirculated) with plain buffer at 60 mm Hg for 5 min and one group was removed for initial determinations. Two additional groups were perfused in a closed, recirculated working (10 cm H_2O left atrial filling pressure) or Langendorf (0 cm H_2O LAFP) system for 30 min with a substrate-free Krebs-Henseleit bicarbonate buffer equilibrated with 95% O_2:5% CO_2. Mean peak aortic systolic pressures were 110 and 65 mm Hg in working and Langendorf hearts, respectively. Heart ventricle lipids were extracted and fractionated by column and thin-layer chromatography. A highly significant ($P < 0.001$) increase in rate of $^{14}CO_2$ production was observed in working hearts. Triglyceride content, ^{14}C, and specific activity decreased approximately 50% from initial values in both working and Langendorf hearts. In both groups phospholipid content (lipid P) was unchanged but ^{14}C and specific activity were increased. Thus, increased pressure had no discernible effect on mobilization of myocardial triglycerides and phospholipids. (Research supported by USPHS grants AM-4829, AM-5444.)

61. A Statistical Analysis of Urinary Amino Acid Excretion of Patients with Cystinuria and Their Relatives. J. C. CRAWHALL,* P. PURKISS,* R. W. E. WATTS,* AND E. P. YOUNG, Montreal, Canada, and London, England (introduced by J. C. Beck).

The urinary amino acid excretion of 24 stone-forming patients with cystinuria, who were believed to be homozygous for the condition, was analyzed. Urine was also analyzed from 19 of the parents of these patients and from 23 normal controls. The amino acid/creatinine ratios were calculated for cystine and the three basic amino acids, and two equations were derived based on these parameters by the method of canonical variate analysis. The urinary amino acid/creatinine ratios for each subject could then be expressed by two functions. The results were plotted between two axes such that groups were defined which were characteristic of homozygote, heterozygote, and normal subjects. The homozygotes were clearly defined by this procedure, but there was considerable overlap between the heterozygotes and normal controls. The same statistical treatment was then applied to the analytical results on 72 other family members to see if the three possible genetic groups could be defined in this way. Three of these other family members fell within the homozygous group but had not formed renal calculi. Previous investigators have described two patterns of inheritance of cystinuria based on the identification of normal or abnormal levels of amino acid excretion in the heterozygotes, but in this investigation of other family members no clear distinction between normal and abnormal levels was found. Possible explanations for this phenomenon would be that there are more than two genetic defects giving rise to cystinuria or that the urinary excretion of amino acids is modified by genetic factors other than those giving rise to cystinuria.

62. Transcellular Membrane Potential in Human Skeletal Muscle: A Study of Membrane Depolarization Accompanying Severe Illness. J. N. CUNNINGHAM, JR.,* N. W. CARTER, F. C. RECTOR, JR., AND D. W. SELDIN,** Dallas, Texas.

Transmembrane potentials (PD) of skeletal muscle were measured in 26 normal human subjects and in 21 patients with severe, debilitating medical disorders. A closed transcutaneous approach to the muscle was made by needle puncture, and PD was measured utilizing standard Ling electrodes. Measurements revealed a PD of 88.8 ±3.8 mv in healthy subjects. The mean PD in 21 in-hospital patients (judged to be severely or chronically ill clinically from a variety of causes) was 66.3 ±9.02 mv. Open deltoid muscle biopsies were performed in seven of the healthy subjects and in 13 of the seriously ill group. The intra-extracellular water partition was estimated by calculating the chloride space from the previously measured PD. The intra-extracellular potassium ratios in the two groups were almost identical. In the group of seriously ill patients with depressed potentials, the intracellular muscle Na^+ concentrations (expressed as meq/liter$_{ICW}$ were uniformly increased and as a group averaged 25% higher than those of the normal patients. The increased intracellular sodium concentrations and depression of PD in severely ill patients might be due either to inhibition of an electrogenic pump or to a selective increase of permeability of the muscle membrane to Na^+. (This work is supported by grant 5-PO1-HE-11662-02, USPHS.)

63. Hypogammaglobulinemia with Impaired Primary Responsiveness but Intact Immunological Memory. F. DAGUILLARD,* J. I. WATSON,* D. C. HEINER,* AND BRAM ROSE, Montreal, Canada.

A 46 yr old woman with repeated skin infections, sore throats, laryngitis, and abscesses was investigated because of low gamma globulin on serum electrophoresis. Both γM and γG were depressed. When tested for ability to form antibodies to several antigens, the patient did not respond to primary antigenic stimuli from *Salmonella* H or O antigens, or with keyhole limpet hemocyanin (KLH). However, she had a good secondary response to numerous agents with which she had had previous contact (tetanus, diphtheria, polio, and specific blood group substances). She was able to produce antibodies of the three major immunoglobulin classes. Because of the similarity to results obtained in several experimental models in which the cells of the primary response could be made selectively nonresponsive, data on this subject are interpreted as evidence of a qualitative difference in the cells of primary immune responses as contrasted with cells of secondary responses. The defect in this subject would correspond to the selective alteration of the X cells in the X-Y-Z scheme of Coons, Sercarz, and Sterzl. In this patient, however, the cause of the alteration of the X cell or primary response cell is not clear. The patient had previously taken colchicine, which might conceivably have played a role. She is under close surveillance for loss of immunologic memory or for return of primary responses to normal.

64. Correlated Biochemical and Pharmacological Observations Concerning the Biosynthesis of Salicylurate from Salicylate. EUGENE D. DAVIDSON* AND LESLIE T. WEBSTER, JR., Cleveland, Ohio.

After low doses, the major excretory product of aspirin in man is salicylurate; common laboratory animals form much less of this glycine conjugate. This species variation stimulated our experiments on the mechanism of salicylate conversion to salicylurate in vitro, and the results support the following sequence: (1) salicylate + ATP + CoA $\rightleftarrows$ salicyl-CoA + AMP + pyrophosphate; (2) salicyl-CoA + glycine $\rightarrow$ salicylurate + CoA. Mitochondrial preparations from liver and kidney of cattle, rhesus monkey, man, dog, and rat were incubated with appropriate substrates and, excepting those from rat liver, catalyzed formation of salicylurate from salicylate. The rate of the first or salicylate activation reaction corresponded to that of the over-all conversion in each case; the rate of the second reaction was greater, and its ratio to the activation reaction varied between different tissues and species. After salicylate administration in vivo maximal rates of salicylurate produced per kilogram had the following relation: monkey $\gg$ man $\gg$ dog and rat. Specific activities for salicylate activation in vitro followed the same order, the highest occurring with preparations from beef liver and kidney. In the series of monkey, man, dog, and rat, the ratio of liver to kidney specific activities for salicylate activation in vitro increased with the species' ability to produce salicylurate in vivo; in one low producer, i.e. the rat, only the renal pathway was demonstrable in vitro. The "salicylate-activating enzyme" from beef liver mitochondria was differentiated from known activating enzymes by partial purification and differential inactivation; results with a human enzyme were similar. Thus, a coupled enzyme system, present in hepatic and renal mitochondria of several mammals, is capable of converting salicylate to salicylurate. Salicylate activation, catalyzed by a newly described enzyme, is rate limiting in this pathway and probably accounts for the species and organ differences in salicylurate production.

65. RNA, Protein, and DNA Synthesis in Epithelial Cells of Colonic Mucosal Excrescences. ELEANOR E. DESCHNER* AND MARTIN LIPKIN, New York, N. Y.

Gastrointestinal epithelial cells normally proliferate for a limited period during their life span. DNA synthesis and mitosis cease as the cells migrate to the surface of the mucosa and undergo functional specialization. In polyposis of the colon and in gastric atrophy, however, epithelial cells incorporate thymidine into DNA at the luminal surface of the mucosa, when they have some of the morphological characteristics of mature surface cells. This study analyzes RNA and protein as well as DNA syntheses in epithelial cells of villous papillomas and other colonic mucosal excrescences, and in histologically normal tissue adjacent to the excrescences. Incubations of specimens of mucosal tissue in culture media with 5-^{3}H-uridine, 4,5-^{3}H-L-leucine, and ^{3}H-thymidine-methyl were carried out and microradioautographs prepared. Measurements of syntheses were further correlated with

histochemical preparations. In specimens of normal mucosa from patients without excrescences, RNA and protein syntheses were greatest in rapidly proliferating epithelial cells located in the lower two-thirds of colonic crypts. In hyperplastic mucosa and in polypoid excrescences, however, RNA, protein, and DNA syntheses in cells located at and near the luminal surface of the mucosa were as great as or greater than in cells in the deeper crypt regions. In some but not all areas of mucosa adjacent to excrescences, RNA and protein syntheses were also greater in surface epithelial cells, and occasional surface cells also incorporated thymidine. Chase experiments using nonradioactive media designed to follow newly formed and labeled RNA demonstrated a shift of nuclear RNA to cytoplasm within 1–3 hr. A more rapid nuclear-to-cytoplasmic RNA shift in hyperplastic cells and a slower shift in villous papilloma cells as compared with normal cells were observed, suggesting differences in the kinetics of rapidly labeled RNA in these lesions.

66. Left Ventricular Performance in Myocardial Infarction. BERNARD DIAMANT* AND THOMAS KILLIP, New York, N. Y.

Assay of left ventricular performance should be useful in evaluating prognosis and therapy of acute myocardial infarction. Therefore, daily indirect measurements of preejection period (PEP) and left ventricular ejection time (LVET) were obtained in patients admitted to a coronary care unit with suspected myocardial infarction. Measurements were obtained from simultaneously recorded external carotid pulse, phonocardiogram, and electrocardiogram. Values were compared with a regression line, corrected for rate, obtained from a control population and expressed as per cent of normal. Of 82 patients studied, final diagnoses were: group A, transmural infarction, 29 patients, 7 deaths; group B, nontransmural infarction, 23 patients, no deaths; group C, no infarction, 30 patients, no deaths. Significant abnormalities were demonstrated in both PEP and LVET in the first 24 hr after myocardial infarction. In comparison with group C, PEP was prolonged in group A ($P < 0.001$ and group B ($P < 0.005$). In comparison with group C, LVET was shortended in group A ($P < 0.001$) and group B ($P < 0.005$). PEP was significantly longer in group A than in group B ($P < 0.02$). Both PEP and LVET in group C were within normal limits. Death occurred from 1 to 5 days after myocardial infarction in seven group A patients. PEP in patients who died was already greatly prolonged on admission, 154 $\pm$33% as compared with a mean value in group A patients who lived of 120 $\pm$19% ($P < 0.05$). History of previous infarct or ECG location of damage could not be correlated with abnormality of PEP or LVET. Thus, indirect measurement of ventricular function reflects presence and extent of myocardial infarction. Analysis of PEP may be useful in evaluating prognosis, since patients who later died from heart failure or shock had marked abnormality within a few hours of the acute attack. (Supported by a contract from the Myocardial Infarction Branch, National Heart Institute.)

67. Effect of Induced Hypotension on Thrombosis in the Extracorporeal Microcirculation of the Rat. PAUL DIDISHEIM,* CHARLES A. OWEN, JR.,* AND E. J. WALTER BOWIE,* Rochester, Minn. (introduced by F. Raymond Keating, Jr.**).

We have previously reported that dipyridamole (Persantine) almost completely prevents electrically induced arterial thrombosis in the rat. To clarify its mechanism of action, a new method, extracorporeal microcirculation, was developed. Platelet thrombosis was induced in this femoral arteriovenous shunt by a 10 sec compression of a segment of the Teflon tubing. Dipyridamole significantly inhibited thrombogenesis in the synthetic vessel ($P < 0.003$). Therefore this effect was not mediated by dipyridamole's vasodilator action. Adenosine and adenosine monophosphate (AMP) were also antithrombogenic. However, at concentrations which inhibited thrombosis, these agents significantly reduced arterial pressure and flow. Two hypotheses to explain their antithrombogenic effect are: (1) direct inhibition of platelet adhesiveness and aggregation in vivo like that which has been demonstrated in vitro; and (2) indirect inhibition by their reduction of arterial pressure and flow, and consequent reduction in magnitude of shear rate increase through the compressed tubing segment, resulting in reduced cellular injury, reduced adenosine diphosphate (ADP) release, and diminished subsequent platelet aggregation. These hypotheses were tested by inducing hypotension without administering an agent which might inhibit platelet function in vivo. 0.1 ml 5% procaine was injected into the cisterna magna of 600 g rats. Bilateral cervical vagotomy was immediately performed. Respirations thereafter were maintained with a respirator. Within 5 min, carotid arterial pressure fell from 123/103 to 38/27 mm Hg (means); femoral arterial flow fell from 7.8 to 3.5 μl/sec (means). However, maximal thrombotic occlusion of the compressed tubing segment did not fall significantly, but actually increased 15%. These data suggest that the antithrombotic action of dipyridamole, adenosine, and AMP is not dependent upon their reduction of arterial pressure or flow, and favor direct inhibition by these agents of platelet adhesiveness and aggregation in vivo. (Research supported in part by grants from the NIH and the Minnesota Heart Association.)

68. Protein A: An Antiphagocytic Factor of *Staphylococcus aureus*. JOHN H. DOSSETT,* GÖRAN KRONVALL,* RALPH C. WILLIAMS, JR., AND PAUL G. QUIE, Minneapolis, Minn.

Protein A is a cell wall constituent of *S. aureus*, which reacts nonspecifically with the Fc portion of human γG globulin. Since sites on the Fc are necessary for phagocytosis of opsonized bacteria, protein A may be antiphagocytic by competing with polymorphonuclear leukocytes for γG receptor sites on Fc. *S. aureus* Cowan I producing large amounts of protein A was compared in phagocytic systems with *S. aureus* Oxford containing no protein A. Serum opsonic requirement for Cowan I was 20 times that for Oxford. The amount of staphylococcal protein A was measured using Oudin tube precipitin reactions with myeloma proteins chosen for their clear reactivity with protein A.

Cowan I grown on mannitol salt agar, a medium which markedly inhibits protein A synthesis, was readily phagocytized, in contrast to organisms of the same strain producing protein A. Patients with staphylococcal osteomyelitis have high serum-agglutinating antibody titers yet show paradoxically low opsonic antibody titers. Ten strains of *S. aureus* from patients with osteomyelitis were compared in phagocytic systems using isolated immune γG. Protein A content per 10^{12} bacteria of each strain was determined. Two of ten strains showed marked resistance to phagocytosis; these organisms contained the highest amounts of protein A. Immune γG from patients with *S. aureus* endocarditis efficiently opsonizes most strains of *S. aureus*. Addition of purified protein A to these phagocytic systems blocked opsonic capacity of isolated immune γG. Blocking by staphylococcal protein A was also demonstrated in a phagocytic system using *E. coli*. When *E. coli* were preincubated with specific antibody opsonin and washed, and protein A was added, phagocytosis was blocked. Blocking of nonstaphylococcal phagocytosis indicated that protein A can interfere with phagocytosis of unrelated opsonized bacteria by combining with the Fc opsonic site. (Research supported by grants AI-06931 and AI-08821 from the NIH and contract DADA-68-17-C-8037 from the U. S. Army Medical Research and Development Command.)

69. Influence of Oral Contraceptives on Angiotensin and Renin Responsiveness. BEN H. DOUGLAS,* RICHARD P. HULL,* AND HERBERT G. LANGFORD,** Jackson, Miss.

Occasional patients receiving oral contraceptive agents develop hypertension. Renin substrate (RS) is elevated, but renin may or may not be elevated. RS is said not to be rate limiting in vivo. If it were rate limiting, increased angiotension production could result with unchanged renin. Groups of 10 rats were given 1× and 10× the human dose per kg of Enovid® for 3 wk (0.1 mg/kg and 1.0 mg/kg, respectively); then their blood pressure responsiveness to 0.12, 0.25, and 0.5 μg/kg angiotensin was tested. The 1× Enovid group's response was identical with that of the control group. The 10× Enovid group's blood pressure response to the highest dose of angiotensin was significantly less than the response of the other groups. The blood pressure response to renin was evaluated in another group. Rat renin was prepared by a modification of the method of Haas and Goldblatt, and amounts were used to give blood pressure responses comparable to those obtained with angiotensin. Only one injection was given per rat. The 1× Enovid rats gave blood pressure responses not significantly different from those of the controls. The 10× Enovid rats' blood pressure response was significantly greater than that of the controls at each dose level. Δ arterial blood pressure (mm Hg): 0.01 GU renin: control (C) 21 ± 2, 10× Enovid (10× E) 30 ± 7; 0.02 GU renin: C 47 ± 4, 10× E 65 ± 7; 0.03 GU renin: C 63 ± 3, 10× E 84 ± 3. These results suggest that (*a*) renin substrate is rate limiting in vivo; (*b*) therefore, methods of renin measurement which are substrate independent may not reflect angiotensin production so well as renin activity methods, and (*c*) increased angiotensin production due to increased substrate may be a mechanism involved in hypertension produced by oral contraceptives. (Research supported by grants from the NIH.)

70. Alterations in Lymphocyte Response to Plant Mitogens in Immunologic Disorders. STEVEN D. DOUGLAS,* ROBERTA M. KAMIN,* AND H. HUGH FUDENBERG, San Francisco, Calif.

Numerous investigations have reported impaired in vitro lymphocyte response in several immunologic diseases. Such studies, however, are primarily based on observation of cultures 3 days after stimulation with phytohemagglutinin (PHA). In the present study the effects of four mitogens (PHA, pokeweed, Concanavalin A, and wax bean glycoprotein) were investigated during a 10 day incubation period. Cells from adult "acquired" agammaglobulinemic (Aγ) patients (eight) and their parents (four) had a diminished thymidine incorporation in 3 day cultures using each of the four mitogens ($P < 0.001$). A late response, however, was observed with PHA or wax bean stimulation at 5–7 days, comparable to that of controls at 3 days. With Concanavalin A, lymphocytes from both Aγ patients and parents had a diminished incorporation throughout the 10 day period ($P < 0.001$). Stimulation with pokeweed mitogen (PWM) distinguished the Aγ cells of patients from those of parents; at 5–7 days parents had a peak comparable to that of controls, whereas the Aγ patients had a diminished peak at 10 days ($P < 0.001$). Furthermore, electron microscope observation of PWM-stimulated lymphocytes from Aγ patients showed only blast cells and small lymphocytes, whereas some plasmacytoid cells were observed in cultures from controls and parents. The PWM response in Aγ may reflect the absence of a subpopulation of cells or failure of specific cytodifferentiation. Although lymphocytes from multiple myeloma patients (six) and macroglobulinemic patients (four) had diminished thymidine incorporation throughout the 10 day period with Concanavalin A ($P < 0.001$), cells from these patients had either normal or delayed responses with PHA, wax bean, and PWM ($P < 0.05$). The observations of late responses demonstrate the need for detailed time studies in assessment of lymphocyte behavior in disease. Abnormal responses to Concanavalin A may be related to alterations in lymphocyte cell membranes. (Research supported by grants from the USPHS, HE-05677 and HE-05997.)

71. Enhancement of Phagocytosis of Pneumococci by the Fixation of Human Antipneumococcal Antibody on Leukocytes. ROBERT H. DRACHMAN* AND LAWRENCE M. LICHTENSTEIN,* Baltimore, Md. (introduced by W. Barry Wood, Jr.**).

These experiments were designed to test whether leukocytes could be passively sensitized with pneumococcal (Pn) immune serum so as to enhance their ability to phagocytize specific Pn. Human subjects were immunized with 92 μg of Pn type II polysaccharide (SSII) and bled when the (previously negative) skin test became positive to 0.005 μg SSII. The immune serum contained specific Pn-agglutinating and mouse protective antibody, and the washed leukocytes of the immunized donor were capable of enhanced phagocytosis of type II Pn. Normal human leukocytes separated by dextran sedimentation were incubated with the immune serum, washed thoroughly, and used in a phagocytic test system containing normal serum and type II Pn. Phagocytosis

was increased from less than 4% to 30–40%. Preincubation of leukocytes with normal or preimmunization sera did not influence phagocytosis, and control experiments ruled out the possibility that the increased phagocytosis was due to direct opsonization of the Pn. The enhancement of phagocytosis was specific for type II; no enhancement was observed with type III or type XXV pneumococci. Heating the serum at 56°C or treatment with Ishizaka's anti-IgE reduced the enhancing effect. We interpret these observations to indicate that antipneumococcal antibody of the reaginic type was formed and that leukocytes carrying this antibody can phagocytize pneumococci of the corresponding type more efficiently. (Research supported by grants from the NIH.)

72. An In Vivo Study of a Defect in DNA Synthesis in Xeroderma Pigmentosum (XP). J. H. EPSTEIN,* K. FUKUYAMA,* W. L. EPSTEIN,* AND W. B. REED,* San Francisco, Calif. (introduced by Lloyd H. Smith).

Previously we demonstrated that UV induces unscheduled DNA synthesis (possibly reflecting DNA repair) in basal (B), malpighian (M), and granular (G) epidermal cells and upper dermal cells in human and hairless mouse skin in vivo. In this study we examined the incorporation of ^{3}H-TdR into DNA of UV-irradiated skin of XP and other skin cancer-prone conditions in vivo. Eight normal controls, three XP, one Rothmund-Thompson's syndrome, one progyria, one basal cell nevus syndrome, two patients with sun damage, and one with X-ray injury were exposed to 13.6×10^6 ergs/cm^2 of short UV. 15 min later 10 μc of ^{3}H-TdR were injected into irradiated and nonirradiated sites. Biopsies obtained 1 hr later were prepared for radioautography. Unscheduled synthesis (USS) was demonstrated by a sparse labeling of 3–15 grains per nucleus. Practically no USS occurred in any nonirradiated sites. After UV, one-third to two-thirds of epidermal (G, M, B) and upper dermal cells showed USS, with the striking exception of those with XP and to some extent X-ray damage, which showed, respectively, 10–59 and 22–244 labeled cells per 1000 cells. The results indicate that unscheduled DNA synthesis is reduced in X-ray damage and markedly limited in XP. These findings may represent a defect in DNA repair as suggested by in vitro studies.

73. Analysis of the Increase of Sweat Rate Brought About by Recruitment or Enhancement of Secretion. JUAN CARLOS FASCIOLO,* BECKY B. JOHNSON,* AND GREGORY TOTEL,* Urbana, Ill. (introduced by Robert E. Johnson**).

Sweat rate in one area of skin may increase by recruitment of fresh glands, enhanced output of already active glands, or both. The relative contribution of each process can be calculated from sodium concentration and sweat rate. Sodium outputs for the two processes are: ($[Na_R] \cdot V_R$) and ($[Na_E] \cdot V_E$), where $[Na_R]$ is sodium concentration in sweat accounted for by recruitment, $[Na_E]$ by enhancement, V_R is volume accounted for by recruitment, and V_E by enhancement. Total volume is V_R plus V_E. $[Na_R]$ equals that of the initial sweat sample, whereas during enhancement $[Na_E]$ increases with sweat rate and can be calculated for various rates. If sodium reabsorption by eccrine sweat glands is a rate-limited process, reabsorption by a population can be calculated from sweat rate and sodium concentration, assuming that maximal reabsorption is reached, sodium in the precursor fluid is 145 μEq/ml, and no significant water reabsorption occurs (the Cage-Dobson hypothesis). When sodium outputs per unit area are plotted against sweat rates, a straight line results. For increased sweat due to recruitment, the X intercept is 0 and the slope $[Na_R]$. For enhancement, the X intercept is positive and the slope $[Na_E]$, always greater than $[Na_R]$. Values for sweat rate vs. sodium output may follow lines for recruitment or enhancement, or may fall between these lines if both processes are at work. Experimental evidence has been derived from sweat collected in unventilated capsules after subdermal or intradermal injections of methacholine and in subjects under moderate heat stress. (Supported by the NSF, the NASA, and the University of Illinois Research Board.)

74. Uptake and Release of Guanethidine and Bethanidine by the Adrenergic Neuron. O. T. FEAGIN,* J. R. MITCHELL,* D. G. SHAND,* AND J. A. OATES, Nashville, Tenn.

The hypotensive action of guanethidine (G) and bethanidine (B) is antagonized by tricyclic antidepressants such as desipramine (DMI) and protriptyline (P). DMI and B block the adrenergic neuron membrane transport system for norepinephrine (NE). NE and G were shown to inhibit competitively the uptake of ^{3}H-B into rat heart slices, indicating that G and B are concentrated in adrenergic neurons by the NE pump. ^{3}H-G and ^{14}C-B were each given i.v. to three volunteers before and during a course of 10 mg t.i.d. In control studies, both G and B were excreted in an initial rapid phase and a second slow phase. The amount of G and B remaining in the body at the beginning of the slow phase was reduced by 53% and 52%, respectively, by P treatment. To confirm the assumption that the slow phase represented release of drug from the adrenergic neuronal pool, three subjects were given 10 mg of D-amphetamine (A) t.i.d. for 3 days starting on the 8th day after G. In each case, a marked increase in G secretion was interpreted as indicating displacement of G from adrenergic neurons by A. Metabolism of G and B was unaffected by P. The data indicate that the selective blockade of adrenergic nerves by G and B depends on their concentration in the neuron by the uptake-reuptake system for the neurotransmitter NE.

75. Effects of Altered Diagnostic Standards on the Changing Incidence of Pulmonary Diseases. ALVAN R. FEINSTEIN AND ELLEN B. MILSTONE,* New Haven, Conn.

We did this research to determine whether the changing annual incidence rates of certain pulmonary diseases might be affected by altered diagnostic standards, rather than by differences in nature or in medical management. In examining the case records of the New Haven Hospital for the years 1921, 1941, and 1961. we tabulated the occurrence of diverse respiratory diseases, and we searched for the supporting clinical and paraclinical evidence in each patient who received a diagnosis of pulmonary tuberculosis, emphysema, or lung cancer. During the three cited years, the annual diagnosis rates per 1000 patient admissions were: tuberculosis, 59.7, 9.5,

and 3.1; emphysema, 16.7, 2.0, and 4.1; carcinoma, 1.2, 1.9, and 3.0; and all types of respiratory disease, 169.7, 57.0, and 52.7. Over the interval of 40 years, the admission rates dropped sharply for diagnoses of bronchitis, pulmonary embolism-infarction, lung abscess, and pleurisy, but rose sharply for pneumonia, asthma, and congestive heart failure. In 1921, 40% of the 50 diagnoses of tuberculosis were based only on symptoms and/or physical signs; the tubercle bacillus was demonstrated in only eight (16%) patients; and 44% of patients did not have a chest X-ray taken. For emphysema, 12 (86%) of the 14 diagnoses in 1921 were based only on clinical evidence. According to modern diagnostic criteria, only 24% of the tuberculosis diagnoses in 1921, and 14% of the emphysema diagnoses, were "justified." By 1961, roentgenography and other paraclinical tests were being used increasingly, and the rates of "justified" diagnosis had risen to 77% for tuberculosis and 57% for emphysema. The results suggest that major changes in the annual incidence rates of pulmonary diseases have occurred because the same clinical ailments receive different diagnostic names with changes in the intellectual and technologic methods for identifying "disease."

76. Feasting and Fasting: Alternation of Insulin and Substrate in the Regulation of Gluconeogenesis in Man. PHILIP FELIG,* ERROL MARLISS,* JOHN WAHREN,* AND GEORGE F. CAHILL, JR., Boston, Mass.

Inhibition of splanchnic glucose output may occur in both hyperinsulinemic (post glucose infusion) and hypoinsulinemic (3–6 wk fasting) circumstances. In fasting, substrate availability has been suggested as rate limiting in view of markedly diminished arterial concentration and net splanchnic uptake of amino acids (AA), particularly alanine, despite unaltered extraction ratios. To characterize the mechanism of decreased gluconeogenesis in these diverse situations, two groups were studied. In six normal postabsorptive subjects, arterio-hepatic venous differences (A-HV) were determined for glucose, lactate, and 20 individual AA, before and after infusion of 0.5 g/kg glucose. Basal A-HV was —10 ±2 mg/100 ml for glucose, 135 ±40 μmoles/liter for lactate, and 117 ±16 μmoles/liter for alanine (which accounted for half of the total AA uptake). After glucose administration, arterial immunoreactive insulin rose by 30 ±11 μU/ml, A-HV for glucose was +21 mg/100 ml, lactate uptake (28 ±75) was insignificant, total AA extraction fell by 25%, and alanine uptake decreased to 79 ±7 (P vs. basal <0.0125, paired t). Arterial alanine concentration did not change, nor could significant reduction in uptake of other AA (proline, valine, phenylalanine) be accounted for by changes in arterial levels. In six obese subjects, infusion of 10 g L-alanine produced a peak increase in peripheral blood glucose of 3 ±1 mg/100 ml in the postabsorptive state and 12 ±1 mg/100 ml after 3–6 wk fasting, representing 14% and 39% of the maximal theoretical increment assuming complete conversion to glucose. In two fasted subjects also given 50 μc U-^{14}C-alanine, 35% of the label was recovered as blood glucose. *Conclusions:* In "feasting" (post glucose), insulin blocks gluconeogenesis by directly inhibiting hepatic uptake of precursor substrate. Conversely, in fasting with hypoinsulinemia, the enzymatic mechanism for gluconeogenesis remains intact and perhaps facilitated, but total output is limited by decreased substrate levels. The data also provide direct evidence that AA-induced hyperglycemia is due to conversion of the administered AA to glucose.

77. Stimulation of Active Chloride Secretion in Small Intestine by Cholera Exotoxin. MICHAEL FIELD,* DAVID FROMM,* CRAIG K. WALLACE,* AND WILLIAM B. GREENOUGH, III,* Boston, Mass., and Baltimore, Md. (introduced by Philip S. Norman).

Active Cl^- secretion can be stimulated in isolated rabbit ileal mucosa by cyclic 3′,5′-AMP or theophylline. The role of this mechanism in producing small intestinal fluid loss in cholera has been investigated. A dialyzed *Vibrio cholerae* filtrate (CF) was added to the luminal side of short-circuited rabbit ileum stripped of serosa and muscularis and mounted between Lucite half chambers. Heat-inactivated CF was added to controls. The short-circuit current (SCC) began to rise 0.5 to 1 hr after addition of CF (1 mg/ml), reaching a plateau 1 to 2 hr later. After peak SCC had been reached, Na^+ and Cl^- fluxes across 11 CF-treated and control tissues were measured with radioisotopes. Results in μeq/cm² per hr were (means ±SE): CF resulted in an increase in SCC from 2.8 ±0.3 to 5.5 ±0.5 ($P < 0.001$), a decrease in net mucosa (M) to serosa (S) Na^+ flux from 1.0 ±0.4 to 0.0 ±0.3 ($P < 0.01$), a reversal of net M-to-S Cl^- flux of 1.5 ±0.4 to a net S-to-M flux of 2.6 ±0.3 ($P < 0.001$). Theophylline, added 3 hr after filtrates, increased SCC by 0.6 ±0.1 μeq/cm² per hr in CF-treated, but by 2.7 ±0.3 in control tissues. Addition of CF to ileal loops in vivo 2 hr before mounting in vitro resulted in the same ion flux changes that followed addition of CF in vitro ($n = 5$). Effects of CF on transmural potential difference (PD) and volume flow (VF) in vivo were determined simultaneously in cannulated ileal loops ($n = 10$). The PD began to increase 1 to 1.5 hr after addition of CF and reached a plateau about 2 hr later (↑ 3.1 ±0.8 mv); changes in VF paralleled changes in PD. In six controls PD and VF did not change. We concluded that small intestinal fluid loss results from stimulation of active Cl secretion in mucosal cells by a nondialyzable heat-labile factor in *V. cholerae* filtrate.

78. Renal Response to Volume Expansion during Metabolic Alkalosis. BURTON P. FINE,* SANGCHAN S. SATRASOOK,* ZENAIDA M. VERGEL,* EDDIE S. MOORE,* AND CHESTER M. EDELMANN, JR.,* Bronx, N. Y. (introduced by Henry L. Barnett**).

In previous studies we found that the renal bicarbonate threshold in infants is low, explaining the "physiologic acidosis" of the period. We have shown that the threshold in puppies similarly is low, but that it is elevated significantly under the stimulus of extracellular volume contraction. Maintenance of metabolic alkalosis is currently attributed to an associated contraction of the "effective" blood volume, whereas renal repair of alkalosis is attributed to expansion. The present studies were designed to investigate the effects of extracellular volume expansion on H^+ excretion, in order to explore further the mechanisms involved in maintenance of the low bicarbonate threshold in young animals. Puppies 6 to 8 wk old were rendered volume contracted, hypochlor-

emic, and alkalotic by acute gastric suction and intravenous replacement with sodium and potassium bicarbonate. Pa_{CO_2} was kept between 30 and 40 mm Hg. When the bicarbonate threshold was reached, each dog was expanded with either saline, a mixture simulating his ECF, or salt-poor albumin. Values of cumulative net acid excretion in 90 min were: saline expansion, —1941, —559, and —303 μEq; "ECF" expansion, —529 μEq; albumin expansion, +213, +748, and +1255 μEq. Saline expansion caused the expected sodium diuresis and partial correction of the alkalosis by dilution. Despite a reduction in the filtered load of bicarbonate, net base excretion increased. With albumin expansion, no sodium diuresis was observed. Despite an increased filtered load of bicarbonate, net acid excretion resulted. These studies suggest that H^+ excretion and the renal bicarbonate threshold are modulated by factors influencing tubular avidity for sodium, and not the state of the extracellular volume per se.

79. Dipalmitoyl Lecithin in Lung Lavage of Normal Nonsmokers and Smokers. THEODORE N. FINLEY AND J. A. LADMAN,* Albuquerque, N. M.

Using 300 ml of saline in lavaging one lobe of eight nonsmokers, the total lavage volume recovered averaged 207 ml with a sediment of 0.3 ml. The upper 50% of the sediment was white and acellular, the lower 50% was brown and cellular; 40–99% of the cells were macrophages. In eight smokers similarly lavaged, 165 ml was recovered and the sediment volume averaged 0.4 ml; 90–100% of its volume was cellular and 99% of the cells were macrophages. We analyzed both layers for lipids; dipalmitoyl lecithin (DPL) is specifically reported. In normal subjects five white layers and two brown layers containing over 90% macrophages were analyzed. In normal smokers two white layers, those with sufficient volume for analysis, and seven brown layers were analyzed. In the normal nonsmokers studied, the volume of white and brown sediment analyzed was 0.13 and 0.12 ml, respectively. In the smokers these volumes were 0.05 and 0.43 ml. By volume, DPL averaged 16 mg/ml and 8 mg/ml in the white and brown layers in nonsmokers, and 17 mg/ml and 5 mg/ml, respectively, in smokers. The DPL of nonsmokers averaged 2.1 mg in the white layers and 0.95 mg in the brown layers for a total of 3.05 mg. The DPL total was practically the same in smokers, 3.10 mg, 0.85 mg in the white layers and 2.25 mg in brown layers. In smokers DPL predominated in the cellular phase; in nonsmokers it predominated in the noncellular phase. This difference in distribution may result from a movement of DPL into the cellular phase in response to smoke exposure. (Supported in part by NIH grants HE-12571-01 and 1-RO1-GM-14435-03, and by the Council for Tobacco Research.)

80. Regional Myocardial Blood Flow. NICHOLAS J. FORTUIN,* LEWIS BECKER,* SHIGEKOTO KAIHARA,* AND BERTRAM PITT,* Baltimore, Md. (introduced by Richard S. Ross).

Studies of regional myocardial blood flow using radioactive microspheres support Fulton's theory of internal redistribution of coronary blood flow. The distribution of 15 and 50 μ radioactive microspheres has been studied in 30 and 14 anesthetized dogs, respectively. The spheres, which are injected into the left atrium, do not recirculate and do not interfere with coronary hemodynamics. After sacrifice, the heart is divided into right ventricle (RV), septum (S), left ventricular endocardial half (LVEndo), and left ventricular epicardial half (LVEpi). The radioactivity is determined and results are expressed as ratios of counts per gram of one area compared with another. There is no difference in distribution of 15 and 50 μ spheres between LV and RV (1.57 ±0.38 vs. 1.45 ±0.29) and S and LV (0.96 ±0.14 vs. 0.97 ±0.15). However, there is a significant difference in distribution between LVEndo and LVEpi (1.12 ±0.13 vs. 1.45 ±0.22; $P < 0.001$). Preliminary studies in the arrested heart on cardiopulmonary bypass indicate that spheres of both sizes distribute equally and in increased concentrations in the endocardium. The differences observed in the distribution of the two sizes of spheres between LVEndo and LVEpi can be explained by Fulton's theory, which postulates a reservoir function of the subendocardial plexus. During diastole, spheres of both sizes enter the endocardial plexus as a function of flow. During systole, there is an Endo-Epi pressure gradient and blood is redistributed toward the epicardium through collateral channels smaller than 50 μ. The 50 μ spheres are entrapped in the endocardium, while 15 μ spheres flow with blood out toward the epicardium. (Research supported by National Heart Institute contract PH-43 67-1444.)

81. Continuous Blood Sampling as a Method for Studying 24 Hour Secretion Rates of Protein Hormones. ANDREW G. FRANTZ,* PAUL KILLIAN,* AND DONALD A. HOLUB,* New York, N. Y. (introduced by Donald F. Tapley).

Valid estimation of daily secretion rates of many protein hormones is hampered by their rapid disappearance from blood and lack of significant urinary excretion. A new approach to this problem has been devised utilizing a double-lumen Teflon catheter which permits continuous withdrawal of blood at very slow (2.5 ml/hr) and constant rates for 24 hr periods. Miniaturization of components and lack of external connections allow subjects normal patterns of exercise, feeding, and sleep. By this technique growth hormone (HGH) secretion has been studied to date in over 20 normal individuals, whose mean plasma levels have been measured during periods of varying duration throughout the day. Integrated 24 hr plasma levels have shown marked variation in different subjects, with means of 0.97 (range 0.24–1.4) mμg/ml for men and 3.04 (range 1.1–5.8) mμg/ml for women. Higher evening levels have occurred in some but not all subjects. Metabolic clearance rates (MCR) have been studied by both single injection and constant infusion techniques. The former method yields curves on occasion which are markedly nonlinear and cannot be analyzed on the basis of one or two compartment models. Extremely short apparent half times (<10 min) may occur at varying levels of plasma hormone. Constant infusion of unlabeled HGH at several dose levels in hypopituitary subjects has yielded consistent values for MCR ranging between 190 and 330 ml/min. MCR determinations in normal subjects, less certain because of endogenous production, appear to overlap this range but may considerably exceed it. Preliminary estimates of normal HGH secretion are 0.12 to 0.8 mg/day for men and 0.6 to 2.9 mg/day for

women. Insulin measurements in these subjects have also been performed and will be discussed. The method appears to have applications for any substance with an irregular secretory pattern and a short half time in blood. (Supported by NIH grants AM-11294 and TI-AM-5397, and American Heart Association grant 68-111.)

82. Correction of the Biochemical Defect of the Hurler and Hunter Syndromes in Culture by Diffusible Factors. JOSEPH C. FRATANTONI* AND ELIZABETH F. NEUFELD,* Bethesda, Md. (introduced by Joseph E. Rall).

Previous studies showed that cultured fibroblasts derived from the skin of patients affected with two mucopolysaccharidoses, the Hurler and Hunter syndromes, incorporate $^{35}SO_4$ into intracellular mucopolysaccharide (MPS) in a linear fashion, which differs from the normal in that no steady state is reached. If the cells are first labeled with $^{35}SO_4$, then chased with cold sulfate, the rate of decrease of intracellular ^{35}S-MPS content is less in diseased cells. From data of this type, we postulated that the defect in these disorders is one of inadequate MPS degradation. When Hurler and Hunter cells are mixed with each other, the kinetic patterns of radioactive MPS accumulation or decay revert to near normal. Such a correction is also observed when the studies are performed on cells in the presence of culture medium which had been in contact with cells of the different genotype. These observations suggest that each cell type releases a diffusible factor necessary for the correction of the other, and form the basis of an assay for the purification of these factors. Medium which has been incubated with heavily grown Hurler cells is assayed on Hunter cells, and vice versa, while medium preincubated with the same genotype as the assay cells serves as control. The corrective substance in each case is nondialyzable, and is precipitable with 70% ammonium sulfate and heat labile. It is excluded from Sephadex G-100 and retarded on G-200. Increased degradation of intracellular MPS by the diseased cells was demonstrated in the presence of partially purified material from the appropriate cell lines. The Hurler and Hunter corrective factors thus appear to be specific macromolecules whose nature should shed light on the mechanism of the disorders.

83. Hypoerythropoietinemia and Anemia. EUGENE P. FRENKEL* AND CHARLES C. DOUGLAS,* Dallas, Texas (introduced by Elias Strauss**).

In renal disease the degree of the hypoproliferative anemia is relatively proportional to the degree and duration of the decreased renal function. An opportunity to evaluate the dichotomy between erythropoietin (EPF) formation and excretory renal function occurred in a man extensively irradiated over the abdomen in 1959 (2148 rad renal dose by moving-strip technique). Subsequently a normochromic normocytic anemia (hematocrits 22–25 v/100 v) with reticulocytopenia developed and persisted 4 yr. Renal studies revealed normal urine and IVP, BUN 19, creatinine 1.3, and a clearance of 80. The following characterized the anemia as hypoproliferative: There was relative marrow erythrocytic hypoplasia. ^{51}Cr erythrocyte survival was normal. Ferrokinetics revealed normal plasma iron clearance and turnover and per cent utilization. ^{59}Fe uptake occurred in marrow; the liver and spleen activity were normal. The marrow transit time was 3.7 days in spite of a hematocrit (hct) of 23. Subsequent androgen therapy induced a rise in hct to 33 with a rise in the RBC mass to normal. Androgens were stopped to evaluate erythropoietin responsiveness, and phlebotomies were performed reducing the RBC mass 40% and the hct to 20. Reticulocytopenia persisted, and the marrow transit time was unchanged (3.4 days). Serial urinary EPF measurements demonstrated virtually no activity during control periods and no rise after phlebotomy. Subsequent androgen therapy induced a second increase in RBC mass. Thus, extensive abdominal irradiation with only minimally decreased renal function caused a profound hypoproliferative anemia. That the anemia was due to decreased EPF is documented by the initial reticulocytopenia and prolonged marrow transit times, the failure of phlebotomy-induced anemia to alter the marrow transit time, reticulocytes, or urinary EPF production, and finally by two documented responses to androgen therapy.

84. Defect in B-Thalassemic Cell-Free System: Unresponsiveness to Ribosomal Subunits and an Inhibitor of Protein Synthesis. JOSEPH E. FUHR,* CLAYTON NATTA,* PAUL A. MARKS, AND ARTHUR BANK,* New York, N. Y.

The genetically determined decrease in globin synthesis in B-thalassemia is due to a defect associated with polyribosome function. These studies show that a cell-free system prepared from thalassemic reticulocytes differs from normal in (1) not being stimulated by ribosomal subunits, and (2) containing an inhibitor of protein synthesis. A concentrated cell-free system was obtained by precipitation at pH 5.4 of enzymes, tRNAs, and polyribosomes necessary for protein synthesis. In 12 studies, purified ribosomal subunits (40S to 60S) prepared from thalassemic reticulocytes, added to the nonthalassemic reticulocyte cell-free system (nonthal pH 5), caused an average of 85% stimulation of amino acid incorporation, which is comparable to that observed with addition of nonthal ribosomal subunits. By contrast, addition of these subunits to the thalassemic cell-free system (thal pH 5) caused either no stimulation or inhibition of up to 50%. Evidence for the presence of an inhibitor of protein synthesis in thal pH 5 includes the following: (1) Incubation of mixtures of nonthal pH 5 and thal pH 5 in varying proportions results in much less amino acid incorporation than expected; (2) dialysis of thal pH 5 before incubation increases amino acid incorporation of the thal pH 5 by 40% or more; (3) addition of dialysate of thal pH 5 decreases nonthal pH 5 amino acid incorporating activity. Heme is probably not the inhibitor, since its addition to the thal pH 5 increases amino acid incorporation. These studies suggest that in B-thalassemia, the presence of an inhibitor may interfere with normal subunit function, presumably at the level of protein chain initiation, and these phenomena may be related to the primary defect in globin synthesis. (Research supported by grants TI-AM-5231 and GM-14552 from the NIH, and GB-4631 from the NSF.)

85. Hypoxanthine-Guanine Phosphoribosyltransferase Deficiency: Activity in Normal, Mutant, and Heterozygote Cultured Human Skin Fibroblasts. WILFRED Y. FUJIMOTO* AND J. EDWIN SEEGMILLER,** Bethesda, Md.

Hypoxanthine-guanine phosphoribosyltransferase (PRT) is not detectable (<0.04% of normal activity) in erythrocyte hemolysates of patients with the Lesch-Nyhan syndrome, an X-linked hereditary neurological disorder with excessive uric acid production. Yet cultured skin fibroblasts from such patients show PRT activity of around 3% of normal. Somewhat higher activities are found in fibroblasts from gouty patients whose erythrocyte hemolysates show an incomplete PRT deficiency. Although PRT activity is normal in erythrocyte hemolysates of obligate heterozygotes, low, intermediate, and/or normal levels are found in heterozygote fibroblasts. The enzyme in fibroblasts of both mutant types is more heat labile than the normal enzyme. Radioautographs, after growth on ^{3}H-hypoxanthine of fibroblasts cultured from carriers for the Lesch-Nyhan syndrome, show the two cell types predicted by the Lyon hypothesis, one with and one without PRT activity, as well as a third type with intermediate degrees of PRT activity. This third cell type is seen both before and after transfer from a high to a low cell density culture. When normal (PRT^+) and Lesch-Nyhan (PRT^-) fibroblasts are grown together at high cell density in 1:1 ratio for 24 hr, replated, and grown at low cell density for 24 hr, then grown for another 24 hr with ^{3}H-hypoxanthine, these three cell types are still seen on radioautographs. Only about 15% of the cells appear to be PRT^-, rather than the expected 50%. Recently, cellular interaction, termed "metabolic cooperation," has been described, whereby apparent enzyme activity appears in mutant cells which are in contact with normal cells. Such "metabolic cooperation" apparently occurs in heterozygote cultures. The persistence of intermediate activity after removal from cell contact suggests mediation by macromolecules (PRT or RNA?) rather than by uptake of enzyme product, inosinic acid. Failure of heterozygotes to show clinical evidence of PRT deficiency may reflect attenuation of abnormal metabolism of defective cells by such metabolic cooperation.

86. Synthesis of Bovine α-, β-, and γ-Globin Chains in Tissue Culture. THOMAS G. GABUZDA,* LILY S. CHEUNG-CHUI,* RUTH K. SILVER,* AND HUGH B. LEWIS,* Philadelphia, Pa. (introduced by Allan J. Erslev**).

The report to follow is an evaluation of the potential use of in vitro tissue culture of bone marrow as a method for investigating changing patterns of activity of structural genes for globin chains, such as those which accompany embryonic development. Suspensions of newborn calf marrow were incubated at 37°C in the presence of tissue culture medium and fetal calf serum with or without added erythropoietin. ^{3}H-leucine was added 21 to 45 hr before termination of the cultures, which were carried out as long as 21 days with periodic medium changes. Cell lysates containing carrier hemoglobin were partially purified on columns of Sephadex G-75 and then γ-, β-, and α-globin chains were separated by CM-cellulose chromatography in 8 molar urea. A highly radioactive protein with the chromatographic localization of the bovine γ-chain was consistently found in 10 to 21 day cultures without labeling of the β- or α-chain peaks. Its synthesis was independent of the presence of erythropoietin. However, in 3 day cultures erythropoietin enhanced the formation of all three types of globin chains. The results suggest persistent and stable synthesis of the fetal γ-globin chains in prolonged cultures. However, further confirmation of the identity of this labeled protein is required, particularly since α- and β-chain synthesis decays within only a few days of culture. Erythropoietin does not grossly discriminate between globin chain types and thus does not appear to play a primary role in globin chain commitment in erythroid stem cells. (Research supported by NIH grant HD-01794.)

87. Immunologic Studies on Two Erythropoietic Fractions. EDWARD GARDNER, JR.,* RUSSELL R. MOORES,* CLAUDE-STARR WRIGHT,** JASPER P. LEWIS,* AND LINDA L. SMITH,* Augusta, Ga.

The immunogenic response of rabbits to erythropoietically active human urinary preparations of erythropoietin is influenced by the composition of these preparations. Two erythropoietic fractions, designated dialysate and retentate, obtained by membrane dialysis of a urinary concentrate from cellulose chromatography were used to immunize two groups of rabbits. One group of seven rabbits received the dialyzable fraction (dialysate), and a second group of eight animals the nondialyzable fraction (retentate). Five animals in the first group responded by the production of neutralizing antibodies. The second group of animals, immunized similarly with the nondialyzable fraction, produced no neutralizing antibodies, although this fraction contained 3 times as much activity and 3 times as much protein as the dialyzable fraction. An additional experiment with a second group of rabbits immunized with the nondialyzable fraction from another human urinary source of erythropoietin substantiated the finding that neutralizing antibodies to this erythropoietin fraction were not readily produced. Moreover, the nonneutralizing antisera from the group of animals immunized with the nondialyzable fraction potentiated erythropoietin when allowed to react in vitro before injection into the assay animal. There are indications that the potentiating effect of the antisera increases after continued immunization. These data are compatible with the concept of an erythropoietin-producing enzyme as previously reported in this and other laboratories. A second inhibitor of erythropoietin cannot be ruled out by these data. (Research supported by NIH grants HE-10591-06 and FR-0061.)

88. The Effects of Nitrogen Dioxide and Nitrite Ion on Hydrogen Peroxide Metabolism in Alveolar Macrophages (AM) during Phagocytosis. J. B. L. GEE,* C. L. VASSALLO,* P. BELL,* AND R. E. BASFORD,* Pittsburgh, Pa. (introduced by J. D. Myers**).

Previous studies indicated that AM (1) contain high concentrations of catalase; (2) exhibit peroxidative metabolism, which is increased during phagocytosis; (3) contain reduced glutathione (GSH), a known antioxidant. Since peroxidative metabolism has been implicated as a bactericidal mechanism in other biological systems (e.g. polymorphonuclear leuko-

cyte), we studied the effects of nitrogen dioxide (NO_2) and one of its products in aqueous solution, nitrite ion (NO_2^-), on peroxidative metabolism and GSH in the AM. AM were harvested by pulmonary lavage and incubated in glucose-Ringer's containing 15% serum. Measurements of $^{14}CO_2$ production from ^{14}C-formate (catalase-dependent peroxidatic reaction) and other ^{14}C-labeled substrates and estimates of particle entry (light microscopy) were obtained under control conditions and during phagocytosis of heat-killed *S. albus*. Catalase activity and GSH concentrations were measured by the perborate and glyoxylase methods, respectively, on freeze-thawed AM extracts. Data indicate that (1) inhibition of AM catalase activity by aminotriazole (which reacts with catalase-H_2O_2 compound I) was reduced by 85% after preincubation with either 1 mM NO_2 or NO_2^-; (2) $^{14}CO_2$ production from ^{14}C-formate was reduced by approximately 50% in the presence of either 0.5 mM NO_2^- or 1 mM NO_2 in control and phagocytosing AM; (3) higher concentrations, exceeding 10 mM, of either NO_2 or NO_2^- were required to stimulate glucose oxidation and to oxidize intracellular GSH; (4) concentrations of up to 100 mM NO_2^- and 15 mM NO_2 did not diminish particle entry. Thus, low concentrations of either NO_2 or NO_2^- decrease catalase-dependent H_2O_2 metabolism in the AM. Higher concentrations of NO_2 or NO_2^- are required to modify glucose utilization and intracellular GSH. This indicates that low concentrations of oxidant air pollutants can modify an important biochemical pathway in an important pulmonary defense cell.

89. The Three-Dimensional Structure of Bradykinin. JACK M. GEORGE* AND LEMONT B. KIER,* Columbus, Ohio (introduced by James V. Warren**).

There is little reported information regarding the three-dimensional structure (conformation) of small biologically active polypeptides. This has impeded understanding of their molecular mechanism of action and the design of medically useful antagonists. This study was designed to describe three-dimensional conformation of essential functional groups of bradykinin. As conformations of minimum energy are preferred in aqueous solution, these were calculated for glycine, alanine, serine, proline, phenylalanine, and arginine in peptide form. The extended Huckel molecular orbital method was used with the aid of a computer, and interactions among all nuclei and valence electrons were considered. Available X-ray crystallographic data for polyamino acids agree with the calculated conformations. The conformation of the peptide bond is known (Pauling and Corey), and Anfinsen showed that linear sequence of amino acids determines conformation of the polypeptide. On this basis, models of amino acids were assembled to form bradykinin. Side chains of adjacent amino acid residues were not close enough to violate van der Waals contact distances (Ramachandran). Guanidinyl groups of the two terminal arginines and aromatic side chains of the two phenylalanines have been shown to be essential for biologic activity, and the model of bradykinin describes an arc with these four groups within 3 A of a common plane and with a distance of 21 A between the two guanidinyl groups. Over 60 analogues of bradykinin have been synthesized and tested for biologic activity. We constructed models of two active and ten inactive analogues, and an important finding was that the conformations of models of active analogues were more like that of the natural hormone than was the case for inactive analogues. The results of this study suggest the topography of the hormone presented to the receptor site and will facilitate the design of useful antagonists.

90. Dynamics of the Nitro Blue Tetrazolium Reaction in Granulocytes, with a Simple Micromethod for Detecting Chronic Granulomatous Disease. ROBERT H. GIFFORD* AND STEPHEN E. MALAWISTA,* New Haven, Conn. (introduced by Sherman M. Weissman).

The diagnosis of chronic granulomatous disease of childhood (CGD) depends upon specialized laboratory techniques that are not widely available. We have developed a simple, rapid, histochemical micromethod for screening large numbers of patients for this disorder and for simultaneously studying the dynamics of the nitro blue tetrazolium (NBT) reaction within a population of granulocytes. A single drop of blood is placed on a cover slip and incubated at 37°C for 25 min in a moist Petri dish. The cover slip is then washed, leaving a high density of adherent, motile granulocytes. These cells are exposed to a drop of pooled human sera with NBT (0.05%) and latex particles (both or neither), and then incubated an additional 20 min, air dried, fixed, and stained with safranin. Exposure to NBT converts numerous granulocytes into large, distinctive, degenerate-appearing cells containing collections of blue formazan precipitates (hence, "formazan cells"). Two children with known CGD repeatedly made no typical formazan cells, although at very high magnification tiny blue cytoplasmic foci could be found. This method allows certain additional observations not possible in other systems: (1) Phagocytosis does not change the percentage of formazan cells (about 80%). (2) Cells given NBT, but producing no formazan, ingest many more particles than either formazan cells or control cells incubated without NBT. Thus, NBT stimulates phagocytosis in nonformazan cells. (3) NBT 0.12% consistently produces fewer formazan cells than NBT 0.05% (the former produces about 40% formazan cells); this inhibition is reversible when the concentration of NBT is lowered. In summary, we have developed a simple screening test for CGD, especially useful in the very young because it requires only minute amounts of blood. In doing so, we have clarified some puzzling aspects of the NBT reaction in granulocytes.

91. Abnormal Albumin Metabolism in "Idiopathic Edema": Its Relation to Sodium Metabolism. JOHN R. GILL, JR.,* THOMAS A. WALDMANN, CATHERINE S. DELEA,* AND FREDERIC C. BARTTER,** Bethesda, Md.

Excessive sodium and water retention in women without cardiac, renal, or hepatic abnormality is a frequently encountered disorder. The cause(s) of the altered electrolyte metabolism is unknown, and this has led to the designation "idiopathic edema." In 14 women with "idiopathic edema," mean serum albumin, 3.27 ±0.31 g/100 ml (SD), was significantly lower than the 3.70 ±0.295 g/100 ml found in eight normal women ($P < 0.01$). Study of iodinated albumin metabolism indicated that plasma volume, 37.08 ±5.98 ml/kg, was subnormal (normal, 42.2 ±5.0 ml/kg) ($P < 0.05$), so that cir-

culating albumin, 1.20 ±0.18 g/kg, was lower than normal (1.56 ±0.17 g/kg) ($P < 0.01$). The total exchangeable pool of albumin, 3.15 ±0.72 g/kg, was also lower than normal (3.62 ±0.40 g/kg) ($P < 0.05$). The fractional catabolic rate, 0.136 ±0.025, was increased (normal, 0.110 ±0.016) ($P < 0.01$). In six patients a low synthetic rate was also present. On a sodium intake of 250 mEq/day, mean aldosterone secretion rates in the patients, 134 ±66 μg/day, were higher than those in the controls (46 ±32 μg/day) ($P < 0.01$); treatment with an aldosterone antagonist on balance regimen resulted in a mean net loss of sodium of 355 ±121 mEq in the patients as compared with 81 ±102 mEq in the controls ($P < 0.01$). These observations suggest that "idiopathic edema" may be, in part, a disorder of albumin metabolism, characterized by increased catabolism, and sometimes by decreased synthesis as well, with hypoalbuminemia, hypovolemia, and hyperaldosteronism. Changes such as these could be important factors in the excessive sodium and water retention.

92. Essential Role of Platelets in Maintaining Vascular Integrity of Organs during Normothermic Perfusion In Vitro. MICHAEL GIMBRONE, JR.,* RAMZI COTRAN,* JAMES H. JANDL, JUDAH FOLKMAN,* AND RICHARD H. ASTER, Boston, Mass.

Interstitial edema and endothelial degeneration invariably accompany prolonged perfusion of organs at 37°C in vitro. The following studies were undertaken to determine whether these changes result from inadequate numbers of viable platelets in the perfusates now in use, inasmuch as platelets are known to "support" vascular integrity in vivo. In 11 experiments, lobes from dog thyroids were perfused for 5 hr in parallel. One lobe of each gland was perfused with heparinized, autologous platelet-rich plasma (PRP), the other with autologous platelet-poor plasma (PPP). Glands perfused with PRP accumulated less edema ($P < 0.01$), had a lesser increase in vascular resistance ($P < 0.05$), and trapped fewer ^{51}Cr-labeled RBC ($P < 0.01$) and less ^{125}I-albumin ($P < 0.05$) than did glands perfused with PPP. Endothelium examined by electron microscopy was intact in most (74%) small blood vessels after PRP perfusion, whereas the majority of small vessels (71%) were damaged or necrotic after perfusion with PPP. Four pairs of lobes were reimplanted after perfusion; all four lobes perfused with PPP developed gross purpura within 15 min, but four lobes perfused with PRP developed neither gross nor microscopic hemorrhage. It is concluded that platelets function to preserve the vascular integrity of organs perfused at 37°C in vitro. Thus, platelets may prove useful, perhaps essential, for long-term organ preservation. In vitro perfusion of organs appears to provide a unique tool for the study of platelet function and may provide definitive information regarding the nature of platelet-endothelium interaction.

93. The Diagnosis of Essential Hypertension by the ^{3}H-Norepinephrine Uptake Test. STANLEY E. GITLOW,* MILTON MENDLOWITZ,** AND LAURA M. BERTANI,* New York, N. Y.

Patients with essential hypertension demonstrate vascular hypersensitivity to infused norepinephrine (NE) as well as abnormal metabolic handling of test doses of ^{3}H-NE. The ^{3}H-NE uptake test requires the determination of that part of 8 μg D,L-β-^{3}H-NE administered intravenously over a 1 min period which is excreted in the urine as tritium within 24 hr. The usefulness of this test in the diagnosis of essential hypertension was examined by a study of 20 normal subjects, 4 patients with surgically proved pheochromocytomas, 3 patients with renal hypertension, and 30 patients with essential hypertension without azotemia or congestive heart failure. The normal subjects excreted 52.82% of the test dose (range, 38.05–62.96%; SD ±7.78%), while those with essential hypertension excreted 75.60% (range, 67.71–91.00%; SD ±5.96%). Mild azotemia (BUN < 30 mg/100 ml) failed to invalidate differentiation of these groups by this test. The patients with pheochromocytoma fell into the hypertensive range before surgery and returned to normal postoperatively. Those patients with renal or renovascular causes for their hypertension excreted normal amounts of the test dose. Two subjects developed preeclampsia with severe hypertension followed by long periods of normal blood pressure during which they excreted abnormally elevated amounts of the ^{3}H-NE test dose. This simple, rapid, and harmless test procedure may well be of considerable diagnostic usefulness in differentiating the various types of human hypertension. (Supported by USPHS grant HE-06546.)

94. Pancreozymin: Sites of Secretion and Effects on Pancreatic Enzyme Output in Man. V. L. W. Go,* ALAN F. HOFMANN, AND W. H. J. SUMMERSKILL,* Rochester, Minn.

The distribution of pancreozymin (PZ) in the small intestine of man is unknown, and its effect on total pancreatic enzyme output has not been quantified. To investigate these, pancreozymin release was stimulated by perfusing different regions of the small intestine with isotonic solutions of essential amino acids (at pH 6.0 and using a marker). The response was determined by our method for measuring pancreatic outputs of lipase, amylase, and trypsin. Regional perfusions of the duodenum and, at distances beyond the ligament of Treitz, of the jejunum (at 30 cm and 80 cm) and ileum (at 130 cm) were carried out in 33 healthy fasting volunteers. Total outputs of each enzyme were similar ($P > 0.1$) after perfusion of the duodenum and both jejunal sites, and were the same as those evoked by maximally tolerated exogenous stimulus of hog pancreozymin by vein ($P > 0.1$). By contrast, perfusion of the ileum caused no secretion; enzyme output did not differ from that during basal steady-state conditions (perfusion of isotonic saline). Intravenous infusions of the essential amino acid mixture also did not influence basal pancreatic enzyme output. When both the endogenous (intraduodenal essential amino acids) and exogenous (hog pancreozymin by vein) stimuli were delivered simultaneously, total pancreatic enzyme output greatly increased ($P < 0.05$) above the "maximal" output resulting from either alone or reported earlier by others. It is concluded that PZ is secreted throughout the duodenum and jejunum but not from the ileum. Maximal secretory capacity of the pancreatic enzymes is not attained by conventional stimuli. These data call for new assessments of pancreatic function in health, and consideration of pan-

creatic integrity in disease or resection of the small intestine. (Research supported by NIH grant AM-6908.)

95. Use of a Specific Serum Antigen for Evaluation of Viral Hepatitis and for Detection of Infectious Blood Donors. DAVID J. GOCKE* AND NEIL KAVEY,* New York, N. Y. (introduced by Stanley E. Bradley**).

A specific antigen has been demonstrated in the sera of patients with viral hepatitis. In addition, this antigen has been found in some blood donors and correlated with development of hepatitis in recipients of their blood. The antigen was detected with a two-dimensional immunodiffusion system, employing sera from four patients who had received multiple transfusions as antisera (two hemophilia, one thalassemia, one sickle cell disease). 70 patients with acute viral hepatitis were studied. Of those tested in the first 12 days after the onset of symptoms, 36 of 45 (80%) were positive. This included 28 of 34 patients with serum hepatitis and 8 of 11 patients with infectious hepatitis tested in the first 12 days. In 19 patients, the presence of the antigen was confirmed on more than one occasion and changes in titer were related to the course of the disease. In 12 patients, conversion to negative occurred from 6 to 34 days after the onset. Decline in titer of the antigen paralleled restoration of hepatic function. In 2 unusual patients, positive reactions persisted for more than 6 months. The antigen was never detected in 31 patients with various acute and chronic forms of liver disease other than viral hepatitis, 70 patients with various hematologic disorders, 69 patients with diseases lacking hepatic involvement, and 318 normal subjects. The antigen was detected in sera from 3 of 256 blood donors. To date, 2 of the 3 recipients of blood from these donors have developed typical clinical and laboratory signs of viral hepatitis associated with appearance of the antigen. None of 30 recipients of 238 units of negative donor blood developed viral hepatitis after transfusion. Thus, this approach appears promising for the evaluation and definition of acute viral hepatitis. More importantly, it may provide a means of detecting potentially infectious blood donors. (Research supported by a grant from the John A. Hartford Foundation.)

96. Diminished Colony-Forming Ability of Cultured Fibroblasts in Diabetes Mellitus and Aging. SAMUEL GOLDSTEIN,* JOHN W. LITTLEFIELD, AND J. STUART SOELDNER,* Boston, Mass.

The ability of an individual cultured cell to form a colony tests its capacity to survive and multiply. Recent work in our laboratory has compared the colony-forming ability of fibroblasts cultured from skin explants of subjects bearing a high genetic risk of developing overt diabetes mellitus with those derived from normals from a similar age group. The percentage of fibroblasts from "high risk" subjects which were able to form colonies ($7.0 \pm 2.6\%$, $n = 13$) was significantly less than that of cells from normals ($12.1 \pm 1.0\%$, $n = 11$) ($P < 0.001$). This decrease in growth capacity was not apparent when the life spans of cell strains in the two groups were compared in mass culture, perhaps owing to metabolic interaction between "hardy" and "weak" cells. The colony-forming experiments above were done at about 20 cell generations after explantation, and then continued at 10 generation intervals. The percentage of cells able to form colonies has shown a downward trend for both groups, the difference between the "high risk" and normal groups being maintained at 30 generations ($6.1 \pm 0.9\%$ versus $9.5 \pm 0.8\%$, $P < 0.01$) and at 40 generations ($4.4 \pm 1.0\%$ versus $8.3 \pm 1.1\%$, $P < 0.02$). These data indicate that the effect of the diabetic gene(s) is to reduce the growth capacity of individual fibroblasts in vitro. Thus this disorder is apparent at the cellular level, and persistently so. The data also indicate a reduction in growth capacity as a function of aging in cells from all subjects. The basis for this effect of either the diabetic gene(s) or aging is unknown. However, the reduced growth capacity of individual cells could contribute to the decreased glucose tolerance and increased vulnerability of both diabetic and aged subjects. (This work was supported by Children's Bureau project 906 and USPHS grants CA-04670-09 and AM-09478-04.)

97. Third Factor: Inhibitor of Na-K-ATPase? H. GONICK,* H. J. KRAMER,* W. L. PAUL,* AND E. LU,* Los Angeles, Calif. (introduced by M. I. Grossman).

Acute volume expansion is known to inhibit proximal tubular reabsorption of sodium, probably by release of a natriuretic hormone ("third factor"). Since there is an impressive body of evidence linking Na-K-ATPase to active sodium transport, we were interested in seeing whether "third factor" exerted its effect on this transport enzyme. Accordingly, isotonic saline was infused intravenously into 13 rats to achieve expansion of extracellular volume to 10% of body weight. Controls consisted of 11 rats infused with glucose without volume expansion. Sodium reabsorption was reduced to 92.2% in the experimental group, as contrasted with 99.5% in the controls. Activity of Na-K-ATPase was determined in homogenates of whole kidney, cortex, and medulla, as well as the heavy and light microsomal fractions, by the method of Chignell and Titus. No significant differences in enzyme activity were noted between experimental and control kidneys. Sera from expanded and nonexpanded animals were fractionated on Sephadex G-25 and each fraction was assayed for its inhibitory effect on renal Na-K-ATPase. Several distinct areas of inhibition were found in both expanded and nonexpanded sera. However, there was a striking difference in inhibitory activity in the fraction with a V_E/V_O ratio of 2.5 (28% vs. 0). This was the only fraction which yielded a coincident UV absorption maximum at 280 mμ. We conclude that "third factor" acts as an inhibitor of Na-K-ATPase but that this effect is not demonstrable in vivo by the usual assay techniques, presumably because of dissociation of inhibitor by dilution.

98. Increased Myocardial Myosin ATPase Activity Associated with an Increased Myocardial Contractility in Hyperthyroid Guinea Pigs. M. JAY GOODKIND,* GEORGE E. DAMBACH,* AND ROBERT J. LUCHI, Philadelphia, Pa.

Increased myosin ATPase activity is associated with an increased rate of contraction in skeletal muscle. Myocardial contractility expressed as velocity of shortening is increased in the hyperthyroid animal. If the contractile mechanisms

of skeletal and cardiac muscle are similar, myosin ATPase activity of myocardium from hyperthyroid animals should also be increased. Guinea pigs were made hyperthyroid by the administration of sodium L-thyroxine (100 μg/day) for 2–3 wk. Hyperthyroid animals lost weight as compared with the weight gain of normal controls, and demonstrated hypertrophy of ventricular myocardium with a ventricular weight of 1.8 g compared with 1.4 g average weight for normal controls. Contractile responses of right ventricular papillary muscles from euthyroid and hyperthyroid guinea pigs were studied in Krebs-Henseleit solution (2.5 mM Ca^{++}) oxygenated with 95% O_2–5% CO_2. Isometric developed tension per gram of muscle and maximum rate of rise of tension were increased and time to peak tension decreased from normal in the myocardium of hyperthyroid animals over a frequency range from 6 to 180 beats/min. Myosin was extracted and myosin ATPase activity determined in ventricular myocardium from four groups of euthyroid guinea pigs and from three groups of hyperthyroid animals (four guinea pigs per group). Myocardial myosin ATPase activity averaged $8.71 \pm 0.13 \times 10^{-7}$ moles P per mg per min and $10.19 \pm 0.52 \times 10^{-7}$ moles P per mg per min for the euthyroid and hyperthyroid guinea pigs, respectively. The 17% greater activity in the hyperthyroid group was significant at $P < 0.02$. This study in hyperthyroid guinea pigs shows for the first time an increase in cardiac myosin ATPase activity occurring in association with an increased myocardial contractility. (Research supported by a grant from the American Heart Association.)

99. An Effect of Aldosterone on Na^+ Transport in the Absence of Oxidative Metabolism. DAVID GOODMAN,* JAMES ALLEN,* AND HOWARD RASMUSSEN, Philadelphia, Pa.

Two different hypotheses have been proposed to account for the effect of aldosterone upon sodium transport in toad bladder. Both propose that aldosterone acts by increasing the synthesis of a specific protein, but differ as to the function of this protein: Either the protein is involved in Na^+ entry across the mucosal border of the cell; or the protein stimulates the capacity of the energy-yielding reactions from pyruvate to succinate, producing thereby an increased supply of ATP to drive the Na^+ pump. A major experimental finding supporting the latter alternative is that under N_2, aldosterone alone does not stimulate Na^+ transport, but vasopressin will induce a significant increase. However, our present experiments show that when bladders are incubated under N_2, after pretreatment with aldosterone, the response to a standard dose of vasopressin is nearly twice that seen in controls. There was no clear difference between the basal rate of transport under N_2 between bladders pretreated with aldosterone and control bladders. After vasopressin treatment the ratio of the maximal rate of Na^+ transport to basal rate was 3.5 in bladders pretreated with aldosterone, and 1.7 in non-pretreated bladders. Thus our experiments demonstrate a significant effect of aldosterone upon Na^+ transport in the absence of O_2, and thereby eliminate the need for obligatory coupling of aldosterone-stimulated Na^+ transport to oxidative metabolism. They imply that a common ATP pool supplies energy for both aldosterone- and vasopressin-induced transport. Also measurement of changes in adenine nucleotide levels under these conditions rule out a primary effect of aldosterone upon energy production. An alternative model will be presented to account for the action of aldosterone.

100. The Immunity Produced in Human Volunteers by the Administration of Meningococcal Polysaccharides. EMIL C. GOTSCHLICH,* IRVING GOLDSCHNEIDER,* MALCOLM S. ARTENSTEIN,* AND TEH YUNG LIU,* Washington, D. C., and Brookhaven, N. Y. (introduced by Maclyn McCarty**).

The majority of meningococci which cause disease belong to sero groups A, B, and C. The group-specific antigens of group A and of group C are polysaccharides; the former a polymer of *N*-acetyl-*O*-acetyl mannosamine phosphate, the latter a polymer of *N*-acetyl-*O*-acetyl neuraminic acid. These polysaccharides were isolated in a high molecular weight form by a method employing Cetavlon to precipitate the polysaccharides from the whole cultures. The final preparations contained negligible amounts of protein or nucleic acid. Over 90% of the weight of these materials could be accounted for by the respective monosaccharide unit, acetyl, sodium, and moisture. All preparations had average molecular weights over 100,000. These polysaccharides contained less than 1% of biologically active endotoxin and had no other demonstrable toxicity for mice or guinea pigs. 204 volunteers were injected intradermally with 50 μg of either polysaccharide. No toxic reactions were observed. Within 2 wk all individuals with two exceptions formed antibodies as measured by passive hemagglutination or by bactericidal activity against group A or group C meningococci. The antibodies belonged to the immunoglobulin classes A, G, and M. Vaccination with the group C polysaccharide also induced specific local nasopharyngeal immunity, as shown by the following experiment. A group of army recruits undergoing basic training and including individuals vaccinated with group A polysaccharide or group C polysaccharide and uninjected controls was observed for a 6 wk period by fortnightly throat cultures. A significantly lower percentage of individuals of the population vaccinated with the group C polysaccharide acquired group C meningococci during the observation period.

101. Peroxidase Arthritis: A Model of Immunologically Mediated Inflammation Permitting Ultrastructural Cytochemical Localization of Antigen. RICHARD C. GRAHAM, JR.,* AND SARAJAYNE LIMPERT SHANNON,* Cleveland, Ohio (introduced by John R. Murphy).

A model of immunologically mediated arthritis, in which the antigen can be detected by ultrastructural cytochemistry, has been produced in rabbits by repeated intra-articular injections of either horseradish peroxidase or bovine lactoperoxidase. The peroxidase antigen catalyzes the formation of an electron-opaque product at the site of the peroxidase molecules. Arthritis occurred both in rabbits previously immunized against the peroxidase used and in rabbits with no contact with the protein other than the intra-articular injections. In the latter group, specific antibody appeared in the

serum as the arthritis developed, whereas in the former group, serum antibody was present before the intra-articular injections. Repeated intra-articular injections of saline or autologous serum, or single injections of antigen, produced no changes in either group of animals. Light and electron microscope examination of the arthritis revealed proliferation of synovial lining cells and extensive subsynovial infiltration by macrophages, plasma cells, fibroblasts, lymphocytes, and varying numbers of polymorphonuclear leukocytes. New collagen formation was observed. Synovial lining cells and subsynovial macrophages ingested large amounts of peroxidase, which was segregated in large cytoplasmic vacuoles. Cellular uptake was much greater than that seen in normal, unimmunized animals, suggesting either cellular alteration or the ingestion of peroxidase-antibody complexes. The histological appearance of synovial tissue in peroxidase arthritis closely resembles that in human acute rheumatoid arthritis. Since the fate of antigen (and antigen-antibody complexes) can be followed by ultrastructural cytochemistry, peroxidase arthritis provides a useful model for the study of immunologically mediated inflammation. (Research supported by research grant and Career Development award [to R. C. Graham] from the NIH.)

102. Vasopressin (ADH) and the Inhibition of Water Diuresis in Adrenal Insufficiency. HOWARD H. GREEN,* AVERY R. HARRINGTON,* AND HEINZ VALTIN, Hanover, N. H.

If ADH were the sole or major mechanism which inhibits acute water diuresis in adrenal insufficiency, then this inhibition should not occur in adrenalectomized rats with hereditary hypothalamic diabetes insipidus (DI), which lack vasopressin. The responses to intragastric water loads (5% of body weight) were determined in the same female DI rats before adrenalectomy (adx), after adx, and after adx plus adrenal cortical hormones. Before adx, 80% of the water load was excreted as dilute urine (93 mOsm/kg) after 1 hr. After adx, only 50% was excreted as more concentrated urine (149 mOsm/kg). The diuresis was restored to 84% when prednisolone was given 2 hr before the water load, but the urine was diluted to only 135 mOsm/kg. In order to assess restoration by the dual criteria of urine flow and urinary dilution, subsequent results were expressed as free water clearance (C_{H_2O}). Before adx, there was a highly significant, direct correlation between spontaneously varying osmolal clearance (C_{osm}) and C_{H_2O}. Adx abolished this relation and reduced C_{H_2O}. Prednisolone, 0.1 mg s.c. 2 hr before water loading, raised both C_{osm} and C_{H_2O} over the quantities seen in adx alone, but did not restore the correlation between the two. Giving 25 μg of aldosterone together with 0.1 mg prednisolone almost completely restored the correlation between C_{osm} and C_{H_2O} to what it had been in the same DI rats before adx. We conclude that ADH is not essential to the blunted water diuresis in adrenal insufficiency, although ADH may abet the inhibition in adx normal individuals; and that both gluco- and mineralocorticoids are required to fully restore the water diuresis. (Supported by NIH grants AM-08469-GM and 5-K3-GM-21,786.)

103. Elevated Erythrocyte Phosphoribosylpyrophosphate in X-Linked Uric Aciduria: Importance of PRPP Concentration in the Regulation of Human Purine Biosynthesis. MARTIN L. GREENE* AND J. EDWIN SEEGMILLER,** Bethesda, Md. (introduced by James A. Shannon).

In mammalian tissues, 5-phosphoribosyl-1-pyrophosphate (PRPP) is a substrate for the amidotransferase catalyzing the rate-limiting reaction of de novo purine biosynthesis, and for the phosphoribosyltransferases which catalyze nucleotide formation from purines and pyrimidines. An increased turnover of PRPP in patients with gout has been suggested. The erythrocyte, which lacks an intact de novo purine biosynthetic pathway, provides a model system for studying certain aspects of PRPP metabolism. We have detected markedly elevated erythrocyte PRPP concentrations in patients with X-linked uric aciduria (Lesch-Nyhan syndrome) and complete deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (PRT). PRPP concentrations (in units of 10^{-9} moles per ml packed cells, $\pm$SE) were 3.1 ± 0.5 in 10 normal subjects, 2.7 ± 0.5 in 14 patients with primary gout, 2.2 and 2.4 in 2 subjects with type I glycogen storage disease, 4.6 ± 1.3 in 3 patients with gout and incomplete PRT deficiency, and 38.8 ± 4.0 in 7 patients with virtually complete PRT deficiency and Lesch-Nyhan syndrome. Intravenous 2,6-diaminopurine or oral adenine promptly reduced elevated erythrocyte PRPP concentrations in the latter patients. Washed erythrocytes incubated in vitro generated free PRPP from glucose or fructose, but initial rates of accumulation of free PRPP were greater in PRT-deficient cells than in normal controls. Methylene blue increased the rate of PRPP accumulation. Hypoxanthine or guanine prevented PRPP accumulation in normal but not in PRT-deficient cells; other purines and pyrimidines prevented accumulation in both cell types. The elevated PRPP concentration in erythrocytes lacking PRT most probably results from their inability to use PRPP in the PRT-catalyzed reaction, thus increasing the amount of PRPP available for alternative reactions. In other tissues, increased availability of PRPP provides more substrate for PRPP amidotransferase, offering one explanation for the accelerated purine synthesis in patients with PRT deficiency. These data suggest that the availability of intracellular PRPP is an important factor in the regulation of purine biosynthesis in man.

104. Stimulation of Gut Electrolyte Secretion by Prostaglandins, Theophylline, and Cholera Exotoxin. W. B. GREENOUGH, III,* N. F. PIERCE,* Q. AL AWQATI,* AND C. C. J. CARPENTER, Baltimore, Md.

Adenosine-3',5'-cyclic monophosphate (cAMP) and *Vibrio cholerae* exotoxin (CT) each increase chloride transport from serosa to mucosa in isolated rabbit ileal mucosal segments. We have compared, in both in vivo and in vitro gut preparations, the effects of CT with those of theophylline, a phosphodiesterase inhibitor, and two prostaglandins (PGA_1 and PGE_1) both of which are known to alter tissue cAMP levels. In dogs with chronic 65 cm Thiry-Vella jejunal loops, PGA_1, PGE_1, and theophylline were infused into the superior mesenteric arteries via indwelling catheters. The jejunal loops were simultaneously perfused with isotonic electrolyte

solution with nonabsorbable marker (PSP), and net fluid and electrolyte transport were measured. PGE_1 and PGA_1 infusion at 2 μg/min for 90 min reduced net jejunal absorption significantly. Infusion rates of 8, 24, and 100 μg/min caused successively greater rates and durations of net fluid loss. 100 μg/min caused net isotonic fluid secretion (mean ±SE) of 21.1 ±8.2 (PGE_1) and 24.0 ±11.0 ml/loop per hr (PGA_1), with altered electrolyte transport persisting more than 3 hr after termination of infusion. Theophylline infusion at 5.5 mg/min did not alter net jejunal absorption, but at 22.2 mg/min it caused net isotonic fluid secretion of 24.0 ±11.2 ml/loop per hr. Mean fluid loss after supramaximal intraluminal challenge with CT resulted in net fluid secretion of 57.4 ±8.1 ml/loop per hr. The composition of fluid produced in response to each of the agents was identical, and no histologic evidence of gut mucosal damage was found. In rabbit ileal mucosa stripped of serosa and muscularis, mounted between Lucite half chambers and bathed with oxygenated bicarbonate-Ringer's, cAMP, theophylline, PGE_1, PGA_1, and CT all had effects on short-circuit current (SCC) which were similar in magnitude and direction. These data indicate that the CT-induced gut fluid loss and CT-induced changes in SCC across gut mucosa can be simulated by agents which alter tissue cAMP levels.

105. Neutralization of Endogenous Glucagon by High-Titer Glucagon Antiserum. N. Grey,* J. McGuigan,* and D. Kipnis, St. Louis, Mo.

Antisera to glucagon (ASG) suitable for radioimmunoassay have been produced, but the weak antigenicity of glucagon has resulted uniformly in ASG of low titer. By covalent coupling of glucagon to hemocyanin with diethylmalimadate, 24 moles of glucagon per 100,000 mol wt hemocyanin, we have been able to enhance substantially the immunogenicity of glucagon, making possible the production of antisera of sufficient titer to induce acute glucagon deficiency in rats in vivo. The glucagon-hemocyanin complex was emulsified in Freund's adjuvant, and injected into foot pads of rabbits (2 mg glucagon) twice at 2 wk intervals and, thereafter, at 3 month intervals. Antibodies were readily detected within 6 wk and antisera were harvested after two or four injections. On the basis of binding of ^{125}I-labeled glucagon, ASG titers of 150–750 mμg glucagon/ml were obtained. The metabolic significance of endogenous glucagon was examined in two experimental situations: (*a*) 48 hr fasted rats, adrenalectomized at the beginning of the experiment to avoid fluctuations in circulating epinephrine, received either ASG or normal rabbit serum (NS), and blood samples were obtained at frequent intervals from a catheter in the right atrium. NS rats showed no significant change in blood sugar, whereas ASG rats exhibited a decrease to 88%, 82%, 69%, 79%, and 63% of base-line levels at 15, 30, 45, 60, and 75 min, respectively. (*b*) Fed rats, pretreated with ASG and then given arginine (0.5 g/kg) intravenously, exhibited the same fall in blood sugar as rats receiving arginine alone. These results indicate that (1) high-titer ASG can be produced by covalent coupling of glucagon to hemocyanin, and (2) glucagon plays a more important role in the maintenance of carbohydrate homeostasis in the fasted than in the fed state. Potent ASG should be a useful tool for examining other consequences of acute glucagon deficiency and for assessing structure-function relations.

106. Mode of Action of Atromid-S on Cholesterol Metabolism in Man. Scott M. Grundy,* E. H. Ahrens, Jr.,** Gerald Salen,* and Eder Quintao,* New York, N. Y.

Cholesterol balance studies of 3–6 months' duration were carried out in 17 patients treated with Atromid-S (Clofibrate, CPIB) in order to determine its mode of action. Three patients were normocholesterolemic; 14 had hyperlipoproteinemia (types I–V). Placebo studies were carried out routinely. All normo- and hyperlipoproteinemic patients, except the two patients with fat-induced hyperglyceridemia (type I), had significant reductions in plasma concentrations of cholesterol and/or triglycerides and striking increases in fecal excretion of endogenous neutral steroids during Atromid-S treatment. Decreases in fecal bile acids were common but smaller. In most cases increments in fecal excretion of total endogenous steroids greatly exceeded decrements of plasma cholesterol content. The drug caused no change in cholesterol absorption in any patient. Using isotopic techniques, we investigated whether the increment in steroid excretion was derived from newly synthesized cholesterol or from tissue stores: 12 patients were pulse labeled with radioactive cholesterol intravenously at the beginning of their studies. The drug caused a decreased slope of the decay curve of plasma cholesterol specific activity in 5 patients, no change in 5, and a slight increase in 2. Since in 10 of 12 patients the curves failed to show an enhanced rate of decay, i.e. increased slope, we conclude that an increase in cholesterol synthesis had not occurred. Indeed, the decreased slope in 5 cases implies that synthesis was actually depressed (as suggested by animal and in vitro studies). Therefore, the increments in excretion of endogenous steroids during drug treatment were derived mainly from tissue stores. This conclusion is supported by the clinical observation that xanthomata regress during drug administration. Thus, Atromid-S lowers cholesterol through an advantageous combination of two actions: (1) increasing efflux from storage sites leading to a reduction of total body cholesterol, and (2) inhibiting the anticipated compensatory increase in cholesterol synthesis. (Research supported by USPHS grant HE-06222-08 from the National Heart Institute, and by USPHS grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources.)

107. Left Ventricular Pressures in Acute Myocardial Infarction. Paul Hamosh,* Ibrahim M. Khatri,* and Jay N. Cohn, Washington, D. C.

32 patients with acute myocardial infarction (MI) were studied less than 24 hr after onset of symptoms. Group I consisted of 11 uncomplicated cases, group II of 8 patients with signs of mild to moderate pulmonary congestion, and group III of 13 patients in shock. Left ventricular pressures were measured at the bedside through a specially designed catheter introduced percutaneously into the femoral artery. Central venous pressure (CVP) was determined through a

catheter in the right atrium, and cardiac output (CO) by the indicator dilution technique. No complications attributable to the procedure were noted. In group I, left ventricular end-diastolic pressure (LVEDP) ranged from 6 to 22.5 mm Hg (mean 14.5 mm Hg). The three patients with normal LVEDP did not show EKG changes of transmural infarction. In groups II and III LVEDP was significantly higher ($P < 0.01$); group II, 19–42 mm Hg (mean 29.5 mm Hg); group III, 14–40 mm Hg (mean 24.8 mm Hg). CVP tended to vary with LVEDP ($r = 0.56$, $P < 0.01$): group I, mean 9.5 mm Hg; group II, 10 mm Hg; group III, 12.5 mm Hg. Mean cardiac index was 3.30 liters/min per m^2 in group I, 2.96 liters/min per m^2 in group II, and 1.35 liters/min per m^2 in group III. Heart rate averaged 73 in group I, 98 in group II, and 108 in group III. These data show that (1) left ventricular failure almost invariably accompanies transmural MI; (2) LVEDP over 25 mm Hg may coexist with few pulmonary symptoms; (3) patients in shock have lower CO but not necessarily higher LVEDP than patients without shock. CO is maintained in MI apparently by left ventricular dilatation and tachycardia. Although shock may reflect more severe LV failure, inadequate compensatory dilatation could contribute to low CO in some patients. (Supported in part by a grant from the NIH.)

108. Familial Defects in Fibrin Stabilization. JAMES W. HAMPTON,* Oklahoma City, Okla. (introduced by Stewart Wolf**).

The stabilization of fibrin has been attributed to a transamidase enzyme which is activated by thrombin and promotes cross-linkages in the fibrin molecule. The enzyme has been presumed to be missing from the blood of individuals with severe hemorrhagic disorders accompanied by functional deficiencies of the fibrin-stabilizing factor. An opportunity to explore the pattern of hereditary transmission of this defect was afforded in a study of individuals from four families. The relative degree of functional fibrin-stabilizing factor (factor XIII) deficiency was studied in five patients from three different families. Three boys and two sisters of one of the boys had marked defects in clot stabilization with similar degrees of deficiency in percentage of normal plasma factor XIII activity. All had very low activity, less than 10% of normal, and cross-correction mixtures of their plasmas confirmed the similarity of their defects. The family of one of the boys, originally reported by Britten, was studied in detail. The plasma of presumed "heterozygotes" in his family, including his parents, contained from 20 to 60% factor XIII activity. None of these heterozygotes have displayed symptoms of excessive bleeding. Plasma transfusions in this boy raised the factor XIII activity to greater than 10% of normal, and the activity gradually fell over the next 10 days. A second peak of factor XIII activity appeared at 14 to 16 days and gradually fell. Our findings support the hypothesis that the fibrin stabilization defect of less than 10% activity represents an autosomal recessive disorder. The presence of 50% activity in the asymptomatic father of the propositus of one family appears to exclude the possibility of cross-linkage in that particular family. (Research supported by grant HE-12316 from the NIH.)

109. Local Immunoglobulin Synthesis in Lower Urinary Tract Infection. W. LEE HAND,* JAMES W. SMITH,* THOMAS E. MILLER,* JACK A. BARNETT,* AND JAY P. SANFORD, Dallas, Texas.

Local production of secretory IgA has been suggested as an important host defense against infection. This immunoglobulin is normally present in urine. Since the bladder has the same embryological origin as the intestinal tract, which is known to have cells containing IgA, it appeared logical to study local immunoglobulin synthesis, including IgA, by both the normal and the infected lower urinary tract. Bladder infection was produced in male rabbits by placing a loose ligature around the bladder neck and injecting *E. coli* 075 into the bladder. After sacrifice, in vitro incorporation of ^{14}C-labeled amino acids was employed to quantitate protein and immunoglobulin synthesis by bladder, regional lymph nodes, and spleen. In six control animals <5% of protein synthesized by bladder was IgG, IgA, or IgM. 10 infected animals were studied at times from 3 to 129 days. In seven animals infected for at least 10 days, mean protein synthesis by the bladder was 3 times and IgG synthesis was 25 times greater than normal. IgG synthesis accounted for 27% of protein synthesized by bladder; IgA and IgM synthesis were only slightly increased above normal (<5% of total protein). Increases in total protein and IgG synthesis, with little increase in IgA or IgM (<5% of total protein), occurred in spleens and nodes from infected animals. Specific antibody produced by bladders, spleens, and lymph nodes of infected animals was determined by precipitation with somatic antigen of *E. coli* 075. Demonstrable antibody was IgG, accounting for 12–38% of this immunoglobulin produced by bladders but only 0–7% of that produced by spleens or nodes. These studies demonstrate that in rabbits with lower urinary tract infection the marked increase in local immunoglobulin production is predominantly IgG, while little IgA or IgM synthesis occurs. The specific antibody produced by the bladder is IgG. (Supported by USPHS grants HD-00851, T1-AI-00030, 1-F3-AI-40,467-01, and a VA clinical investigatorship.)

110. Transformation of Human Blood Monocytes into Macrophages. JON M. HANIFIN* AND MARTIN J. CLINE, San Francisco, Calif.

We have developed techniques for maintaining normal human monocytes in culture for periods of more than 2 months. After 7–14 days the cells underwent a marked morphologic transformation. Cell diameters increased 5- to 10-fold, ultrastructure became characteristic of macrophages, and time lapse cinemicrography demonstrated characteristic slow movement and unique cytoplasmic extensions. Nuclei became round or ovoid with prominent nucleoli, and many multinucleate forms appeared. At least some of these macrophages were capable of DNA synthesis. After 72 hr exposure to ^{3}H-thymidine, 0.4–2.7% of cells were labeled, with occasional asymmetric labeling of multinucleate cells. No mitoses were seen, either with or without colchicine. Multinucleate cells probably resulted from endomitosis and/or cell fusion. 100% of macrophages were phagocytic for bacteria and yeast, and they ingested many more particles per cell than did monocytes. As with monocytes, phagocytosis was reduced by glycolytic inhibitors but not by anaerobic condi-

tions. We studied monocytes from patients with beryllium granulomas and tuberculosis and a patient with Sezary's syndrome. All showed the usual transformation to macrophages capable of normal phagocytic activity.

111. Immunologic Distinction between Serum and Infectious Hepatitis. R. LESLIE HARGROVE,* GRAHAM H. JEFFRIES, AND ALFRED M. PRINCE,* New York, N. Y.

An antigen (SH), located on small virus-like particles, has been detected during the incubation period and early clinical course of posttransfusion serum hepatitis. To determine whether this antigen was specific for disease caused by the virus of serum hepatitis (virus B), we tested for the presence of SH antigen in early sera from more than 400 patients with hepatitis of different kinds, using the Ouchterlony technique. The specificity of the test was controlled in each case by carrying out reactions of identity with reference SH antigen. SH antigen was found in each of eight cases induced by Krugman's long-incubation-period (MS-2) strain of hepatitis virus. It was absent in corresponding sera from two cases of the short-incubation disease (MS-1). The antigen has not been detected in more than 60 cases of childhood or epidemic "infectious" hepatitis. These findings suggest that the SH antigen is specific for infection by the causative agent(s) of serum hepatitis (virus B). This suggestion is further supported by the presence of SH antigen in 56% of patients with acute viral hepatitis who are known drug users, and in 52% of cases of hepatitis following transfusion. In a detailed study of 56 unselected adult patients with acute viral hepatitis seen at The New York Hospital, 35 of whom had SH antigen in acute serum samples, there was no significant difference in racial and age distribution or duration of hospitalization between those patients in whom antigen was found and those in whom antigen could not be detected. Especially noteworthy was the finding that 37% of hepatitis patients who were SH positive admitted to no parenteral exposure to blood, blood products, or injections of any kind. If the population studied is representative of adult viral hepatitis in general, the virus, or virus group, associated with classical serum hepatitis (virus B) appears to be responsible for most cases of hepatitis in this age group, despite the absence of known parenteral exposure. (Supported by grants from the NIH and the Health Research Council of the City of New York.)

112. The Regulation of Thrombopoiesis. J. A. HARKER,* Seattle, Wash. (introduced by C. A. Finch).

The mechanisms regulating the cytoplasmic maturation and release of platelets from megakaryocytes were evaluated by ^{35}S labeling studies. Injected ^{35}S incorporates into cytoplasmic mucoplysaccharides of developing megakaryocytes and later appears in the circulation bound to the released platelets. In normal rats ^{35}S utilization in platelets was constant over a dose range of 0.1–10 μc ^{35}S per gram of weight when expressed as per cent of the injected dose, and was maximal on the 3rd day after injection. Measurements in animals with stimulated thrombopoiesis (produced by 10 day period of exchange transfusion–induced thrombocytopenia) demonstrated a 4 × normal ^{35}S incorporation into platelets, the maximal utilization appearing a day earlier than normal. Conversely, determinations in animals with suppressed platelet production (induced by 10 day platelet hypertransfusion) showed ^{35}S incorporation reduced to one-fourth of normal, which was maximal a day later than normal. In a test system analogous to the bioassay of erythropoietin, plasma from animals with stimulated, normal, and suppressed thrombopoiesis was injected over a 4 day period into animals whose endogenous thrombopoiesis was suppressed by platelet hypertransfusion, followed by determination of ^{35}S utilization to measure platelet production. Suppressed plasma did not increase the ^{35}S utilization over base line. However, normal plasma induced a doubling of ^{35}S utilization, and stimulated plasma caused an incorporation of ^{35}S 4-fold greater than base line. It is concluded that thrombopoiesis is regulated by a plasma-stimulating factor(s), and that this thrombopoietin affects cytoplasmic maturation as shown by the ^{35}S marrow transit time and incorporation into circulating platelets. (This investigation was supported by USPHS research grants 5-RO1-HE-06242 and HE-11775-01.)

113. Reversal of Cholera Exotoxin–Induced Jejunal Secretion by Cycloheximide. DAVID T. HARPER, JR.,* DAVID I. GRAYER,* JOHN H. YARDLEY,* AND THOMAS R. HENDRIX,** Baltimore, Md.

Cycloheximide, an inhibitor of protein synthesis, will prevent cholera exotoxin–induced fluid production in rabbit jejunal loops. To characterize the mechanism of this inhibition, five groups of rabbits had instilled in isolated jejunal loops: control ("Syncase") broth; broth 1 hr after i.v. cycloheximide 20 mg/kg (cycloheximide-broth); broth followed 2 hr later by i.v. cycloheximide (broth-cycloheximide); filtrate of a broth culture of *Vibrio cholerae* (cholera exotoxin); cholera exotoxin followed 2 hr later by i.v. cycloheximide when fluid production in the loops was well established (cholera-cycloheximide). After broth or exotoxin had been in the loop for 1 hr, net fluid movement during the subsequent 9 hr was monitored by measuring PSP concentration in a recirculating perfusate of Ringer's lactate. Final perfusate volume was also measured. The control broth group showed progressive concentration of PSP and final net fluid absorption of 0.25 cc/cm loop. The cycloheximide-broth and broth-cycloheximide groups showed more rapid concentration of PSP beginning 4½ hr after cycloheximide, and greater final net fluid absorption of 0.65 cc/cm and 0.4 cc/cm, respectively. The cholera exotoxin group showed marked dilution of PSP and final net fluid secretion of 2.25 cc/cm. The cholera-cycloheximide group showed final net fluid secretion of only 0.4 cc/cm, with an inhibition of secretion beginning 2½ hr after cycloheximide administration. Cycloheximide in this group caused mild to moderate epithelial cell cytoplasmic vacuolization and nuclear swelling, more marked in crypts than on villus tips. Unidirectional sodium fluxes in jejunal loops 6 hr after cycloheximide showed reduction in lumen-to-blood flux, but still greater reduction in blood-to-lumen flux. *Conclusions:* Cycloheximide (1) reverses established cholera exotoxin–induced fluid production in in vivo rabbit jejunal loops, and (2) increases net absorption from control loops. Flux studies suggest that both effects are the result of a greater inhibition of secretion than of absorption. (Re-

search supported by NIH graduate training grant AM-09095 and NIH research grant AI-08187.)

114. Augmentation of Tumor Antigenicity by Chloroquin and Alkylating Agents. WILLIAM J. HARRINGTON AND SHARON P. POCHRON,* Miami, Fla.

The most curable of disseminated cancers, gestational choriocarcinoma, owes this feature to its complement of allograft antigens. If similarly potent antigens could be conferred upon other tumors, they should be likewise curable. We have demonstrated that ionizing radiations and radiomimetic alkylating agents induce formation of new antigens in normal and malignant cells. Immunization with isolated populations of these cells selectively enhances damaging effects of these agents. The new antigens appear to represent mutations which undergo repair. Chloroquin inhibits DNA polymerase, a participant in repair, and reportedly increases the responsiveness of resistant tumors to ionizing radiations or alkylating agents. The effects of chloroquin on induction and persistence of the new antigens were studied. (1) Four groups of adult male Holtzman rats were immunized with rat bone marrow in adjuvant. The donors were normal, busulfan-treated, chloroquin-treated, or combination-treated rats. Each group was then given busulfan (2 mg/kg per day, 6 times), chloroquin (25 mg/kg per day, 6 times), or their combination. Augmented marrow depression from busulfan was noted in animals immunized with marrow from busulfan or combined-treatment donors. Its ultimate severity was greater in the latter group, as was its persistence. (2) Six patients with leukemia or lymphoma have been studied. Excellent and continuing remissions have been accompanied by augmented immunologic responses to new antigens induced in the malignant cells. Improved responsiveness of tumors to ionizing radiations and alkylating agents, through use of chloroquin, involves, in part, host factors, and provides further leads to exploitation of immunologic mechanisms in cancer therapy. (Supported by NIH grant 5-PO1-AM-09001.)

115. Collagenase in Synovial Fluid: Its Possible Role in Rheumatoid Arthritis. EDWARD D. HARRIS, JR.,* AND STEPHEN M. KRANE, Boston, Mass.

A collagenase which degrades native collagen at neutral pH has been partially purified from cultures of rheumatoid synovium in vitro. However, little activity is detected in fresh synovial homogenates, and this collagenase is inhibited by serum. Therefore, to establish a role for collagenase in the articular destruction accompanying rheumatoid arthritis (RA) one must demonstrate active enzyme extracellularly (e.g., in synovial fluid) despite the presence of serum-type inhibitors. A component of synovial fluid inactivated by trypsin (but not by hyaluronidase or by heating to 56°C) inhibited synovial collagenase. However, synovial fluids from 4 of 13 patients with RA had inhibitory activity (corrected for protein concentration) less than 50% of normal. In these four fluids collagenolytic activity was found in fresh centrifuged specimens. Heating such fluids resulted in loss of collagenase activity and an increase in the apparent inhibitory capacity. In individual subjects, the collagenase activity in synovial fluid, inversely proportional to the titer of inhibitor, varied with fluctuations in clinical activity of the RA. After digestion with hyaluronidase, one of these fluids was subjected to gel filtration on Sephadex G-150 and Bio-Gel P-200. Two peaks of collagenase activity were found. One was eluted in a position similar to that of synovial collagenase, was inhibited by serum, and cleaved collagen molecules in solution at 27°C to quarter and three-quarter segments as shown by electron microscope examination of segment long spacing aggregates. The other collagenase peak appeared to be of higher molecular weight and was not inhibited by serum, a fact which suggests its possible relation to the leukocyte enzyme recently described. We now postulate that in chronic active RA sufficient collagenase may be released from proliferating synovial cells and possibly leukocytes to overcome the natural inhibition of synovial fluid proteins, enabling articular destruction by collagenase to occur. (Supported by grants from the NIH and the Massachusetts Arthritis Foundation.)

116. HeLa Glucose-6-Phosphate Dehydrogenase. PETER HATHAWAY* AND JACK LAROC,* New York, N. Y. (introduced by Kurt Hirschhorn).

S. Gartler's finding that 18 established cell lines had glucose-6-phosphate dehydrogenase (G-6-PD) type A and phosphoglucomutase (PGM) type 1 was quite contrary to expectation based on known gene frequencies, and led him to suggest that all these lines had a common origin, most likely HeLa contamination. Because we felt that there might be other reasons for finding what looks like G-6-PD type A in these lines, we decided to look more closely at HeLa cell phenotypes. We evaluated G-6-PD using precipitates from 3/5 saturated ammonium sulfate at 4°C in starch gel electrophoresis. A stock buffer, in which the total molarity of Tricine and Tris was 0.9 and EDTA was 0.02 M at pH 8.3, was diluted 1:20 for the gel (11.5 g electrostarch per 100 ml) and 1:8 for the wick chambers. A+ is clearly distinguished from, and runs more rapidly than, HeLa G-6-PD. A— and HeLa are not distinguished by mobility. However, G-6-PD A— stains much less intensely than HeLa. Moreover, Yoshida has already distinguished A— from G-6-PD HeLa by CM-Sephadex chromatography. Preliminary studies of PGM do not distinguish HeLa from type 1-1 by mobility, but show a marked shift in the distribution of activity when HeLa is compared with other type 1-1 tissues. This shift is also demonstrable on isoelectric fractionation. In two systems, then, HeLa is different to one degree or another from the alleles normally studied in human material. The data could support Gartler's hypothesis, but for reasons different from his. On the other hand, they are not incompatible with other hypotheses rejected by him. (Supported by USPHS grants HD-04134 and HD-02552.)

117. A Possible Mechanism for the Interaction of Phospholipid Membranes with Isolated Leukocytic Granules. JACEK HAWIGER,* ROBERT G. HORN,* ROBERT D. COLLINS,* AND M. GLENN KOENIG, Nashville, Tenn.

A cell-free system containing isolated PMN leukocytic granules and artificial phospholipid membranes (spherules)

has been previously utilized to study factors responsible for degranulation of leukocytes during phagocytosis. Two types of phospholipid spherules, lecithin and inositol phosphatide, caused activation of the lysosomal marked enzymes, beta glucuronidase and beta *N*-acetyl glucosaminidase. Electron microscope examination revealed marked disruption of the granules. When granules were incubated with phospholipid spherules at pH 7.0, structural disintegration of the granules occurred, and lysosomal enzymes were activated but remained largely sedimentable with the membrane fragments. A decrease of pH from 7.0 to 5.0 or below solubilized the active enzymes. The granule-spherule interaction did not appear related to the formation of phospholipid breakdown products. The activation of lysosomal enzymes by phospholipid spherules was not affected by sodium fluoride, an inhibitor of lipolytic enzymes in leukocytes, or albumin, which inhibited the granule-disrupting activity of lysolecithin. Only protamine sulfate, a basic protein known to form complexes with phospholipids, abolished the ability of spherules to activate lysosomal enzymes. These data suggest that the mechanism of interaction of phospholipid membranes with leukocytic granules may involve the formation of a complex between the phospholipid spherules and a protein in the granule membrane. This may lead to steric changes in the membrane, and activation of lysosomal enzymes. Subsequent release of these enzymes in soluble form requires a drop in pH. Thus, degranulation of leukocytes during phagocytosis may be induced by unmasking free, active phospholipid groups of the vacuolar membrane which can interact with a protein in the lysosomal membrane. (Supported by grants AI-03082, AI-00323, and HE-10048 from the USPHS.)

118. Evidence That Prostaglandin E_1 and Vasopressin Act at a Chlorpropamide-Sensitive Site in the Toad Bladder. RICHARD M. HAYS AND JULIE R. INGELFINGER,* New York, N. Y.

Prostaglandin E_1 (PGE_1) is a potent inhibitor of vasopressin in the toad bladder. Though it has been suggested that PGE_1 and vasopressin compete for the active site of adenyl cyclase, the evidence is not conclusive. We have utilized chlorpropamide, a sulfonylurea that reduces urine volume in diabetes insipidus, to determine whether vasopressin and PGE_1 do act at a common site. We have recently observed that chlorpropamide enhances the effect of vasopressin, but not cyclic AMP, on osmotic water flow (Δw) across the bladder; it therefore appears to increase the sensitivity to vasopressin of adenyl cyclase or some closely related step. The present studies were designed to determine whether PGE_1, like vasopressin, acts at the chlorpropamide-sensitive step. In the first set of experiments, Δw was determined in paired bladder halves mounted on bungs. One half was incubated for 30 min in Ringer's solution containing 3×10^{-4} M chlorpropamide; the control was in Ringer's alone. PGE_1 (10^{-7} M) was then added to both serosal solutions, and 30 min later, vasopressin (6 mU/ml) was added to both solutions. Instead of the usual enhancement in Δw, the chlorpropamide-treated bladders showed a $13 \pm 5\%$ (SE) lower Δw than controls ($P < 0.05$). In the second series, 10^{-7} M PGE_1 reduced Δw 64% in bladders initially given 3×10^{-4} M chlorpropamide plus 6 mU/ml vasopressin, but only 38% in bladders initially given vasopressin alone ($P < 0.01$). Thus, in both series, chlorpropamide intensified the inhibitory effect of PGE_1. Our findings indicate that PGE_1 and vasopressin both act at a chlorpropamide-sensitive locus in the cell. Though this locus remains undefined, the results are consistent with the view that PGE_1 and vasopressin compete for the active site of adenyl cyclase, while chlorpropamide increases the sensitivity of the enzyme to both agents. (Research supported by a grant from the NIH.)

119. The Dynamic Morphology of the Left Ventricle during Exercise in Man. RICHARD H. HELFANT,* JOEL H. MANCHESTER,* MICHAEL V. HERMAN,* EDMUND H. SONNENBLICK, AND RICHARD GORLIN, Boston, Mass.

Although the hemodynamic effects of exercise are well known, the morphologic response patterns of left ventricular contraction have remained undefined. Patients with no demonstrable evidence of cardiac disease as judged by standard left and right catheterization hemodynamics and selective coronary angiography were studied. Cardiac output, left ventricular pressure, and heart rate were determined immediately before cine ventriculography, performed in the right anterior oblique projection with a power injection of 40–60 ml of 75% meglumine diatrizoate into the pulmonary artery. The hemodynamic and ventriculographic measurements were then repeated after 5 min of supine leg exercise on a bicycle ergometer. After exercise, the expected increases in heart rate, cardiac output, and stroke volume occurred, with little change in left ventricular end-diastolic pressures. However, using each patient as his own control, frame-by-frame ventriculographic analysis revealed a small increase in average end-diastolic volume (average 10%) in the exercise state. The most striking change with exercise was an increase in the waist of the left ventricle in end diastole as measured by bisecting the mid aortic valve to apex length or taking the average width derived mathematically. This resulted in an increase in the ratio of end-diastolic width-to-length ratio (average 38%), indicating a more spherical ventricular chamber during exercise. The increased width-to-length ratio persisted in half the subjects, while in the remainder the ratio rapidly reverted to the elongated form. In summary, the morphologic response of the left ventricle to exercise is characterized by a small increase in end-diastolic volume and by the assumption of a more spherical chamber leading to more efficient translation of linear motion to ejection. This may have been due to an augmentation of venous return. (Research supported by a grant from the NIH.)

120. Functional Significance of the Distribution of Na-K-ATPase within the Kidney. ERNESTO K. HENDLER,* JORGE TORRETTI,* EDWARD WEINSTEIN,* AND FRANKLIN H. EPSTEIN, New Haven, Conn.

The specific activity of Na-K-ATPase in the red medulla of the kidney far exceeds its concentration in both the cortex and the white medulla. In the rat, Na-K-ATPase activity in homogenates of red medulla is twice that in cortex and 3 times that in white medulla. In dog kidneys, Na-K-ATPase is 4 times as high in whole homogenate and microsomes of red medulla as in the cortex. The discrepancy is emphasized by the fact that it is not paralleled by analogous differences

in Mg-ATPase, succinic dehydrogenase, glucose-6-phosphatase, or 5'-nucleotidase between red medulla and cortex. K_m for Mg-ATP (0.9 mM) and Na^+ (26 meq/liter) are similar in medulla and cortex, suggesting that the same enzyme is present in both regions. When tritiated ouabain is infused into one renal artery of dogs, renal tissue becomes labeled in a way suggesting that the cardiac glycoside is firmly bound to Na-K-ATPase. Binding of ^{3}H-ouabain follows the distribution of Na-K-ATPase, being highest in red medulla, lower in cortex, and least in white medulla. Both binding and unilateral sodium diuresis persist for hours after the infusion of ouabain is stopped. Intra-arterial infusions of ouabain that inhibit renal Na-K-ATPase completely when kidney tissue is assayed in vitro block only about 50% of sodium reabsorption from the glomerular filtrate. Concentrating ability ($T^c_{H_2O}$) is, however, almost completely eliminated. These results suggest that the activity of Na-K-ATPase is considerably higher in the thick ascending limb of Henle's loop that constitutes the bulk of the red medulla than in proximal tubules and collecting ducts. The mechanism of sodium absorption may therefore be different in the distal from that in the proximal tubule, Na-K-ATPase playing a larger role in the ascending loop of Henle and the distal convolution.

121. Steroid-Dependent Changes in Copper and Zinc Metabolism. R. I. HENKIN, S. MERET,* AND J. B. JACOBS,* Bethesda, Md.

Serum and urinary copper and zinc were measured by atomic absorption spectrophotometry in 50 normal volunteers (NV), 7 patients with adrenal cortical insufficiency (ACI), 4 patients with Cushing's syndrome (CS), and 14 cats before and after adrenalectomy. In NV, serum copper and zinc were 105 ±4 μg/100 μl and 96 ±4 μg/100 μl (mean ±SEM), respectively; urinary copper and zinc were 53 ±12 μg/day and 373 ±27 μg/day, respectively. Administration of 50 mg prednisone to eight NV for 5 days decreased serum Zn significantly (82 ±4 μg/100 μl) and increased urinary copper and zinc excretion more than 2-fold. In patients with CS serum, zinc was significantly below normal (61 ±13 μg/100 μl); urinary copper and zinc excretion was elevated above normal. In the venous effluent from the diseased adrenal there were further depressions of zinc (45 ±12 μg/100 μl) and further increases of cortisol as compared with peripheral levels. Adrenalectomy or treatment which lowered plasma cortisol was associated with return of serum and urinary copper and zinc to normal. By contrast, in patients with ACI, serum copper and zinc were significantly elevated above normal (132 ±6 μg/100 μl, 154 ±22 μg/100 μl), while urinary copper and zinc were below normal (14 ±2 μg/day, 337 ±55 μg/day). Treatment with carbohydrate-active steroids (CAS) returned serum and urinary copper and zinc to normal. In adrenalectomized cats, serum copper and zinc were significantly elevated above normal 1 wk after adrenalectomy associated with significant decreases in plasma cortisol and corticosterone. These data demonstrate an inverse relation between plasma concentrations of cortisol and zinc and a positive relation between plasma cortisol and urinary copper and zinc. These relations, useful in the diagnosis of hyper- and hypofunction of the adrenal cortex, suggest that CAS are important in the regulation of copper and zinc metabolism.

122. Antigenically Related Virus-like Particles in Sera of Patients with Hepatitis and in Simian Sera. RICHARD J. HIRSCHMAN,* N. RAPHAEL SHULMAN,** LEWELLYS F. BARKER,* AND KENDALL O. SMITH,* Bethesda, Md.

An antibody found in serum of multitransfused individuals was described by Blumberg et al. as precipitating a serum antigen (Australia antigen) that appeared to be inherited, and was rare in normal Americans but was common in patients with infectious hepatitis and, as found by Prince, in patients with serum hepatitis. Bayer, Blumberg, and Prince reported virus-like particles in antigenic sera. Using an antibody with Australia antigen specificity, we observed agar gel precipitation with randomly collected sera from 2 of 39 patients involved in a probable common-source outbreak of infectious hepatitis, 11 of 112 patients with infectious hepatitis in an endemic area, and 2 of 24 patients with serum hepatitis. Antigen was detected in sera collected from 13 of 22 individuals exposed to hepatitis-containing blood products. Antigen usually appeared transiently, 60 to 90 days after exposure to serum hepatitis, and disappeared before the onset of jaundice, but in 2 cases antigen persisted 17 and 34 months. Multiple exposures to antigen appeared necessary for development of precipitating antibody, which was found in 8 of 29 hemophiliacs. Chimpanzees have been suspected of transmitting infectious hepatitis to human beings. Of 128 chimpanzees tested, 2 had the antigen, which persisted for 10 months in one, and 2 had antibody. One of 14 gibbons tested had the antigen, but neither antigen nor antibody was found in 50 baboons, 33 orangutans, 139 vervets, 169 rhesus monkeys, 30 normal marmosets, and 8 marmosets with experimental hepatitis. Aggregates of uniform 20 mμ virus-like particles were found in precipitates formed by addition of human antibody to antigen-containing human, chimpanzee, and gibbon sera, but not in similarly treated control sera. These findings suggest that the antigen is a virus associated with both infectious and serum hepatitis in man and possibly with the same disease in higher primates.

123. The Influx of Plasma Lipoproteins and Cholesterol into Normotensive and Hypertensive Arteries. WILLIAM HOLLANDER AND DIETER M. KRAMSCH,* Boston, Mass.

The present study was undertaken to clarify the relation between experimentally induced hypertension and atherosclerosis. Coarctation of the thoracic aorta was produced in dogs and resulted in severe hypertension above the coarcted site and normotension below the coarcted site. The influx of plasma lipoproteins and cholesterol into the arteries was determined from 6 hr to 5 days after the intravenous administration of alpha and beta lipoproteins doubly labeled with ^{125}I and ^{3}H-cholesterol. The injected lipoproteins were isolated and identified radiochemically in the plasma and arterial wall by differential density ultracentrifugation, paper electrophoresis, and immunoelectrophoresis. In 10 hypertensive coarcted animals fed a standard kennel diet for 3 to 5 yr, the influx of plasma lipoproteins and cholesterol into the arteries above and below the coarcted site was comparable to the influx rates found in the corresponding arterial segments of control dogs. The content of cholesterol, triglycerides, and phospholipids in the arteries and plasma also was comparable

in the control and experimental groups. None of the animals developed atherosclerosis. 10 other coarcted dogs and their paired controls were fed an atherogenic diet of cholesterol and thiouracil for 3 months to 2 yr. The hypertensive coarcted dogs developed the same degree of atherosclerosis above and below the coarcted site as was observed in the corresponding arterial segments of the control dogs. In both groups there also were comparable increases in the lipid content of the arteries and plasma. Lipid accumulation in the arteries was associated with an increased influx of plasma lipoproteins and cholesterol into the arteries. The influx rates were the same in the hypertensive and normotensive animals. In conclusion: The development of atherosclerosis and the influx of plasma lipoproteins and cholesterol into normal and diseased arteries of dog are not influenced by the level of blood pressure. (Research supported by a grant from the NIH.)

124. Addict Hepatitis: Toxic or Viral? A. W. HOLMES,* HOWARD ROSENBLATE,* REUBEN EISENSTEIN,* AND DAVID BALDWIN,* Chicago, Ill. (introduced by Richard B. Capps**).

Viral hepatitis is a common disease of narcotic addicts. 10 to 15% of addicts are reported to have biochemical abnormalities consistent with acute hepatitis. Another 60% have distinct though far less impressive biochemical abnormalities which are usually attributed to chronic hepatitis, perhaps modified in some way by continued drug use. We have studied 30 heroin addicts entering our hospital for withdrawal. None of these individuals was admitted because of overt evidence of hepatic disease. 2 patients had biochemical evidence of acute hepatitis, and needle biopsy of the liver confirmed this diagnosis. 21 (70%) had one or more abnormal hepatic tests, but these were not of the severity seen in viral hepatitis. 7 patients had normal hepatic tests. Only 1 of the 28 patients without clinical hepatitis had a normal liver biopsy. The lesion in the others consisted of small foci of hepatocellular necrosis with associated lymphoid cells. Impressive portal collections of lymphocytes and occasionally plasma cells were also present. The smooth endoplasmic reticulum (SER) was strikingly hypertrophied, as might be expected in individuals using drugs metabolized by hepatic microsomes. In foci of necrosis, there seemed to be a progression from cells with hypertrophied SER to eosinophilic bodies. The ultrastructural changes of viral hepatitis were readily recognized in the two addicts with biochemical and light microscope abnormalities suggesting acute hepatitis, but were not seen in the remainder of our patients. The liver lesion most commonly seen in narcotic addicts thus may not be due to persisting, chronic, or modified viral hepatitis, but may represent instead a toxic injury resulting from protracted intense stimulation of the hepatic SER.

125. Changes in Intracellular Distribution of Lysosomal Enzymes during Phagocytosis by Human Polymorphonuclear Leukocytes. BEULAH HOLMES,* JUDY SATER,* GLENN RODEY,* BYUNG PARK,* AND ROBERT GOOD,** Minneapolis, Minn. (introduced by Robert A. Ulstrom).

During our studies of degranulation of human leukocytes, it became apparent that lysosomal enzymes of these cells do not simply redistribute intracellularly during phagocytosis. After incubation of leukocytes with and without heat-killed bacteria, there was a striking decrease in the total amount of lysosomal hydrolases recoverable from phagocytizing leukocytes. For example, the β-glucuronidase of incubated control cells was distributed 64% in the granules, 25% in the nuclear fraction, 8% in the cytoplasm, and 3% in the medium. The total enzyme of the homogenate of cells incubated with bacteria was 55% less than the control homogenate and was distributed 51% in the granules, 17% in the nuclear fraction, 9% in the cytoplasm, and 23% in the medium. If the total from control cells was used in calculating the distribution of β-glucuronidase of phagocytizing cells, there was 23% in the granules, 8% in nuclear fraction, 4% in the cytoplasm, and 10% in the medium, a loss of 41% from the granule fraction. Cells from patients with chronic granulomatous disease (CGD) have been shown morphologically to exhibit delayed degranulation after phagocytosis of bacteria. With one exception, the total enzyme content of homogenates of leukocytes from these patients was not significantly altered by incubation with bacteria. The total lysozyme content of CGD leukocytes was reduced in amount comparable to that of normal leukocytes. The decrease in all granule-associated hydrolases except lysozyme was significantly less than that in normal leukocytes. Our data indicate that lysosomal hydrolases of human leukocytes are degraded or inactivated after release from granules during phagocytosis. These observations add critical support to the conclusion previously reached on morphologic grounds that an essential anomaly of the leukocytes in CGD is the failure of normal degranulation after phagocytosis.

126. Erythrocyte Fragmentation: A Mechanism of Red Cell Destruction in Sickle Cell Anemia. CHRISTOPHER P. HOLROYDE,* BENGT LUNDH,* AND FRANK H. GARDNER,** Philadelphia, Pa.

Our observations on the blood of patients with sickle cell (SS) anemia, using interference contrast, phase, and light microscopy, demonstrate numerous particles shown to contain hemoglobin. Particles range from 1.0 to 7.0 μ in length and consist largely of microspheres from 1.0 to 2.0 μ in diameter. Beadlike and rodlike structures are seen, as are spiculated forms. Intermittent exposure to decreased O_2 tension greatly increases particle formation (fragmentation), and lends support to observations on single SS cells by Padilla et al. indicating that this phenomenon is associated with the sickle-unsickle cycle. To quantitate fragmented particles, a reproducible technique with a >95% yield has been developed. 50 μ^3 of washed, defibrinated whole blood are transferred to a microcapillary tube and spun through a silica gel column for 30 sec at 15,000 g. Whole cells sediment, while concentration of particles occurs at the silica-plasma interphase, from which they are removed; they are diluted, and counted in a Neubauer chamber. Particles are seen as highly refractile objects exhibiting vigorous Brownian movement, easily distinguishable from contaminating red cells. Fragmentation closely parallels the degree of sickling in vivo as measured by dry-slide counting of venous blood. It is inhibited by continuous incubation at a high Po_2 or by failure to return the rigid sickled cell to its unsickled state. Young cell populations

fragment most readily. Seemingly, the most critical factor for fragmentation is the total time a cell is sickled, rather than the frequency of sickling. Associated parameters of red cell damage are: excess K^+ leak with inability to return to baseline values on reoxygenation; increased irreversibly sickled, acanthotic, and osmotically fragile spherical forms. Fragmentation occurs out of proportion to the small rise in plasma-free hemoglobin, to imply an additional mechanism of red cell destruction distinct from direct mechanical fragility. (Supported by a grant from the NIH.)

127. Evidence for a Proteinpolysaccharide Inhibitor and Mineral Particles in Micropuncture Fluids (C_{fl}) from Calcifying Cartilage. DAVID S. HOWELL, JULIO C. PITA,* JUAN F. MARQUEZ,* FRANCISCO J. MULLER,* AND VICTOR PARDO,* Miami, Fla.

A study was first made to determine whether a proteinpolysaccharide (PPL) fraction which inhibits Ca phosphate particle growth could be separated and quantitated in C_{fl}. To accomplish this goal, C_{fl} and various cartilages (homogenized suspensions) were processed in most respects according to the method of DiSalvo and Schubert, scaled down for ultramicro (20 nl) samples. A PPL-(R_2) fraction was isolated from C_{fl}, and upon recycling, R_2 showed potent inhibitory action on mineral accretion in vitro. The R_2-to-total sample ratio of hexuronate was 5 times higher in C_{fl} than in whole growth plate or articular cartilage suspensions from the same animals. Results were interpreted to indicate that (1) C_{fl} represents a physiological compartment containing a PPL profile different in composition from that in adjacent solid phase; and (2) a unique property of C_{fl} proteinpolysaccharide is its ability to prevent unregulated mineral extension. In other experiments, normal C_{fl} sediments after ultracentrifugation at 100,000 *g* were diluted 1:50 and incubated 5½ hr in synthetic lymph which was either metastable in respect to hydroxyapatite, or lacking in Ca and phosphate ions. Experiments wherein C_{fl} sediments were reisolated and tested in an in vitro system for crystal growth indicated that immature Ca phosphate particles in C_{fl} rather than an organic nucleating agent were the cause of rapid mineral phase separation. Electron micrographs of normal C_{fl} sediments dried on carbonized grids revealed spherical particles about 300 A in diameter, consistent with amorphous calcium phosphates. A similar study of untreated rachitic cartilage C_{fl} containing the same amount of protein and hexuronate failed to show these particles. Both the inhibitory proteinpolysaccharide and mineral particles are considered to be part of a physiological mechanism for calcification in vivo. (This work was supported by NIH grant AM-08662.)

128. Ultrastructure and Enzymatic Deficiency of Fibrocyte Cultures in Type II Glycogenosis (GSD) and Metachromatic Leukodystrophy (MLD). GEORGE HUG, WILLIAM K. SCHUBERT,* AND SHIRLEY SOUKUP,* Cincinnati, Ohio.

Glycogen-filled vesicles in hepatocytes in a patient with GSD disappeared after 18 days of infusion with fungal α-glucosidase but not after 18 days of infusion with crystalized human salivary amylase. Arylsulfatase A (ASA) prepared from beef brain and infused in a patient with MLD did not have any detectable effect. To establish a patient-independent system for the study of these two fatal lysosomal diseases, skin culture fibrocytes of the two patients and of "normal" controls were examined enzymatically and with the electron microscope. Acid maltase was determined by measuring the appearance of glucose from maltose at pH 4 and expressing the activity as μmoles maltose split per mmole nitrogen of fibrocytes per minute. ASA was determined by measuring the appearance of 4-nitrocatechol from *p*-nitrocatechol sulfate at pH 4.5 and expressing the activity as μmoles 4-nitrocatechol formed per mmole nitrogen of fibrocytes per minute. The activity in four normal cultures was: maltase, 0.45–1.4; ASA, 3.1–10.7. The activity of GSD fibrocytes was: maltase, 0; ASA, 1. The activity of MLD fibrocytes was: maltase, 2.3; ASA, 0. Electron microscope examination of GSD fibrocytes showed membrane-surrounded vacuoles filled with glycogen to a varying degree. These resembled the glycogen-filled abnormal lysosomes characteristic of hepatocytes in GSD. Electron microscope examination of MLD fibrocytes showed electron-opaque lamellar inclusions resembling similar structures in the patient's liver and brain. Electron microscope and enzymatic identification of abnormalities in cultured fibrocytes from GSD and MLD should provide in vitro systems for studying disease mechanisms and for evaluating therapeutic agents without hazard to the patients. (Research supported by the Children's Hospital Research Foundation and USPHS grant FR-00123.)

129. Adrenergic Effects on the Pulmonary Circulation. ROLAND H. INGRAM,* JAN P. SZIDON,* AND ALFRED P. FISHMAN,** Chicago, Ill.

During recent years the role of local factors and that of gravity in the regulation of the pulmonary circulation have been extensively studied. The role of the autonomic nervous system remains unsettled. In the present study the effects of hypothalamic and sympathetic stimulation (SNS) were examined in two preparations: (1) an isolated lobe of dog perfused in vivo with pulsatile flow, and (2) the open-chest dog equipped with suitable transducers for the simultaneous measurement of pressure and diameter in the main pulmonary artery (MPA). In the isolated lobe, SNS produced increased stiffness of large pulmonary arteries without a change in vascular resistance, whereas norepinephrine (NE) infusions increased both stiffness and resistance. In the open-chest dog, pressure and diameter were linearly related up to MPA pressures of 45 mm Hg. During SNS, stiffness increased by 35% and the unstressed diameter (DR) remained unchanged; during NE infusions, stiffness increased by 14% and DR decreased. Application of NE directly to the wall of the MPA duplicated the effects of SNS. Application of elastase prevented the increase in stiffness with SNS and NE, but DR decreased in both. Histologically, the disposition of outer medial vascular smooth muscle and elastic fibers suggests an in-series, force-multiplier arrangement; in contrast, the appearance of the inner medial layer suggests an in-parallel arrangement. These physiological and anatomical observations indicate that adrenergic nerve stimulation stiffens the pulmonary arterial tree rather than increasing pulmonary vascular resistance. This stiffening of large vessels involves

preferential contraction of the outer layer of pulmonary arterial smooth muscle. In contrast, blood-borne NE increases pulmonary vascular resistance by stimulating smooth muscle in the inner and outer layers of large vessels and by constricting small vessels. (Supported by a grant, HE-06375, from the NIH, USPHS.)

130. Quantitative Relations of Fetal and Maternal Pituitary-Adrenal Systems. BENJAMIN T. JACKSON,* HELMUT F. J. RAUSCHECKER,* AND GEORGE J. PIASECKI,* Boston, Mass. (introduced by Richard H. Egdahl).

Using advanced techniques for intrauterine fetal surgery, the quantitative function of the fetal pituitary-adrenal system and its relations with that of the mother were investigated. Experiments were conducted in a group of hypophysectomized pregnant dogs and in a group of normal pregnant dogs. Cannulas for collecting adrenal effluent were placed in fetuses in utero of 54 to 60 days gestational age. In these fetuses, adrenal effluent was sampled, ACTH (10 mμ) was injected intravenously, and a second adrenal sample was collected. Fetal and maternal peripheral arterial samples were then obtained. Fetuses and their adrenals were weighed. Concentrations of cortisol were determined by a modification of the double-isotope method of Kliman and Peterson, and fetal secretory rates were calculated. Fetal adrenal/body weight ratios were 204 ±45 mg/kg in the hypophysectomy group and 140 ±22 mg/kg in normals. Cortisol data were as follows: *Hypophysectomy group:* concentrations (mμg/ml) fetal adrenal pre-ACTH 290 ±101 and post-ACTH 346 ±99, fetal peripheral 58 ±31, maternal peripheral 13 ±6; fetal secretory rates (mμg/min) pre-ACTH 119 ±16, post-ACTH 149 ±20. *Normals:* cortisol concentrations (mμg/ml) fetal adrenal pre-ACTH 175 ±83 and post-ACTH 262 ±86, fetal peripheral 98 ±25, maternal peripheral 109 ±4; fetal secretory rates (mμg/min) pre-ACTH 53 ±40, post-ACTH 170 ±117. *Conclusions:* (1) Depression of maternal adrenal function by hypophysectomy leads to fetal adrenal hypertrophy. (2) The fetal dog actively secretes cortisol. (3) Fetal cortisol secretory rates pre-ACTH were significantly greater in the hypophysectomy group than in normals, indicating the elimination of suppression of the fetal pituitary by maternal cortisol in the former group; a statistically significant difference in secretion was not found between the two groups post ACTH, a fact which suggests that suppression of the fetal pituitary by maternal cortisol is insufficient to reduce fetal adrenal responsiveness to ACTH. (4) Elevation of cortisol secretory rates in response to ACTH in fetuses in hypophysectomized mothers suggests that ACTH secretion by the fetal pituitary may be the rate-limiting factor in fetal pituitary-adrenal function. (5) The large fetal-maternal gradient of cortisol in the hypophysectomy group suggests that the positive maternal-fetal gradient for cortisol in normal animals is not due to a specifically selective action of the placenta. (This research was supported by NIH grant HD-01272-06.)

131. Immunoglobulin Synthesis by Peripheral Blood Cells. HUGO E. JASIN* AND MORRIS ZIFF,** Dallas, Texas.

Immunoglobulin synthesis by peripheral blood mononuclear cells, which are representative of the recirculating lymphocyte pool, may reflect the immunological status of the individual at any given time. Accordingly, in vitro synthesis of γ-globulin by buffy-coat cells has been measured in normal controls and in patients with noninflammatory diseases, rheumatoid arthritis and systemic lupus erythematosus (SLE). Cells were incubated with ^{14}C-labeled amino acids for 21 hr, supernatants were dialyzed, and the radioactivity incorporated into γ-globulin was determined by immune coprecipitation of carrier protein with specific goat anti-human immunoglobulin sera. Nonspecific radioactivity was determined by coprecipitation with an unrelated antigen-antibody system. No difference in immunoglobulin synthesis was detected between 11 controls (mean, 2234 ±1142 cpm/10^6 mononuclear cells) and 14 patients with rheumatoid arthritis (1617 ±970 cpm). Synthesis was increased over 6 times in 20 patients with SLE (13,285 ±13,319 cpm). 15 showed increased synthesis, and five fell in the normal range. Serum β_{1c}-β_{1a} levels were decreased in 93% of the SLE patients with increased synthesis. Conversely, all five patients with normal synthesis had normal β_{1c}-β_{1a}. In two SLE patients treated with cyclophosphamide, immunoglobulin synthesis by peripheral cells fell as serum β_{1c}-β_{1a} rose to normal. Precipitation with monospecific antisera and radioimmunoelectrophoresis showed that most of the radioactivity was in IgG and IgA; both classes were equally elevated in SLE patients. Partial separation of the cell type responsible for γ-globulin synthesis was achieved by albumin gradient centrifugation. Most of the γ-globulin-synthesizing cells were in the upper cell layer, a fraction rich in large lymphocytes. These results indicate that immunoglobulin synthesis by peripheral blood cells reflects the known enhanced activity of the lymphoid system in SLE. They may provide a sensitive parameter for measurement of the effect of treatment on this immune hyperactivity. (Research supported by the VA and NIH grant AM-09989.)

132. Abnormal Motor Nerve Excitability in Amyotrophic Lateral Sclerosis. RICHARD J. JOHNS AND J. COLIN BROWN,* Baltimore, Md.

Studies of nerve conduction velocity in motor neuron diseases have been unrevealing. Accordingly, techniques were developed which permit quantitative assessment of nerve excitability in man—the functional relation between nerve stimulus intensity and the response amplitude. Measurements of motor nerve excitability in 10 patients with amyotrophic lateral sclerosis revealed four abnormalities: (1) A slight increase in stimulus intensity caused either a large increase in the amplitude of the evoked muscle action potential or no change. (2) Amplitudes of intermediate size could not be obtained by varying the stimulus intensity. (3) In some instances the entire muscle action potential response was evoked in this "all-or-none" manner. (4) There was a random steplike variation in the amplitude of the muscle action potential to stimuli of the same intensity, and the probability of eliciting the larger potential was increased by increasing the stimulus intensity. Ephaptic excitation of damaged axons appears to be the most likely explanation of these findings. (This research was supported in part by NIH grants NB-00894, GM-10895, and FR-00035.)

133. Characterization of Oxygen Receptors in the Human Umbilical Artery. JUDITH JONES,* EDGAR ENGELMAN,* JAMES WALDMAN,* AND KENNETH L. MELMON, San Francisco, Calif.

Vascular response to oxygen has been incompletely characterized. Of the substances (histamine, serotonin, prostaglandins, bradykinin, and oxygen) which constrict human umbilical arteries in vitro, oxygen and bradykinin have been implicated as mediators of neonatal circulatory adaptation. Our experiments suggest that oxygen and bradykinin exert their vasoconstrictive effect through a common receptor mechanism, by showing that (1) exhaustion of response to oxygen and bradykinin occurs simultaneously while responses to other drugs are maintained, and (2) selective competitive inhibition of the response to oxygen and bradykinin is produced by salicylates. Rings from 40 human umbilical arteries were mounted in a tissue bath containing Krebs-Ringer solution (pH 7.3) equilibrated with 2% oxygen, 93% nitrogen, and 5% CO_2. 39 arteries constricted to graded doses of bradykinin (0.05–0.2 μg/ml), and although the response was delayed, 26 similarly constricted to increasing concentrations of PO_2. All arteries were constricted by histamine and serotonin. In four rings, the responses to repeated doses of oxygen and bradykinin were lost simultaneously while a normal response to the other agents was maintained. Sodium salicylate (5×10^{-3} M) abolished oxygen- and bradykinin-induced constriction, but this inhibition could be overcome by increasing the concentration of either agent and again inhibited by increasing the concentration of antagonist (sodium salicylate). Responses to histamine or serotonin were not significantly altered by 5×10^{-3} M sodium salicylate, a fact which suggests specific inhibition of kinin and oxygen by salicylate. The findings suggest that oxygen and bradykinin may share a common physiological receptor in this preparation, in that oxygen releases bradykinin or that oxygen and bradykinin each release an unknown vasoactive substance. The delay in constriction after exposure to oxygen suggests secondary rather than primary effects of oxygen. Characterization of oxygen receptors in this study is in accord with previous demonstrations of oxygen-dependent kinin production and the suggestion that kinin has a role in neonatal circulatory adaptation. (Research supported by grants from the NIH.)

134. Effects of Glucagon and Parathyroid Hormone on Plasma and Urinary 3′,5′-Adenosine Monophosphate in Man. N. I. KAMINSKY,* A. E. BROADUS,* J. G. HARDMAN,* H. E. GINN,* E. W. SUTHERLAND,* AND G. W. LIDDLE,** Nashville, Tenn.

3′,5′-Adenosine monophosphate (cyclic AMP) is the intracellular mediator of the actions of many hormones. This nucleotide occurs in urine, but it has not previously been quantified in human plasma. The present study examined the relation between plasma and urinary cyclic AMP and the effects of parathyroid hormone and glucagon on plasma and urinary levels of the nucleotide. Under basal conditions, plasma cyclic AMP concentrations in normal human subjects were 10–20 nanomoles/liter, and most of the cyclic AMP in the urine could be accounted for on the basis of glomerular filtration. In response to intravenous glucagon (0.05–0.2 μg/kg per min) normal subjects exhibited dose-related increases in plasma and urinary cyclic AMP up to 30 times basal levels. Renal clearance of cyclic AMP approximated that of inulin. Experiments in dog and studies in collaboration with J. H. Exton in rat indicated the liver to be the major source of glucagon-induced increases in plasma and urinary cyclic AMP. Urinary cyclic AMP has previously been shown by Chase and Aurbach to rise in response to parathyroid hormone. We have found that normal subjects respond to 200 units of parathyroid hormone, infused intravenously for 30 min, with a 4-fold increase in plasma cyclic AMP and a 40-fold increase in urinary cyclic AMP. Renal clearance of cyclic AMP far exceeded that of inulin. In three anephric patients plasma cyclic AMP concentrations showed little or no increase in response to infusions of parathyroid hormone. *Conclusion:* Glucagon, principally by its action on the liver, effects a rise in plasma cyclic AMP which leads to increased excretion of the nucleotide in the urine. Parathyroid hormone acts on the kidney to induce the release of cyclic AMP both into the circulation and directly into the urine.

135. Induction of Alkaline Phosphatase by the Obstructed Liver. MARSHALL M. KAPLAN* AND ADRIANA RIGHETTI,* Boston, Mass. (introduced by Samuel Proger**).

This study investigates the cause of the elevated serum alkaline phosphatase (AP) in obstructive liver disease and presents evidence that it is due to induction of liver AP with leakage of this induced enzyme into the blood. Male Wistar rats were subjected to bile duct ligation and sacrificed at frequent intervals up to 24 hr, and AP was assayed in serum and liver extracts. AP activity in the liver began to increase within 2 hr after ligation and reached a peak in 12 hr that was 7 times that of sham-operated animals. The AP in ligated liver was confined to membrane fractions and was identical with that found in control animals when both were examined by electrophoresis on polyacrylamide gel. After ligation the serum AP increased 2.5-fold, but the rise lagged behind that in the liver by several hours. The serum AP isozymes were separated by polyacrylamide gel electrophoresis and quantitated by densitometry. The elevated serum activity was due to the appearance of a new isozyme identical with that found in the liver. The increase in liver AP could not be accounted for by retention of AP by the obstructed liver, since it was 240 times greater than the amount of AP excreted in the bile of rats with cannulated bile ducts over a similar period of time. The increase was dependent on de novo protein synthesis. Cycloheximide, in a dose that inhibited the incorporation of ^{14}C-leucine into the liver protein by 80%, inhibited the increase in liver AP by 95% and that in the serum by 80%. Pretreatment with actinomycin D gave similar results. These data suggest that bile duct ligation induces liver AP and that leakage of this induced enzyme into the serum produces the AP elevation in obstructive liver disease. (Research supported by grants from the NIH and the Boston Medical Foundation.)

136. Evidence for Young and Old Human Platelets. SIMON KARPATKIN,* New York, N. Y. (introduced by A. Johnson**).

Human platelets have been separated into two extreme density populations by centrifugation in inert specific density media. A large-heavy platelet population with sp gr >1.055 and a light-small population with sp gr <1.046 were obtained, each representing 15–20% of the total population volume. The average volume per platelet of the separated large-heavy and light-small platelet populations was 12 and 5 μ^3, respectively. When data are expressed per gram wet weight or per milligram protein, the large-heavy platelet population had a 1.3-fold greater adenine nucleotide and P_i content, 2-fold greater glycogen content, 4.2-fold greater rate of glycogenolysis, 2.6-fold greater rate of glycolysis, 5.7-fold greater rate of glycogen synthesis, 2.9-fold greater rate of protein synthesis, and 2.5-fold greater resistance to osmotic shock. In vivo $DF^{32}P$ survival curves of large-heavy and light-small platelets isolated from rabbits indicated that large-heavy platelets were "young platelets" which progressed with age to light-small platelets, "old platelets." Platelet function studies revealed that after addition of ADP, thrombin, or epinephrine, platelet aggregation time was 3.0-, 4.5-, and 3.3-fold shorter with young platelets, which released 3.7-, 7.6-, and 8.1-fold more ATP respectively than did old platelets. After platelet aggregation by thrombin or epinephrine, young platelets released 6.0- and 3.8-fold more ADP into the media. After aggregation by ADP, old platelets consumed 5.9-fold more added extracellular ADP than did young platelets. Young and old platelets had equal ability to develop platelet factor 3 activity; however, young platelets aggregated by ADP, thrombin, or epinephrine released 9.1-, 8.5-, and 12.7-fold more platelet factor 4. These data reveal the presence of young large-heavy platelets, with greater metabolic potential and platelet function, which progress to older light-small platelets with diminished metabolic potential and platelet function.

137. Phenobarbital Prevents Chemical Porphyria. LEON KAUFMAN* AND HARVEY S. MARVER,* San Francisco, Calif. (introduced by Rudi Schmid).

The basic defect in genetically determined and chemically induced porphyrias is similar and reflected by increased levels of hepatic δ-aminolevulinic acid (ALA) synthetase. This study demonstrates that inducers of porphyria are metabolized on the smooth endoplasmic reticulum (SER) of the liver and that augmentation of this detoxification prevents porphyria. Progesterone, allylisopropylacetamide (AIA), and dihydrocollidine (DDC) administered to rats induced striking increases in hepatic ALA synthetase (10–30× normal) and an outpouring of urinary ALA and porphobilinogen. Pretreatment with phenobarbital (PB) abolished these effects. This suggested that PB, known to stimulate microsomal mixed-function oxidases, increased the rate of detoxification of porphyrogenic compounds. To test this hypothesis, crystalline 2-^{14}C-AIA and 2,6-^{14}C-DDC were synthesized and administered to rats, and plasma disappearance, hepatic clearance, and intracellular distribution were studied. PB caused a 10- to 20-fold increase in the rate of plasma disappearance; hepatic clearance increased 3- to 6-fold, reflecting marked augmentation in disappearance from cytosol, chromatin, and SER. Moreover, PB pretreatment enhanced the rate of formation of more polar metabolites on SER (>10×). These data indicate that (1) inducers of porphyria are metabolized to more polar compounds via mixed-function oxidases of SER; (2) the parent compounds and not their metabolites are active inducers of ALA synthetase; (3) PB, and presumably other compounds that enhance SER activity, prevent chemical porphyria by increasing the rates of detoxification of inducers. The relevance of these studies to the pathogenesis of human genetic porphyria is further evidenced by demonstration that sera of some porphyric patients induce ALA synthetase in cultured embryonic liver. This protective action of PB is paradoxical because of its known toxicity in genetic porphyria. However, we previously reported that PB, though ineffective in normal animals, exacerbates acute porphyria because of genetic inability to respond normally to drug stimulation of SER. That enhancement of detoxification by SER protects against the most potent porphyrogenic compounds further supports this concept. (Supported in part by NIH grants AM-11296 and AM-11275.)

138. Urine Osmolality in Experimental Pyelonephritis. DONALD KAYE AND HEONIR ROCHA,* New York, N. Y.

The effect of bilateral pyelonephritis on urine osmolality was studied in rats. Animals were inoculated with (1) 10^6 *Escherichia coli* into the medullas of both kidneys, (2) 10^6 enterococci intravenously (i.v.), or (3) 10^6 *Staphylococcus aureus* i.v. Maximum urine osmolality was determined on urine aspirated from the bladders and numbers of bacteria were determined in each kidney. The mean maximum urine osmolality of normal rats was 2350 mOsm/kg water. Inoculation with *E. coli* caused reversible pyelonephritis with sterilization of the kidneys within 12 wk. By 1 day after injection the mean maximum urine osmolality decreased to 1300 mOsm; it remained at this level for 3 wk and then rose to normal by 12 wk. Injecting heat-killed *E. coli* into the medullas as a control caused only a transient decrease in concentrating ability, with return to normal by 3 days. After injection of enterococci and staphylococci, the mean maximum urine osmolalities decreased over 3–4 days to about 1400 and 700 mOsm respectively. In enterococcal infection the urine osmolalities remained 1400 mOsm or less for at least 12 wk, whereas in staphylococcal infection the osmolalities gradually rose. Therapy of *E. coli* pyelonephritis with sodium colistimethate, and enterococcal or staphylococcal infection with ampicillin, rapidly reduced bacterial titers in the kidneys, with an associated rise in urine osmolality to normal or near normal. Presence of a concentrating defect was correlated with presence of bacteria in the kidneys. With antimicrobial therapy or with self-limited infection, the rate of increase in osmolality could be directly correlated with the rate of decrease of bacterial titers. The increase in osmolality with antimicrobial therapy was not correlated with improvement in histological abnormalities. These studies demonstrate that experimental pyelonephritis is associated with a concentrating defect that can be rapidly reversed and therefore is not related to permanent renal damage. (Supported by the Health Research Council of the City of New York, grant I-489, and the NIH, grants AI-07581 and TI-AI-255.)

139. The Effect of Vitamin K and Warfarin upon the Biosynthesis of Factors II and VII by Liver Tissue In Vitro. ROGER K. KIPFER* AND ROBERT E. OLSON,** St. Louis, Mo.

On the basis of studies of intact rats and the isolated perfused rat liver, it has been proposed that vitamin K and the coumarin drugs react with a regulatory protein which influences the biosynthesis of four coagulation proenzymes (factors II, VII, IX, and X) at the ribosomal level. Studies have been carried out to compare the effects of vitamin K and warfarin upon the biosynthesis of factors II and VII in the intact rat, in the isolated perfused rat liver, and in surviving tissue slices. Biosynthesis of these factors in vivo could be arrested by administration of warfarin (3 mg/100 g) or cycloheximide (0.75 mg/100 g), after which the half times for factors II and VII were, respectively, 1.0 ±0.3 hr and 6 ±1 hr. When normal rat liver was perfused in vitro with Krebs-Ringer buffer containing 3% bovine albumin, amino acids, glucose, and 30 μg/ml of warfarin, factor II synthesis was abolished, but factor VII synthesis was unaffected, yielding 12% of normal values in the medium in 2 hr. Neither factor was synthesized in perfused livers from vitamin K–deficient rats until vitamin K_1 was added in vitro, after which factor II reached levels of 5% in 2 hr whereas factor VII increased to 10% in the same time. When liver slices from vitamin K–deficient rats were incubated with vitamin K_1, factor VII but not factor II could be detected after 3 hr, in agreement with Pool and Robinson. The effects of vitamin K and warfarin upon the biosynthesis of factors II and VII are thus qualitatively similar but quantitatively so different as to suggest that they are under the control of separate regulatory systems. (Supported by NIH grant AM-09992.)

140. Enzymic Aggregation in Studies of Normal and Mutant Enzymes. HENRY N. KIRKMAN, Chapel Hill, N. C.

The Tel Hashomer variant of glucose-6-phosphate dehydrogenase (G-6-PD) migrates as two bands during electrophoresis in several gel systems in which only single bands are seen with G-6-PD from other men. This observation seemed puzzling, in view of the current evidence that the structure of human red cell G-6-PD is determined by a single locus on the X chromosome. The slower electrophoretic component was shown to have an abnormally high molecular weight by its relative movement during ultracentrifugation in sucrose gradients, chromatography in Sephadex, and electrophoresis in gels of different concentrations. Conversion of isolated slow to fast component occurred on dilution and incubation in 10 mM mercaptoethanol. With both crude and 36,000-fold purified Tel Hashomer G-6-PD, the slow component reappeared after incubation of the fast component at higher concentrations of enzyme and lower concentrations of mercaptoethanol. Prior treatment with iodoacetate blocked reformation of the slow component. The Tel Hashomer variant, therefore, seems unusually capable of assuming a higher molecular weight through the formation of disulfide bridges with itself. At enzymic concentrations greatly in excess of those used in the Tel Hashomer study, pure, normal G-6-PD has also been found to form a complex of unusually high molecular weight. Protein concentrations above 1 mg/ml are generally necessary for studies of a pure protein in the analytical ultracentrifuge. For many enzymes, however, such concentrations are several orders of magnitude greater than those occurring either in vivo or during gel electrophoresis. When aggregation occurs during purification and ultracentrifugation at these concentrations, as with human G-6-PD, the resultant estimate of molecular weight can be different from those obtained by other methods and can be inappropriate for interpretation of hybridization and isozymic patterns. This effect may contribute to occasional uncertainties and controversies concerning the subunits and genetic control of proteins.

141. Relative Contribution of New and Old Collagen Degradation to Urinary Hydroxyproline. LEROY KLEIN,* Cleveland, Ohio (introduced by Max Miller).

It has been shown that collagen can exist in an isotopic steady state. This experimental approach was used to evaluate the relative contribution of new and old collagen degradation to urinary hydroxyproline. 12 female weanling Fischer rats were labeled with ^{3}H-L-proline 11 times over 6 wk (total of 330 μc/rat). 18 to 22 months after labeling, groups of three animals each were fasted for 0 to 23 days. The following indices of collagen metabolism were compared: specific activity (SA) and amount of urinary hydroxyproline (hypro), of hypro from salt-soluble, citrate-soluble, and insoluble collagen of skin, and insoluble collagen of femur. Both before and after fasting, constant SA of hypro within multiple extractions of salt- and citrate-soluble collagen indicated the presence of an isotopic steady state. Animals fasted for 23 days in comparison with controls had a lower body weight (mean, 131 vs. 263 g), lower urinary hypro (range, 166–250 vs. 365–445 μg/day), higher SA of urinary hypro (range, 2.2–2.9 vs. 0.5–0.7 dpm/μg hypro), higher SA and lower amount of 0.45 M NaCl-soluble collagen (mean, 0.90 vs. 0.47 dpm/μg hypro, 0.12% vs. 0.75% of total collagen). For all animals, SA of hypro was 3.0–3.9 for skin and 10.2–13.9 for bone. The SA of proline from fasted animals increased over the controls for serum proteins (mean, 0.49 vs. 0.11) and liver (0.69 vs. 0.27). The 4-fold increase in SA of urinary hypro of fasted rats simultaneous with an 84% decrease in new collagen synthesis indicates that in the fed old rat a maximum of 25% of the total urinary hypro is derived from old collagen. The SA of urinary hypro approached that of skin, suggesting that collagen was mainly lost from skin. (Research supported by grants from the NIH.)

142. A Polypeptide Derived from the Second Component of Human Complement (C'2) Which Increases Vascular Permeability. MARTIN R. KLEMPERER,* FRED S. ROSEN, AND VIRGINIA H. DONALDSON, Boston, Mass., and Cincinnati, Ohio.

The esterase derived from the first component of human complement (C'1s) increases vascular permeability when injected intradermally into humans. This effect is not histamine dependent, and the mediator of this response is not known. When C'1s, in an amount which did not demonstrably increase vascular permeability, was incubated with purified human C'4 and C'2 at 37°C, the generation of a permeability

factor could be demonstrated by intradermal injection of the mixture into individuals pretreated with intravenous Evans blue. Mixtures of C'1s and C'4, C'1s and C'2, C'4 and C'2 lacked this permeability factor activity. The permeability factor was distinct from the anaphylatoxins derived from C'3 and C'5 in that it was heat stable (resisted boiling for 30 min) and was not blocked by antihistamine. The permeability factor could be isolated from mixtures of C'1s, C'4, and C'2 by chromatography on Sephadex G-25. The active material was included in the gel and could be recovered from a sharply defined peak with low absorbancy at λ280 but high absorption at λ210. Inclusion in Sephadex G-25 suggests a molecular weight of 5000 or less. Permeability factor activity was destroyed upon incubation with trypsin for 10 min at 37°C. When very highly purified human C'2 was treated with trypsin for 30 sec at 37°C and immediately acidified, chilled to 0°C, and chromatographed on Sephadex G-25, a peptide was included in the gel. This material had physicochemical and biological properties similar to those of the peptide generated by the interaction of C'1s, C'4, and C'2. (Research supported by grants from the NIH and the American Heart Association.)

143. In Vivo Metabolism of C'3 and C'4 in "Hypocomplementemic" Chronic Glomerulonephritis. PETER F. KOHLER,* MARTIN P. HUTT,* AND CONRAD RILEY,* Denver, Colo. (introduced by David W. Talmage**).

Complement (C') abnormalities are unusual in chronic glomerulonephritis (CGN). Three patients with low serum hemolytic C' were studied, and each was found to have a different component alteration consisting of (1) decreased C'3 and C'5 in an 18 yr old female, (2) low C'3 and C'4 in a 12 yr old boy, and (3) low C'1q, C'3, C'4, and C'5 in a 10 yr old boy. All had proteinuria of 6 months duration or longer, had normal renal clearance studies, and were asymptomatic. To determine the basis for the varying component patterns, the metabolism of radioiodine-labeled purified human C'4 and/or C'3 was studied using single- or paired-label techniques. Fractional catabolic and synthetic rates were determined by analysis of plasma and urine radioactivity for periods of 5 to 7 days. In all three subjects the daily fractional catabolism of plasma pool C'3 was increased from 1.4 to 3.6 as compared with 0.34 to 0.55 in controls. Similarily, the daily C'4 fractional catabolism, in the two subjects studied, was 0.86 and 0.88 as compared with 0.34 and 0.37 in controls. The C'3 and C'4 synthetic rates either were normal or increased, except for the patient with the lowest serum C'3, whose synthesis was 8.2 mg/kg per day (controls 16.1 to 29.2). The increased C' protein catabolism was the primary cause of the lowered serum C'3 and C'4 and suggests an ongoing immunopathogenic process involving the C' system. These metabolic findings in combination with the varying serum component patterns distinguish this form(s) of "hypocomplementemic" CGN from the group designated "progressive glomerulonephritis," which has been characterized by an isolated deficiency of C'3 secondary to impaired synthesis rather than increased in vivo utilization. (Research supported by NIH grant 1-RO1-HD-03381-01.)

144. Estradiol in Human Plasma: Demonstration of Elevated Levels in Gynecomastia and in Cirrhosis. STANLEY G. KORENMAN,* LAURENCE E. PERRIN,* AND THERESA MCCALLUM,* Torrance, Calif. (introduced by Karlman Wasserman).

Using the radioligand binding assay system previously described, unconjugated estradiol was measured in 3 to 5 ml of human plasma. Samples were prepared by ether extraction and discontinuous solvent elution from Celite columns. 15 samples could be run daily with ease. Blanks were usually 0 and never exceeded 13 pg/ml. Estradiol concentrations in postmenopausal women ranged from <5 to 21 pg/ml and in two hypophysectomized women were <5 and 6. Individual samples obtained from cycling women showed that during days 1–10, the mean estradiol concentration was 64 ±3.6 pg/ml. Values then rose and became more variable with a mean of 127 ±10.9 pg/ml. In normal men the mean estradiol concentration was 22.6 ±2.9 pg/ml, and in seven men with severe cirrhosis in the presence or absence of gynecomastia it was 42.8 ±6.6 pg/ml ($P < 0.025$). In gynecomastia of diverse noncirrhotic etiology, estradiol averaged 34.2 ±2.5 pg/ml, also significantly different from normal ($P < 0.025$). *Conclusions:* For the first time a convenient, sensitive, and precise assay system is available for estradiol measurement. Plasma concentrations in normal men and women are consistent with their physiologic state and the stage of the menstrual cycle. These data first demonstrate the truth of the clinical aphorism that circulating estrogens are elevated in cirrhosis. Although gynecomastia per se is associated with an increased mean estradiol level, values were lower than those found in the follicular phase in women. (Research supported by grants from the American Cancer Society and The Population Council.)

145. Isolation and Characterization of Phytohemagglutinin Receptor Sites. STUART KORNFELD* AND ROSALIND KORNFELD,* St. Louis, Mo. (introduced by Carl V. Moore**).

Phytohemagglutinin (PHA) has the capacity to agglutinate erythrocytes and to stimulate normal but not chronic lymphocytic leukemia (CLL) lymphocytes to undergo mitosis. Since the binding of PHA to cell surface receptor sites is the initial step in the sequence of events leading to mitogenesis, we prepared purified ^{131}I-PHA and performed binding studies with various cell types. CLL lymphocytes bound significantly less PHA than normal lymphocytes (5.7×10^5 vs. 1.3×10^6 receptor sites per cell); further, the binding decreased as the patient's WBC increased. The binding affinity for PHA was the same in both cell types ($K_a = 1.2 \times 10^7$ M). Since these results suggested that CLL lymphocytes have a decreased number of PHA receptor sites, we undertook to isolate and characterize these structures. Trypsin treatment of cells reduced the binding of ^{131}I-PHA to erythrocytes by 40% and to lymphocytes by 70%. The trichloroacetic acid–soluble glycopeptide material released from RBC stroma by trypsin inhibited the binding of ^{131}I-PHA to untreated RBC and lymphocytes. Direct binding of the glycopeptide to PHA was demonstrated by gel filtration studies; hence the PHA receptor was contained in this material. Treatment of the glycopeptide with alkaline boro-

hydride released two-thirds of the oligosaccharide chains (those linked *O*-glycosidically to serine and threonine), while the residual glycopeptide (mol wt 4000–6000) containing sialic acid, galactose, *N*-acetyl-hexosamine, mannose, and fucose retained full inhibitory activity. Removal of terminal sialic acid residues with neuraminidase did not destroy inhibitory activity. However, β-galactosidase treatment following, but not preceding, neuraminidase treatment resulted in a 70–90% loss of activity. The only sugar released was galactose. These studies demonstrate (1) that PHA binds to glycoprotein structures on the cell surface with the specificity residing primarily in the galactose moiety of an oligosaccharide chain having the terminal sequence sialic acid—β-galactosyl—R, and (2) that CLL lymphocytes bind less PHA than normal lymphocytes, reflecting an altered cell surface.

146. Microinjection Study of Urate Transport in the Rat Kidney. RONALD A. KRAMP,* WILLIAM E. LASSITER, AND CARL W. GOTTSCHALK,** Chapel Hill, N. C.

Intrarenal transport of urate was studied in anesthetized rats, using the tracer microinjection technique. Small volumes of saline containing ^{14}C-urate and ^{3}H-inulin were injected into proximal and distal tubules on the kidney surface, placed on the renal capsule, or injected intravenously, and excretion of isotopes in the urine was measured. Rats were made diuretic by continuous infusion of 2.5% NaCl. Excretion of urate was almost complete after distal injection (97%) but was less after injection into late proximal (90%) and early proximal tubules (75%). The means of the three groups are significantly different ($P < 0.01$). Comparison of excretion curves revealed greater delay of urate excretion relative to that of inulin after proximal than after distal injection. Probenecid infusion (100 mg/kg body weight i.v.) caused significant inhibition of proximal urate reabsorption, and excretion after early proximal injection increased to 83% ($P < 0.01$). After late proximal injections, total urate excretion was not increased, but a significantly greater fraction of urate was excreted directly, following the pattern of inulin excretion ($P < 0.01$). Probenecid had no effect on the pattern of excretion after distal injections. With or without probenecid, there was no evidence of precession of urate over inulin excretion when the test solution was placed on the kidney surface or injected intravenously. The results indicate that under the conditions of these experiments, urate reabsorption occurs primarily in the convoluted portion of the proximal tubule in the rat, although some reabsorption occurs more distally, presumably in the pars recta of the proximal tubule. Probenecid inhibits urate reabsorption in the proximal tubule.

147. Physiological and Biochemical Evidence Exempting the Renal Medulla in the Experimental Renal Tubular Dysfunction of Patients with Hereditary Fructose Intolerance (HFI). JOSEPH F. KRANHOLD,* DANA LOH,* AND R. CURTIS MORRIS, JR.,* San Francisco, Calif. (introduced by Richard J. Havel).

In patients with HFI, sustained administration of fructose induces an experimental renal tubular disorder (ERTD) with physiologic characteristics of Fanconi's syndrome and prototypic "proximal" renal tubular acidosis (RTA). The occurrence of the causal enzymatic defect in the renal cortex accords with these physiologic characteristics. To investigate possible exemption of the renal medulla in the ERTD, we measured (1) C_{H_2O} during water diuresis and (2) $T^c_{H_2O}$ during combined antidiuresis and osmotic diuresis, immediately before and during ERTD. The findings that C_{H_2O} increased from 12 to 14 ml/min and $T^c_{H_2O}$ remained at 6 ml/min failed to demonstrate dysfunction of the distal nephron during ERTD. The metabolic abnormality induced by fructose is initiated by cellular accumulation of fructose-1-phosphate (F-1-P) in those tissues which are deficient in aldolase activity toward F-1-P and normally convert fructose to glucose exclusively via F-1-P and the triose products of its aldolase cleavage. Hence only in those tissues normally containing the unique enzyme cluster fructokinase, the "B" aldolase isoenzyme ("liver aldolase"), and triokinase can a deficient aldolase activity toward F-1-P lead to F-1-P accumulation. We found significant specific activities of these enzymes in mammalian renal cortex (dog, goat, rat, and man), but not in the renal medulla, which did contain aldolase A (the "muscle aldolase"). The aldolase isoenzymes were identified by their electrophoretic and kinetic characteristics. These physiologic and biochemical data provide strong evidence that the ERTD affects only the renal cortex. Yet a patient with HFI who has not abstained from fructose has medullary nephrocalcinosis and persistent, seemingly classic, "gradient" RTA. Her identical twin has only ERTD. The association of classic RTA and HFI seems more than fortuitous and suggests the possibility that what is ultimately recognized clinically as a renal medullary disease may stem from an enzymatic defect isolated to the renal cortex. (Research supported by grants from the NIH and the American Cancer Society.)

148. Treatment of Red Cell Aplasia with Immunosuppressive Drugs. SANFORD B. KRANTZ,* VINCENT KAO,* AND ELMER B. BROWN,** Bethesda, Md., Chicago, Ill., and St. Louis, Mo. (introduced by Leon O. Jacobson**).

We have previously demonstrated a plasma inhibitor to heme synthesis and an antibody to erythroblast nuclei in a case of red cell aplasia. These results suggested that the patient's disease might be due to a specific antibody to erythroblasts which either prevented their development or destroyed them as they arose. Treatment with 6-mercaptopurine resulted in normal erythropoiesis which persisted without further therapy for 1½ yr. Two additional patients with red cell aplasia (who failed to respond to androgens, corticosteroids, or splenectomy) have now been studied. When their marrows were incubated in vitro, the rate of ^{59}Fe incorporation into heme increased 2- to 6-fold over 72 hr, whereas in normal marrow it declines. This suggests that the marrow cells were freed from an in vivo inhibitor. Addition of erythropoietin to the cultures further increased the rate of heme synthesis above controls. When purified IgG proteins from the patients' plasmas were conjugated with fluorescein isothiocyanate and applied to normal marrow cells, a specific fluorescence of erythroblast nuclei was observed. The patients' plasmas did not retard the rate of heme synthesis by normal marrow cells in vitro, but the purified IgG

fraction of one patient markedly inhibited heme synthesis as compared with the IgG fraction from normal plasma. The patients were treated with cyclophosphamide for 60–80 days until toxicity was evidenced by a decrease in platelet or WBC counts. Treatment was then discontinued. Several weeks later both had a marked reticulocytosis and have since maintained normal erythropoiesis for up to 1 yr without further treatment. Antibody to erythroblast nuclei, and inhibitor to heme synthesis, were no longer present in the plasma 2 months after treatment. These studies support the hypothesis that red cell aplasia may be due to an antibody to marrow erythroblasts, and clearly demonstrate the value of immunosuppressive drugs in selected cases.

149. Conglutinin in Bacterial Phagocytosis: Studies of a Natural Anti-C'3. GÖRAN KRONVALL,* JOHN H. DOSSETT,* PAUL G. QUIE, AND RALPH C. WILLIAMS, JR., Minneapolis, Minn.

Bovine conglutinin has been shown to inhibit complement-dependent (C'3) immune adherence when added in large excess. Since phagocytic mechanisms are mediated by complement as well as directly by some immune γG opsonins, it seemed important to study the effect of phagocytosis of conglutinin, a naturally occurring anti-C'3. Conglutinin was purified from bovine serum by absorption with zymosan, elution with EDTA buffer, euglobulin precipitation, and extraction from the precipitate. Final preparations showed activity/protein ratios between 15,000 and 34,000 and represented a 200- to 500-fold purification. Conglutinating titers of 1:32,000 were present in such purified conglutinin preparations. Phagocytic systems studied consisted of human leukocytes, *Escherichia coli* or *Staphylococcus aureus,* and normal or immune human serum. When conglutinin was added to complement-dependent phagocytic systems of *E. coli* or *S. aureus,* leukocytes, and fresh normal human serum, inhibition of phagocytosis was noted. Concentrations of conglutinin necessary to produce inhibition in phagocytic systems were 200 times the amount required to give conglutination of alexinated sheep erythrocytes. Increasing inhibition was seen with proportionately increasing amounts of conglutinin. When immune γG globulin constituted the major phagocytosis-promoting factor, no inhibition by conglutinin was observed. Blocking of phagocytosis by conglutinin thus appeared to depend on a complement-mediated system. Bovine conglutinin produced little or no antiopsonic effect if bacteria were preincubated with fresh serum before conglutinin was added. Conglutinin therefore seemed to interfere at a stage before C'3 amplified the opsonic potential of antibodies. Bovine conglutinin as a natural anti-C'3 factor—like rheumatoid factor, the natural antibody to γG globulin—is capable of blocking phagocytosis in vitro.

150. Serial Measurements of Left Ventricular Function in Intact Conscious Dogs: Acute Depression and Subsequent Recovery after Experimental Myocardial Infarction. RAJ KUMAR,* WILLIAM B. HOOD, JR.,* JULIO JOISON,* AND JOHN C. NORMAN,* Boston, Mass. (introduced by Walter H. Abelmann**).

Acute myocardial infarction (MI) causes depression of left ventricular (LV) function, but the ability of the ventricle to recover from such an injury remains unknown. This problem was explored by measuring LV function in six intact, conscious dogs before, 1 hr after, and again 7 days after MI. Acute MI was produced using a new technique which entails gradual inflation over an average period of 1 hr of a balloon cuff previously implanted around the left anterior descending coronary artery. Occurrence of anterior wall infarction was detected electrocardiographically and later confirmed by postmortem examination. Left ventricular function was evaluated from the relation between LV developed pressure (LV peak systolic pressure minus LV end-diastolic pressure) and LV end-diastolic pressure during transient aortic occlusion with a balloon catheter. Left ventricular function curves were obtained by plotting LV developed pressure at increasing LV end-diastolic pressures up to 50 mm Hg. Acute MI caused marked depression of LV function measured 1 hr after onset of infarction, but one week later all six animals showed improvement, with return of function toward the control level. Thus at a reference LV end-diastolic pressure of 25 mm Hg on the LV function curves, the pressure developed by the left ventricle was 223 ±18 mm Hg (SD) in the control state, 156 ±20 mm Hg 1 hr after infarction, and 192 ±28 mm Hg 1 wk later. These figures all differ significantly from one another ($P < 0.01$). To rule out rate dependence of LV function, atrial pacing was carried out in four animals at 120 to 180 per min, the range encountered in the study, and did not alter LV function curves significantly. These data show that the marked depression of LV function observed immediately after experimental acute MI undergoes considerable resolution within 1 wk, although functional recovery is incomplete.

151. Anticoagulant and Thrombolytic Effects of a Polypeptide Extracted from Malayan Viper Venom. HAU C. KWAAN AND E. J. FEDOR,* Chicago, Ill.

Recent reports of the induction of a defibrination state in animals, and of a therapeutic thrombolytic effect in human patients, caused by a polypeptide extracted from the Malayan pit viper venom, "Arvin," suggest a new approach to anticoagulation and thrombolysis. Studies were made on the pharmacological characteristics of this agent in healthy animals and in those with induced thrombosis. Assays of blood-clotting factors and fibrinolytic system after an intravenous injection of Arvin showed a prompt decrease in fibrinogen and plasminogen levels with no alteration in platelet count, other clotting factors, and other fibrinolytic factors. Fibrinogen split products appeared in the circulation for about 6 hr, and plasma levels of fibrinogen remained low for 6 days. A species difference in dose response was found corresponding to the species characteristics of the blood-clotting and fibrinolytic profile. Fibrin microthrombi were identified by the immunofluorescence method in small vessels in the liver, spleen, and renal glomeruli immediately after injection of massive doses of Arvin, but were not demonstrable later. They also were not seen with a smaller dose that can produce defibrination. Fibrinolytic activity in the vascular endothelium and other tissues was not affected after systemic or local Arvin administration. A lack of fibrinolytic effect of Arvin on pure fibrin clot or clotted plasma was also shown

in vitro. However, in experimental jugular venous thrombosis in dogs, a return of blood flow through the thrombosed segment was observed within 24 hr of treatment. These findings indicate that Arvin achieved an anticoagulant effect by lowering the blood fibrinogen level and producing fibrinogen split products, but its mode of action in producing in vivo thrombolysis remains obscure.

152. Abnormal Membrane Deformability: A Model for the Hereditary Spherocyte. PAUL L. LA CELLE* AND ROBERT I. WEED, Rochester, N. Y.

Erythrocyte deformability is essential for passage through restricted regions of the microcirculation (3 μ in the spleen). This study examined deformability of hereditary spherocytes (HS) using a micropipette analogue of the microcirculation, and investigated the dependence of deformability on ATP, Ca, and Mg. Normal erythrocytes deform to a standard hemispherical configuration in a 3.25 μ pipette by a negative pressure (P) of 3–5 mm H_2O; 6–20 mm H_2O pressure (P_t) induces the cell to traverse the pipette. ATP-depleted normal erythrocytes have a 1000% increase in P. Ghosts have values for P and P_t similar to those of corresponding intact cells, indicating that resistance to deformation is a membrane property. These changes appear to result from an actomyosin-like sol $\rightarrow$ gel transformation at the inner membrane surface, induced by Ca and inhibited by ATP and Mg. HS cells have mean values of P like those of control P; the mean P_t is higher, 20.5 vs. 11.2 mm. However, in fresh HS blood 2–15% of cells have abnormal P (7–60 mm), and either P_t is >300 mm H_2O or hemolysis occurs at high P_t. Incubation of HS cells with adenosine restores P and P_t to control values. By contrast with normal, HS ghosts reconstituted with 10^{-3} M ATP are incompletely protected against Ca-induced rigidity, and 10^{-3} M ATP does not restore P_t to control in all depleted HS cell ghosts. In terms of the sol $\rightarrow$ gel membrane deformability model, the data suggest that the relations of ATP, Ca, Mg, and membrane protein in HS are qualitatively like the normal, but that at a given ATP concentration HS membranes are less deformable than normal, a fact which will significantly impair HS cell passage through the spleen. (Supported by NIH grant HE-06241-08 and AEC publication UR-49-1048.)

153. Synchronization of the Mitotic Cycle in Acute Leukemia. BEATRICE C. LAMPKIN,* TAKESHI NAGAO,* AND ALVIN M. MAUER, Cincinnati, Ohio.

Cytosine arabinoside (CA) can synchronize the mitotic cycle of cells in tissue culture. In vivo synchronization of leukemic cells was attempted in the following study. 11 studies were done in 10 patients; nine had lymphoblastic leukemia (ALL), one lymphosarcoma with leukemic transformation, and one myeloblastic leukemia (AML). A single rapid injection of CA, 5 mg/kg, was given. Changes in marrow blasts were determined by serial measurements of ^{3}H-T labeling (LI) and mitotic index (MI). By 4 hr, LI, quantity of label per cell, and MI were markedly decreased. In four patients a striking rebound in LI was seen 24 to 84 hr after CA, with lesser but significant increases over pretreatment values in another five. Two patients with ALL did not have rebound increases over base line. The return of MI lagged behind recovery of LI. CA inhibited DNA synthesis as indicated by decreased LI and label per cell. This effect is confirmed by return of LI before MI. The subsequent rebound over pretreatment levels indicated that some cells entered DNA synthesis as others were held in this phase. The release of all these cells from drug effect simultaneously resulted in partial synchronization of the mitotic cycle. In four patients (three ALL and one AML), Vincristine (VCR) was given at a time of maximal synchronization in DNA synthesis. Subsequent changes in marrow studies suggested that a greater number of cells were destroyed by VCR in two patients than would have been expected from VCR alone. VCR was used because of its readily measurable effect, but augmentation of other mitotic cycle–dependent drugs might be achieved by prior synchronization of cells with CA. (Research supported by grants CA-04826 and FR-00123 from the NIH.)

154. Intracardiac Tamponade as a Factor in Acute Right Heart Failure: Its Operation in Constrictive Pericarditis. RAMON L. LANGE, JAMES T. BOTTICELLI,* AND MICHAEL H. KEELAN, JR.,* Milwaukee, Wis.

Acute and severe right heart dilatation reducing pericardial compliance, elevated systemic venous pressure, high resistance to pulmonary blood flow, and low cardiac output (i.e., pulmonary embolism, acute cor pulmonale) are associated with hypotension, respiratory variation in arterial pressure, severe circulatory distress, and significant mortality. Under such circumstances, right ventricular diastolic volume (RVDV) might encroach upon and reduce left ventricular diastolic volume (LVDV). This potential mechanism may be investigated in constrictive pericarditis, where total diastolic volume is limited and phasic respiratory variation in right and left heart filling pressure is seen. The extent of variable right and left ventricular partition of total diastolic volume by movement of the interventricular septum was deduced from aortic pulse contour or pressure as affected by phasic variation in ventricular filling pressures. Variations in end-diastolic right and left ventricular pressures (RVEDP and LVEDP), along with aortic and arterial pressures during tranquil respiration, were measured in 15 patients with constrictive pericarditis. In these, the change $\pm$standard error in arterial pulse pressure was 10.0 $\pm$0.7 mm Hg (range, 7–16; normal, 4.23 $\pm$1.1). The RVEDP was: inspiration, 14.7 $\pm$1.3 mm Hg; expiration, 14.5 $\pm$1.3 mm Hg. The LVEDP was: inspiration, 15.2 $\pm$1.2 mm Hg; expiration, 21.0 $\pm$1.1 mm Hg, causing the pressure (LV $-$ RV) across the interventricular septum to be: inspiration, 0.5 $\pm$0.5 mm Hg; expiration, 6.5 $\pm$0.6 mm Hg. These findings support the hypothesis that impaired LVDV in inspiration is responsible for the variation in arterial pressure by the Starling mechanism. An estimate of the magnitude of the variation in LVDV may be made from the assumed values of LV compliance (16 ml/mm Hg) and the fraction of left ventricular endocardial surface formed by the septal area (20%) and responsive to transseptal forces. A 6 mm Hg variation in transseptal pressure would result in a diastolic volume variation of nearly 20 ml, corresponding to a 10 mm Hg difference in pulse pressure. With a nonadhesive but tense peri-

cardium, RVEDP may exceed LVEDP sufficiently to induce a condition of intracardiac tamponade, causing hypotension and pulsus paradoxus. Inspiratory splitting of the second sound by shortening of LV ejection time, characteristic of constrictive pericarditis, would also reflect this phenomenon.

155. Familial Type II Hyperlipoproteinemia: A Defect of Beta Lipoprotein Apoprotein Catabolism? TERRY LANGER,* WARREN STROBER,* AND ROBERT I. LEVY,* Bethesda, Md. (introduced by Donald S. Fredrickson).

Familial type II hyperlipoproteinemia is characterized by increased levels of apparently normal plasma beta lipoproteins (βLP), autosomal dominant transmission, xanthomatosis, and premature atherosclerosis. It has been suggested that this may be a disorder of beta lipoprotein apoprotein (βLP-P) metabolism. βLP-P turnover was studied by injection of autologous, pure βLP ($d = 1.019$–1.063), isolated by ultracentrifugation and radioiodinated in the peptide moiety with iodine monochloride, into six type II patients and four normal subjects fed isocaloric diets containing <300 mg cholesterol per day. Proteins prepared in this manner behaved identically with screened preparations in dogs. The distribution of βLP-P conformed to a two-compartment model consisting of the intravascular space containing 70–80% of the protein and an extravascular compartment. βLP-P did not appear in other lipoproteins. The level of circulating βLP-P (170 ± 33 mg/100 ml; mean $\pm$SD) in type II was increased as compared with normals (61 ± 9 mg/100 ml). The half time of protein survival ($T\frac{1}{2}$) in type II (4.90 ± 0.19 days) was significantly longer than in the normals (2.96 ± 0.61 days), and the fractional catabolic rate (FCR) in type II (0.219 ± 0.014) was much less than normal (0.509 ± 0.086). The synthetic rate of βLP-P in type II (15.8 ± 2.5 mg/kg per day) was, however, similar to that in normals (14.6 ± 0.6 mg/kg per day). The six type II patients then received cholestyramine (24 g/day) and the mean βLP-P fell to 116 ± 18 mg/100 ml. Repeat studies demonstrated a marked increase in FCR to 0.316 ± 0.038 and reduction in $T\frac{1}{2}$ to 3.60 ± 0.31 days. The synthetic rate remained unchanged (15.9 ± 1.4 mg/kg per day). These observations indicate that over a wide range of βLP-P concentrations, difference in βLP-P levels are due to alterations in the degradative rate of βLP-P, and not to changes in synthetic rate or distribution. Furthermore, the data suggest that the elevated levels of βLP seen in type II hyperlipoproteinemia result from hypocatabolism of βLP-P.

156. Natriuretic Activity in Plasma and Urine of Salt-Loaded Man and Sheep. JOHN H. LARAGH, JEAN E. SEALEY,* AND J. DIANNE KIRSHMAN,* New York, N. Y.

Natriuretic hormone activity was demonstrated in human and ovine plasma and urine which had been fractionated by gel filtration and concentrated up to 1000-fold. Samples were assayed by intravenous injection of 0.1–0.5 ml of the concentrate in nembutalized rats during water diuresis. Assays utilized heterozygous or homozygous rats with hereditary diabetes insipidus. Responsiveness of the assay animals was related directly to preexisting sodium balance and inversely to plasma renin. The samples from man collected after saline infusion or 5 days of a high-sodium diet consistently caused marked increases in both urinary sodium concentration and $U_{Na}V$ in recipient animals, whereas control samples obtained before saline infusion or during sodium deprivation had no effect. Natriuretic activity appeared in samples from six of seven normal subjects, from all six patients with essential hypertension, and from four with primary aldosteronism. The natriuretic response increased $U_{Na}V$ from an average control rate of 1 μEq/min to 2–10 μEq/min for human samples and to 5–20 μEq/min for samples from sheep which had drunk 0.45% NaCl for a week. Natriuretic response can persist for 2 hr or more after a single injection, with only slight or inconsistent changes in urine flow or potassium excretion. The injection of unconcentrated plasma or urine never yielded positive assays. Only those fractions associated with apparent molecular weights greater than 50,000 were natriuretic. The natriuretic agent was inactivated by trypsin, not by thioglycollate. Its properties suggest a protein which inhibits sodium reabsorption in a distal portion of the nephron. (This work was supported by NIH grant HE-01275.)

157. DNA Polymerase in Fetal Rat Liver. JOHN LASZLO* AND PETER OVE,* Durham, N. C. (introduced by R. Wayne Rundles**).

DNA polymerase is the final and possibly the rate-limiting enzyme of DNA synthesis. We have found that its activity generally parallels the rate of DNA synthesis and growth of normal rat tissues and of a series of 12 hepatomas with varying grades of malignancy. Hepatoma DNA polymerase differs from normal liver in its preference for denatured DNA as an in vitro primer. This enzyme was separated by Sephadex G-200 chromatography (peak I) and was distinct from normal enzyme (peak II). The present study shows that DNA polymerase prepared from fetal rat liver (−6 days from birth) has a marked preference for denatured DNA, and has activity even higher than that found in the most malignant hepatomas: 3.8 μg triphosphate incorporated per mg protein per hr vs. 6.3 for Morris hepatoma 3683 vs. 0.1 for normal liver. At the time of birth, peak I activity decreases to levels found for "minimal deviation" Morris hepatoma (0.5 μg). 1 wk after birth, peak I activity is barely detectable, as in normal adult or regenerating rat liver. By contrast, peak II polymerase activity progressively increases until 1 wk after birth and then declines slightly to reach the normal adult level by 21 days. It appears that peak I DNA polymerase is characteristic of fetal rat liver and that it is progressively repressed as peak II is expressed. However, the fetal enzyme pattern may become derepressed during the process of malignant transformation. (Research supported by grants from the NIH and the American Cancer Society.)

158. Total and Nutritional Coronary Flow, the Dichotomy of the Coronary Circulation. GEORGE LEB,* FRANZ DERNTL,* NORA GOLDSCHLAGER,* CHARLES COWAN,* AND RICHARD J. BING, Detroit, Mich.

This work is based on the concept that it is possible to measure nutritional (effective) and total coronary flow by means of rubidium-84. This isotope is a positron emitter, and

its uptake by the heart is primarily dependent on flow. Total coronary flow can be measured by the Fick principle, (myocardial uptake)/(coronary A-V) (requiring catheterization of coronary sinus); nutritional flow is measured by the myocardial uptake of rubidium as a fraction of cardiac output (Sapirstein), (myocardial uptake)/$_0\int^\infty$A; catheterization of the coronary sinus is not required for this determination. Using the coincidence counting method, the activity of the heart can be separated from that of surrounding structures. A single bolus of rubidium-84 is injected intravenously and uptake is measured by a pair of coincidence detectors. Since rubidium is exchanged only through the capillaries and since this process is flow limited, the fractional uptake of rubidium by the myocardium represents nutritional flow. The opening of functional shunts in the myocardium (the increase in ratio of total to effective flow) could be demonstrated in the anesthesized closed-chest dog during the infusion of norepinephrine and nicotine and in the presence of hypoxia. On the other hand, isoproterenol did not result in an opening of functional shunts, as total and effective flow increased proportionately, suggesting that a larger capillary bed is available to accommodate the increase in flow. A similar divergent response has been recently observed in skeletal muscle. The results suggest that functional shunts can occur in the capillary bed of the heart. The method for measuring nutritional flow can be applied to clinical situations. Studies in patients with acute myocardial infarction demonstrated a significant decrease in effective myocardial blood flow as compared with normals; this was independent of changes in cardiac output and stroke work. (Research supported by grants from the NIH and the John A. Hartford Foundation.)

159. Reactivity of Anti-RBC Antibodies with Human Leukocyte Lysosomes. JOHN P. LEDDY AND MARILYN A. FELTON,* Rochester, N. Y.

In 1964 Quie and Hirsch noted that rabbit leukocyte lysosomes and RBC shared common antigen(s). We have isolated lysosomes from normal human leukocytes by the method of Chodirker et al. By hemagglutination-inhibition tests and by absorption studies, such lysosome preparations have consistently shown strong reactivity with γG autoantibody ("non-Rh" specificity) in acid eluates from cases of autoimmune hemolytic disease (AHD), anti-A or anti-B isoagglutinins, and anti-i cold agglutinins. Weaker reactivity was found with anti-D isoantibody and a single "Rh-related" γG autoantibody. Anti-I cold agglutinin and a γG anti-LW isoantibody were not affected. Lysosomes from a group B donor bound anti-B but no anti-A; and group A lysosomes bound anti-A but not anti-B. Anti-i cold agglutinin was absorbed by lysosomes at 5°C but not at 37°C. Absorption of antibodies by lysosomes was partial within 15 min and advanced at 30 min. Effect of lysosome suspensions on the various anti-RBC antibodies was removed by sedimentation of the granules at 25,000 *g* for 10 min before exposure to antibodies, despite persistence of protease activity in such supernatants. The absorption of γG autoantibodies was not altered by the presence of a great excess (2.5–5.0 mg/ml) of normal γG globulin (native or acid treated) added to serve as a buffer against lysosomal proteases during the incubation. Recent studies reveal that rabbit leukocyte lysosomes also absorb human γG autoantibody ("non-Rh" specificity) in the presence of 2.5 mg/ml nonspecific acid-treated γG. Studies with ^{125}I-labeled normal γG show that the rabbit lysosomes hydrolyzed or bound only 5% of the nonspecific γG globulin under these conditions while reducing the autoantibody by 75% or more. Thus, the possibility emerges that the "non-Rh" antigens relevant to human AHD, although undetectable on nonprimate RBC, may have a wide distribution on non-RBC mammalian membranes. The observation that certain antigenic determinants relevant to "erythrocyte" autoantibodies may be shared by the membranes of intracellular organelles such as lysosomes may provide (*a*) a clue to the origin of autoantibodies reactive with autologous RBC appearing during certain viral infections, and (*b*) a possible pathway for the development of positive Coombs tests in systemic lupus erythematosus, in which multiple antibodies to intracellular structures are well known.

160. Hyperbetalipoproteinemia in the Genetic Disease Acute Intermittent Porphyria (AIP). ROBERT S. LEES,* CHULL S. SONG,* RICHARD D. LEVERE,* AND ATTALLAH KAPPAS, New York, N. Y.

Hypercholesterolemia occurs in both experimental and human hepatic porphyria. We have examined the nature of the disturbance in lipoprotein metabolism which underlies this hypercholesterolemia, by measuring the composition and concentrations of the various lipoproteins in the plasma of porphyric subjects. Plasma concentrations of total cholesterol, glycerides, cholesterol content of very low density (VLDL), low density (LDL or beta), high density (HDL or alpha) lipoproteins, and the protein content of LDL were measured in 20 AIP patients and compared with appropriate controls. In the AIP group mean values of total plasma cholesterol and particularly LDL-cholesterol were substantially higher than normal; LDL-protein concentrations and LDL-cholesterol/protein ratios were also increased. LDL-cholesterol was high even in several patients with normal total plasma cholesterol. 16 of the 20 patients had LDL-cholesterol concentrations at least 1 SD above the mean of age-matched controls; none of the four remaining had values lower than control means. Families of three patients were studied; one parent in each had high LDL-cholesterol, as did two siblings of one patient. The extent of the LDL abnormality was unrelated to the magnitude of porphyrin precursor excretion. Five patients with the congenital bone marrow disorder of porphyrin metabolism, erythropoietic protoporphyria, had normal values of all lipid parameters examined. Of six patients with cutaneous hepatic porphyria, a disease considered to be frequently of acquired nature, only one had hyperbetalipoproteinemia. This study demonstrates that hyperbetalipoproteinemia is a common biochemical abnormality in acute intermittent porphyria. This lipoprotein abnormality may be genetically or metabolically related to the known defect in control of hepatic porphyrin synthesis which characterizes AIP; and it may be a useful marker for this genetic liver disease.

161. The Separate Effects of Metabolic Acidosis and Glucose Ingestion on Urinary Calcium ($U_{Ca}V$) and Magnesium ($U_{Mg}V$) Excretion. EDWARD J. LENNON, WALTER F. PIERING,* JEAN BROCK,* AND OPAL A. KELLY,* Milwaukee, Wis.

Glucose ingestion augments $U_{Ca}V$, $U_{Mg}V$, and renal acid excretion ($U_{net\ acid}V$). The same changes have been reported during metabolic acidosis, although not all investigators found increases in $U_{Mg}V$. To test whether glucose ingestion and NH_4Cl acidosis increase renal excretion of divalent cations by a common mechanism, clearance studies were performed on four healthy men before and 10 days after the induction of NH_4Cl acidosis. Each clearance study consisted of ten 20 min periods, four before and six after ingesting 100 g glucose. The effects of glucose alone, metabolic acidosis alone, and acidosis plus glucose were compared with the initial control periods, each subject serving as his own control. Results are expressed as peak changes from control (mean ±SEM, μmoles/min). $U_{Ca}V$ rose significantly after glucose (+4.56 ±1.39, $P < 0.05$) and during acidosis (+4.37 ±1.06, $P < 0.05$). Glucose ingestion also augmented $U_{Mg}V$ (+4.47 ±0.73, $P < 0.05$), but acidosis did not (+0.78 ±0.85, NS). Glucose ingestion during acidosis produced peak changes in $U_{Ca}V$ (+8.54 ±1.93) and $U_{Mg}V$ (+3.28 ±0.69) which were not statistically different from the sum of the separate effects of glucose and acidosis. Glucose ingestion in the control studies caused urinary acidification and increases in $U_{net\ acid}V$, whereas glucose ingestion during acidosis tended to make the urine less acid, and $U_{net\ acid}V$ did not change. *Conclusions:* Glucose ingestion augments both $U_{Ca}V$ and $U_{Mg}V$, whereas NH_4Cl acidosis increases only $U_{Ca}V$. The effects of glucose are independent of urine acidification or increases in $U_{net\ acid}V$ and are additive to those of NH_4Cl acidosis. Thus, glucose ingestion and NH_4Cl acidosis appear to alter divalent cation excretion by different mechanisms. (Research supported by grant 5-MO1-FR-00058 from the USPHS.)

162. Myocardial Adenyl Cyclase: Activation by Thyroid Hormones and Evidence for Two Adenyl Cyclase Systems. G. S. LEVEY* AND S. E. EPSTEIN,* Bethesda, Md. (introduced by J. Wolff).

The mechanism responsible for the hyperdynamic circulatory state in hyperthyroidism has not been defined. Although certain cardiac manifestations resemble those caused by excessive adrenergic stimulation, recent evidence suggests that thyroid hormone exerts an effect on the heart that is independent of the adrenergic system. Since the inotropic and chronotropic effects of norepinephrine appear to be mediated by activation of adenyl cyclase, the possibility that thyroxine and triiodothyronine are also capable of activating adenyl cyclase was examined in the particulate fraction of cat heart homogenates. L-Thyroxine and L-triiodothyronine increased the conversion of $AT^{32}P$ to cyclic 3',5'-$AM^{32}P$ by 60% and 45% respectively ($P < 0.01$). A variety of compounds structurally related to the thyroid hormones, but devoid of thyromimetic activity, did not activate adenyl cyclase; these included 3',5-diido-L-thyronine, L-thyronine, 3,5-diiodotyrosine, monoiodotyrosine, and tyrosine. D-Thyroxine activated adenyl cyclase, and the K_m was identical with that of the L isomer. Although the beta adrenergic blocking agent propranolol abolished norepinephrine-induced activation of adenyl cyclase, it failed to alter activation caused by thyroxine. When maximal concentrations of L-thyroxine (5×10^{-6} M) and norepinephrine (5×10^{-5} M) were incubated together, an additive effect on cyclic 3',5'-AMP production resulted. This investigation demonstrates that (1) thyroid hormone is capable of activating myocardial adenyl cyclase in vitro, and (2) this effect is not mediated by the beta adrenergic receptor. Moreover, the additive effects of norepinephrine and thyroxine suggest that at least two separate adenyl cyclase systems are present in the heart, one responsive to norepinephrine, the other to thyroid hormone. These findings are compatible with the hypothesis that the cardiac manifestations of the hyperthyroid state may, in part, be caused by the direct activation of myocardial adenyl cyclase by thyroid hormone.

163. Measurement of Cardiac Output by Indicator Dilution in the Nonsteady State. GILBERT E. LEVINSON,* MOHAN JESRANI,* AND JAMES J. FIORE,* Jersey City, N. J. (introduced by Francis P. Chinard**).

Rapid change in cardiac output may be an important determinant of the clinical course of such cardiovascular emergencies as acute pump failure, peripheral vascular collapse, hypertensive crises, and arrhythmias. However, conventional methods for measuring cardiac output in the intact subject require constancy of flow and do not permit measurement of transient flow changes or of constantly changing flows. Measurements in such nonsteady states are possible, however, by the continuous-infusion indicator-dilution technique, if a nonrecirculating indicator is used or if recirculating indicator can be quantitatively accounted for. In anesthetized mongrel dogs, with electromagnetic flow probes placed around the ascending aorta, indocyanine green dye was infused at a constant rate into the left ventricle and its concentration measured continuously in aorta and left atrium by catheter sampling. The difference in dye concentration between the two sites, which is a function of the dye infusion rate and of left ventricular output, increased when flow, measured by the sine wave flowmeter, decreased, and vice versa. In 121 experiments in 16 animals, the "continuous-infusion flowmeter" provided quantitative or semiquantitative measurement of constantly changing outputs or of transiently altered outputs associated with such events as hemorrhage, myocardial deterioration, hypoxemia secondary to episodes of apnea, acute pulmonary or systemic hypertension, sudden decrease in venous return, sustained or transient dysrhythmias, and chronotropic, inotropic, and vasoactive drugs. Fleeting changes, such as those with extrasystoles, although not quantifiable by dye dilution, were readily detectable, and changes in flow lasting as little as 5 sec could be measured. The results indicate that this technique will permit observations, which until now have not been possible, during hemodynamic nonsteady states in intact animals and human subjects. (Research supported by a Research Career Development award from the NIH to G. E. Levinson.)

164. Absorption of Amino Acids from the Small Bowel of Hypophysectomized Rats. RUVEN LEVITAN* AND ELI HAVIVI,* Chicago, Ill. (introduced by Malcolm Stanley**).

The effect of hypophysectomy on the absorption of amino acids from the small bowel of hypophysectomized rats was studied 4, 5, 6, 10, and 15 days after the removal of the pituitary both in vitro and in vivo. Small bowel segments were prepared and incubated in Krebs-Ringer-bicarbonate solutions containing 10 μM concentration of a carrier amino acid and 0.5 μc of a ^{14}C-labeled form of the same. The amino acids used were L-glycine, L-leucine, DL-methionine, and DL-ornithine. During the in vivo experiments, a 20 cm segment of upper small bowel was filled for 30 min with normal saline containing 10 μM carrier amino acid and 0.1 μc of the ^{14}C-labeled form. 4–5 days after pituitary ablation, the uptake and transport of all amino acids by the small bowel in vitro and in vivo was enhanced as compared with that in sham-operated and control rats ($P < 0.01$); however, 10–15 days after hypophysectomy, these functions were decreased. Protein biosynthesis in the small bowel was also found to be decreased at that time. The observed increase of amino acid absorption early after hypophysectomy, and its subsequent decrease, follow a pattern found by us for hexoses. The mechanism responsible for these alterations in absorption is not clear. The enhanced absorption may possibly be attributed to semistarvation, which may exist in animals soon after pituitary ablation. 2 wk after hypophysectomy, the bowel is smaller and thinner in operated animals; thus the decreased area and/or hormonal deficiencies may explain the decreased absorption. Since it has been shown that hypophysectomy lowers tissue ribosome content and since growth hormone can increase protein synthesis, we suggest that the decreased amino acid incorporation into protein in the small bowel of hypophysectomized rats is due to growth hormone deficiency.

165. Physiological Contributions of Thin and Thick Loops of Henle to the Renal Concentrating Mechanism. PHILIP D. LIEF,* ANN SULLIVAN,* AND MARTIN GOLDBERG, Philadelphia, Pa.

To investigate the relative contributions of the thick and thin segments of Henle's loop to maximal urine osmolality (U_{max}) and free water reabsorption ($T^c_{H_2O}$), Wistar rats were prepared by bilateral excision of the renal papillary tip. After recovery they were compared with normal rats in studies of U_{max} during hydropenia and $T^c_{H_2O}$ during 5% saline infusion. The mean $\pm$SEM U_{max} for 12 papillectomized rats (1299 $\pm$104 mOsm/kg H_2O) differed significantly from that for 9 normal rats (3287 $\pm$110 mOsm/kg; $P < 0.001$) and from that for 6 sham-operated rats (2676 $\pm$210 mOsm/kg; $P < 0.001$). During saline diuresis, 7 normal and 6 papillectomized rats maintained a stable inulin clearance (GFR) (mean $\pm$SEM: 2.25 $\pm$0.29 ml/min in the normals and 2.32 $\pm$0.19 ml/min in the papillectomized rats; $P > 0.80$). Osmolar clearance (C_{osm}) rose progressively, reaching peak values of 0.30–0.40 ml/min in both groups. $T^c_{H_2O}$ rose linearly with increasing C_{osm}, and showed no evidence of reaching a limiting value in either the normal or the papillectomized rats. The slope of C_{osm}/100 ml GFR versus urine flow per 100 ml GFR, 1.78 in the normal group, was not significantly different from the slope of 1.63 in the papillectomized group ($0.10 > P > 0.05$), indicating no significant differences in $T^c_{H_2O}$ between the two groups. Histological study of the papillectomized kidneys revealed a decrease in the ratio of thin limbs to collecting ducts in the medullary remnant ($P < 0.01$). Thus ablation of the long thin segments of Henle's loop produced a marked defect in U_{max} but little or no abnormality in $T^c_{H_2O}$. We conclude that at low rates of loop sodium delivery, the thin limbs are important in contributing to U_{max}, but at high rates of sodium delivery, almost all of the increment in loop sodium transport occurs in the thick segment. (Research supported by NIH grant HE-00340.)

166. Pathways of Glucose Metabolism in Human and Canine Arteries. ROBERT D. LILLE* AND ARAM V. CHOBANIAN, Boston, Mass.

In view of the relation between atherosclerosis and carbohydrate intolerance and the observation that glucose is the major substrate for energy production in the arterial wall, studies were conducted to investigate pathways of glucose metabolism in human and canine arteries. Aortas were incubated for 1–3 hr with either 1-^{14}C-glucose (1-^{14}C-G) or 6-^{14}C-glucose (6-^{14}C-G) using Krebs bicarbonate buffer (pH 7.4, glucose 5.67 μmoles/ml). The results demonstrated that: (1) Lactate was the major end product of glucose metabolism under both aerobic (95% O_2) and anaerobic (100% N_2) conditions. (2) Conversion of glucose to CO_2 was minimal and represented less than 3% of glucose utilized in human arteries and less than 7% in dog arteries. Oxidation of 1-^{14}C-G to CO_2 was consistently greater than that of 6-^{14}C-G, but the calculated hexose monophosphate shunt (HMPS) activity represented less than 3% of glucose metabolism in both normal and atherosclerotic tissue. (3) Intimal conversion of glucose to lipid, glycogen, protein, and glucosamine represented minor fractions of total glucose uptake. Most of the lipid radioactivity was recovered from the glycerol moiety of phospholipid. (4) Insulin, glucagon, and growth hormone had no consistent effect in vitro on arterial glucose utilization. (5) The electron acceptor methylene blue (10^{-4} M) markedly stimulated (up to 40-fold) the oxidation of 1-^{14}C-G to CO_2 and decreased the conversion of glucose to lactate. (6) Dinitrophenol (10^{-4} M), an uncoupler of oxidative phosphorylation, stimulated 6-^{14}C-G conversion to CO_2 without influencing lactate. (7) Cyanide (10^{-8} M), an inhibitor of the respiratory chain, depressed glucose conversion to CO_2. In conclusion, these in vitro studies indicate that the major pathway of arterial glucose metabolism is via aerobic glycolysis with an unusually high conversion of glucose to lactate. Arterial HMPS and TCA cycle activity appear to be relatively small but can be augmented by appropriate stimuli. (Research supported in part by grants from the NIH.)

167. Gene Loss in Human Teratomas: Evidence for Crossing-Over. DAVID LINDER,* San Francisco, Calif. (introduced by Nicholas L. Petrakis**).

If benign cystic teratomas (dermoid cysts) of the ovary arise from a germ cell which has undergone meiosis, they should be lacking in genes which are present in the patient.

Three independently segregating allelic isozymes in benign cystic teratomas of the human female ovary were compared with normal tissue of the same patient. Dermoid cysts from patients heterozygous for these isozymes are frequently homozygous for that particular gene product. Two of three dermoid cysts are homozygous for glucose-6-phosphate dehydrogenase (G-6-PD), four of six tumors are homozygous for phosphoglucomutase at the PGM_1 locus, and two or more of eight tumors are homozygous for phosphoglucomutase at the PGM_3 locus in women heterozygous for these allelic isozymes. These findings are consistent with the hypothesis that these tumors arise from a germ cell which has undergone meiosis with varying degrees of crossing-over. (Research supported by NIH grants 1-RO1-HD-01487 and CA-10107.)

168. Tracheobronchial Clearance in Chronic Obstructive Lung Disease. RUY V. LOURENÇO,* TRUMAN O. ANDERSON,* AND HAROLD LEVINE,* Chicago, Ill. (introduced by Harry F. Dowling**).

Abnormalities of the mechanisms of clearance of inhaled particulate matter may play a role in the genesis and maintenance of infection in the tracheobronchial tree of patients with chronic obstructive lung disease. Clearance of iron particles, 2 μ in diameter, tagged with gold-198 was studied in 24 patients with chronic obstructive lung disease and in 12 normal subjects. The particles were administered in the form of a monodisperse aerosol, produced in a spinning disk atomizer; both external imaging and counting of the inhaled particles were carried out with a scintillation camera. Quantification of total protein and immunoelectrophoretic analysis of serum protein components were made in bronchial washings obtained during bronchoscopy in 21 patients. Ventilatory function was assessed from standard ventilatory tests, and airway resistance measured in a constant-volume body plethysmograph. Our results indicate that normal subjects and patients with less marked airway obstruction presented an initial deposition of inhaled particles throughout both lungs, with many particles in the periphery; by contrast, in patients with more marked airway obstruction the majority of the particles were deposited centrally, with a few in the periphery of the lungs. Normal subjects and patients with less severe ventilatory dysfunction showed immediate onset of clearance after inhalation, with retention of less than 90% of particles at 45 min; patients whose ventilatory dysfunction was more severe demonstrated a delayed clearance, with retention of 90% or more of the particles at 45 min. In the latter patients, the exudation of serum proteins into the bronchial lumen was diminished. These abnormalities in both the onset of clearance of inhaled particles and the concentration of serum proteins in the tracheobronchial tree may contribute to the decreased defenses against respiratory infection present in patients with chronic obstructive lung disease. (Research supported by NIH grant AI-07612 and an ATS grant.)

169. Elevated Intrarenal Pressure in Essential Hypertension. JEROME LOWENSTEIN,* DAVID S. BALDWIN,** AND HERBERT CHASIS,** New York, N. Y.

Patients with essential hypertension exhibit "exaggerated natriuresis" during saline infusion. In the light of experimental evidence that increased renal interstitial pressure or volume may mediate decreased tubular reabsorption of sodium, we have examined the hypothesis that intrarenal pressure might be increased in essential hypertension. Wedged renal vein pressure (WRVP), taken as a measure of intrarenal pressure, was found to be significantly elevated in patients with essential hypertension (hypertensives, 28.7 $\pm$6.5 mm Hg, $n = 21$; normal, 20.9 $\pm$4.1 mm Hg, $n = 18$; $P < 0.001$). Increased intrarenal pressure was a consequence of greater transmission of elevated arterial pressure to the peritubular capillaries, as evidenced by the finding that calculated glomerular pressure was increased. In nine hypertensive subjects, after rapid infusion of 1 liter of 2.5% saline, sodium excretion increased from 277 to 1600 μeq/min and WRVP from 26.7 to 44.2 mm Hg. In six normotensive subjects, sodium excretion averaged 200 μeq/min before and 414 μeq/min after saline infusion; WRVP averaged 22.5 mm Hg and increased to 30.6 mm Hg. During saline infusion, GFR and arterial pressure increased and afferent and efferent resistance fell, indicating that increased WRVP was caused by further increased transmission of systemic pressure (rather than by increased tubular volume as in mannitol diuresis). Increments in WRVP paralleled changes in sodium excretion, suggesting that increased intrarenal pressure (or its consequence, increased interstitial volume) initiated decreased sodium reabsorption. The exaggerated natriuretic response may be attributed to the elevation of intrarenal pressure which characterizes the hypertensive patient. This increase in intrarenal pressure, and the further elevation during saline infusion, result from greater transmission of systemic pressure to the peritubular capillaries. Elevated intrarenal pressure in essential hypertension, where we have previously demonstrated increased proximal sodium reabsorption, may represent the mechanism by which sodium excretion is maintained in the face of increased reabsorption at some sites in the nephron. (Research supported by grants from the NIH and the Health Research Council of the City of New York.)

170. Uremic Carbohydrate-Insulin Metabolism. EDMUND G. LOWRIE,* J. STUART SOELDNER,* CONSTANTINE L. HAMPERS,* AND JOHN P. MERRILL,** Boston, Mass. (introduced by Kendall Emerson, Jr.**).

Definition of the pathogenesis of uremic glucose intolerance is unclear. Accordingly, seven patients, six of whom were surgically anephric, received an intravenous glucose tolerance test (GTT), glucose-glucagon test, glucose-insulin test, and insulin tolerance test (ITT) after withholding dialysis for 6 days and after 2 wk of intensive hemodialysis (bath glucose, 100 mg/100 ml). After dialysis, mean glucose utilization increased and mean insulin responses improved for all tests. Speed of insulin release did not change, as peak levels occurred at 1 min in three of seven before dialysis and four of seven after dialysis. The proportion of early-released insulin during GTT was improved, as shown by increases in 1 min IRI levels ($P < 0.025$), 1–3 min/1–5 min insulin curve areas ($P < 0.0125$), and the proportion of insulin secreted in the first 10 min ($0 \rightarrow 10/0 \rightarrow 60$) ($P < 0.025$). There was no significant difference in total insulin secreted. Glucose K rate was linearly correlated with $0 \rightarrow 10/0 \rightarrow 60$ after dialysis,

($P < 0.01$), but not before dialysis. This, with larger declines in glucose during postdialysis ITT, suggests that a defect in tissue insulin response was corrected. $T\frac{1}{2}$ of IRI before dialysis (11.6 ±0.6 min) (SEM) was significantly larger ($P < 0.025$) than $T\frac{1}{2}$ of endogenous IRI in normals (8.7 ±1.1 min). After dialysis $T\frac{1}{2}$ (9.8 ±0.3 min) was significantly shorter ($P < 0.0025$) than before dialysis, but not different from normals. In one patient, early insulin secretion was improved but K rate was not, while in another, the reverse was true. The data are consistent with a beta cell defect resulting in a reduced proportion of early-phase insulin and a tissue defect resulting in insulin insensitivity. One or both of these may be corrected by dialysis in any given patient. (This research was supported by the National Institute of Arthritis and Metabolic Diseases.)

171. On the Effect of Nephron Reduction on Bicarbonate Transport in the Mammalian Nephron. HERBERT LUBOWITZ,* MABEL L. PURKERSON,* AND NEAL S. BRICKER, St. Louis, Mo. (introduced by Carl G. Harford).

Recent results derived from HCO_3 titration experiments have indicated that a marked reduction in nephron population is followed by profound alteration in the kinetics of HCO_3 transport. In the present studies, micropuncture techniques were used to assess quantitatively the effects of nephron reduction on the reabsorption of HCO_3 in individual surface nephrons of the rat. Two experimental conditions were employed. In both, the nephron population of one kidney was reduced by approximately two-thirds by ligating branches of the renal artery. In the first set of studies, the contralateral kidney was left in situ. GFR in the remnant kidneys averaged 32% of that in the contralateral organs. Some 90% of the filtered HCO_3 was reabsorbed by the end of the accessible proximal tubule in both kidneys; neither TF/P_{HCO_3} nor TF/P_{Inulin} was significantly different as between the two kidneys. In a second set of studies, the contralateral kidney was removed, thus rendering these animals uremic (BUN +90.1 ±7.1). Under these conditions, proximal tubular TF/P_{Inulin} values were slightly lower than those found in the nonuremic group. However, TF/P_{HCO_3} ratios in the azotemic animals averaged more than twice the values obtained in the nonazotemic rats. Thus when over 60% of the total nephron population was removed, more than 20% of the filtered bicarbonate remained unreabsorbed at the end of the accessible proximal tubule. These data suggest that glomerulotubular balance for bicarbonate is reset in consequence of nephron reduction. They also quantitate the adaptive change in a sodium-dependent proximal tubular transport system in uremia.

172. Racial Polymorphisms in Human Chromosomes. HERBERT A. LUBS* AND FRANK H. RUDDLE,* New Haven, Conn. (introduced by S. Lipsky).

Minor variations in the length of human chromosomes have been described frequently in both normal and abnormal individuals, but the significance of these variations has remained unclear. The present study, which was designed to evaluate these variants, was based on cytogenetic, clinical, and sociological data from 2500 consecutively born infants. Two karyotypes per newborn were prepared initially, and variations in length of individual chromosomes were compared with reference standards within the same cell. For example, an increased length of a short arm in group D or G was scored if a short arm was equal in length to the short arm of E18 in both cells. A marked increase in length was scored if a G short arm was longer than the short arm of E18. A giant satellite was scored if a D or G satellite was larger than the short arm of the same chromosome. Quantitative studies in 30 cells per newborn are in progress in order to characterize those variants more precisely. Although a few minor variants appeared to carry an increased morbidity, the majority did not. Their frequency, however, varied significantly between races (0.05–0.01 level). Many of the more common variants were almost twice as frequent in Negroes as in whites: increased short arm of a D chromosome (20.0% vs. 12.27%) and a G chromosome (4.7% vs. 2.8%), giant satellites in group G (4.7% vs. 2.8%), and long E16 (4.7% vs. 2.4%). Certain less common variants were also more frequent in Negroes; 8 of 9 metacentric C's and 5 of 14 long A1's were observed in Negroes, although the latter constituted only 18% of New Haven newborns. The only individual with a G short arm longer than the short arm of E18 was Negro, and only 3 of 9 unusually long Y's occurred in whites. The majority of these variants, therefore, probably represent racial polymorphisms, and significant clinical correlations were demonstrated in only a few instances. (Research supported by NIH contract 43-67-1463.)

173. Demonstration of Interferon in Human Infections with St. Louis Encephalitis Virus. JAMES P. LUBY,* WILLIAM E. STEWART, II,* S. EDWARD SULKIN,* AND JAY P. SANFORD, Dallas, Texas (introduced by Ralph Tompsett**).

Epidemic St. Louis encephalitis (SLE) in Dallas, 1966, afforded an opportunity to assess interferon in human infections with SLE. Inhibition of cytopathic effect produced by Sindbis virus on human embryonic lung diploid fibroblasts was the primary assay method. 97 sera from 74 patients and 13 urines from 13 patients, all with laboratory-documented SLE, most collected during the first week of illness, had no detectable interferon. Suspensions of different sections of brain, liver, kidney, and spleen from eight patients dying with SLE were assayed. Interferon was detected in suspensions of brain from three patients from whom SLE virus was isolated and who died early in their illness. Interferon was not homogeneously distributed through the brain and there was no clear topographical relation between the presence of SLE virus and interferon. Two patients had SLE virus isolated from brain but had no detectable interferon. These two patients had severe underlying illnesses: leukemia and uremia. The interferon obtained from these tissue suspensions was nonsedimentable at 105,000 *g*, virologically sterile, active against two unrelated viruses (Sindbis virus and vesicular stomatitis virus), but, as is characteristic of interferon, inactive against one of the same viruses (Sindbis virus) when heterologous cells (bat embryo fibroblasts) were used. These data suggest that interferon in SLE is operative locally early

in the course of illness and probably acts to limit the extent of infection. The absence of interferon in serum, urine, liver, kidney, or spleen supports the concept that after the onset of illness, the SLE virus is primarily limited to the central nervous system. (Supported by USPHS grants TO1-AI-00142, TO1-AI-00337, and TO1-AI-00030.)

174. Alteration of the Bactericidal Activity of Polymorphonuclear Neutrophils by an NADH Oxidase Inhibitor. GERALD L. MANDELL,* WALTER RUBIN,* and EDWARD W. HOOK, New York, N. Y.

Polymorphonuclear neutrophils (PMN) from patients with chronic granulomatous disease of childhood have impaired bactericidal activity and are deficient in NADH oxidase. Since hydrocortisone has been shown to inhibit NADH oxidation, experiments were undertaken to determine the effect of hydrocortisone on several parameters of human PMN function. The phagocytic and bactericidal capacity of PMN with or without hydrocortisone (1 mg/ml) was determined by quantitation of cell-free, cell-associated, and total bacteria. Phagocytosis of *Staphylococcus aureus* and *Proteus mirabilis* was unimpaired by the presence of hydrocortisone in the media. In contrast, killing of bacteria was markedly impaired by hydrocortisone. After 30 min of incubation there were 130 times as many bacteria surviving in hydrocortisone-treated PMN as in simultaneously run controls without hydrocortisone. The defect of intracellular killing in the presence of hydrocortisone was not related to impaired degranulation. Quantitative kinetic studies of degranulation revealed no difference in the release of granule-associated acid phosphatase in hydrocortisone-treated and control PMN after phagocytosis. Electron microscope examination of PMN also indicated that the presence of hydrocortisone had no effect on the extent of degranulation after phagocytosis. These observations were confirmed by studies using histochemical techniques to detect lysosomal enzymes. After phagocytosis, hydrocortisone-treated PMN demonstrated 50% less NADH oxidase activity and oxygen consumption than postphagocytic control PMN. In addition, nitro blue tetrazolium dye reduction, which is dependent on normal NADH oxidase activity, was diminished in hydrocortisone-treated PMN. Thus impairment of NADH oxidase activity in normal human PMN by hydrocortisone results in reduced intracellular killing of bacteria, diminished postphagocytic oxygen consumption, and decreased ability to reduce nitro blue tetrazolium. These abnormalities are similar to those seen in the PMN of patients with chronic granulomatous disease of childhood. (Research supported by NIH grants TO1-AI-255 and AI-05940, and contract U-1107 with the Health Research Council of the City of New York.)

175. Ferrous Iron Oxidation by Intestinal Mucosal Homogenates and Its Possible Relation to Iron Absorption. JAMES MANIS,* New York, N. Y. (introduced by David Schachter).

Previous studies of the active transport mechanism for iron with everted gut sacs in vitro and duodenal loops in vivo demonstrated the formation of a ferric iron pool from ferrous iron in the intestinal mucosa of the rat. In the present studies an oxidation of Fe^{++} to Fe^{+++} by sucrose homogenates of intestinal mucosa is seen to occur and suggests enzymatic action. The oxidative activity is heat labile and nondialyzable, and the reaction follows Michaelis-Menten kinetics. The enzyme activity is stable overnight at 4°C and for 6 months at —10°C. Homogenates incubated initially in the absence of Fe^{++} do not produce substances that subsequently oxidize Fe^{++} under the conditions of the enzymatic assay. Uric acid (7×10^{-4} M) in the experimental system does not oxidize Fe^{++} sufficiently to explain the oxidation observed with homogenates. Approximately 35% of the ferric iron produced by the enzymatic reaction can be reduced to ferrous iron upon incubation with α,α-dipyridyl at room temperature for 60 min. Although the enzyme is present throughout the entire small intestine, the duodenum, the site of maximal iron transport, contains more activity per mg of protein than does the distal ileum. Duodenal homogenates from rats fed 4 mg of $FeSO_4$ 3 hr before sacrifice show inhibition of the enzyme as compared with control homogenates. In contrast, ileal homogenates from similarly iron-loaded rats showed no difference from the activity of controls. The iron content of homogenates from iron-loaded rats was not detectable in the final reaction mixture and does not appear to account for the differences in ferrous iron oxidation described.

176. Soluble Human Transplantation Alloantigens. DEAN L. MANN,* G. NICHOLAS ROGENTINE,* JOHN L. FAHEY, AND STANLEY NATHENSON,* Bethesda, Md., and New York, N. Y.

A series of alloantigenic determinants, designated HL-A, are present on membranes of human tissues and function as transplantation antigens. Isolation of the HL-A alloantigens from their membrane-associated state was undertaken to provide materials which could be purified and characterized. HL-A alloantigenic materials were solubilized by papain treatment of spleen and lymphoid cell membranes and were purified by chromatographic and acrylamide gel electrophoretic techniques. The purified materials bearing HL-A alloantigenic determinants were found to be glycoproteins having a molecular weight of 50,000 to 60,000. Materials with differing HL-A determinants were found to have similar amino acid composition. This evidence indicates that differences in HL-A alloantigenic determinants are represented by small changes in amino acid composition or that carbohydrate contributes significantly to HL-A antigenicity. The HL-A alloantigenic determinants are thought to be controlled by a single complex genetic locus consisting of several subdivisions, each controlling a series of allelic HL-A determinants. Soluble HL-A alloantigenic materials were separated into different molecular fractions. One fraction had the "LA" series and another the "4" series of HL-A alloantigens. The molecular separation was particularly significant because of the evidence that the "LA" and "4" series of alloantigens are controlled by different genetic subdivisions. These data together with the results of the compositional studies provide evidence which suggests models for the molecular representation of HL-A alloantigenic determinants on cellular membranes.

177. Chloramphenicol and Protein Synthesis in Bone Marrow Mitochondria. ORLANDO J. MARTELO,* DAVID R. MANYAN,* UNA S. SMITH,* GRACE K. ARIMURA,* AND ADEL A. YUNIS, Miami, Fla.

The mechanism of bone marrow depression from chloramphenicol (CAP) remains unknown. Although CAP is a specific inhibitor of ribosomal protein synthesis in sensitive bacteria, it has little or no effect on ribosomal protein synthesis in the mammalian cell. Recent studies have clearly established that mitochondria isolated from several mammalian tissues are capable of independent protein synthesis and that this process is sensitive to small concentrations of CAP. Thus interference with mitochondrial function may be a major underlying mechanism in CAP toxicity. To test this hypothesis, the effect of CAP was studied in mitochondria isolated from rabbit bone marrow. Mitochondria were incubated at 30°C in the presence of succinate, inorganic phosphate, AMP, NAD, 19 ^{12}C-labeled amino acids, and ^{14}C-leucine or ^{14}C-valine. The incorporation of ^{14}C-labeled amino acid into mitochondrial protein was linear for 2 hr. Ribonuclease was without effect. CAP in concentrations as low as 16 μg/ml resulted in rapid and almost complete inhibition of protein synthesis. Penicillin and streptomycin were not inhibitory. CAP concentrations at which protein synthesis was completely inhibited had no effect on mitochondrial respiration, suggesting that the drug exerts its effect directly on the protein synthetic pathway of mitochondria. Mitochondrial protein synthesis appears to be the only metabolic pathway in mammalian cells which is sensitive to therapeutic concentrations of CAP. Interference with mitochondrial function may represent the biochemical basis for reversible bone marrow depression from the drug, since this lesion is dose dependent. Preliminary electron microscope observations on bone marrow from patients receiving CAP appear to support this hypothesis.

178. Assessment of Cardiac Contractility in Man: Comparison among Different Patients of the Maximum Intrinsic Velocity of the Myocardium and the Relation between the Rate of Pressure Rise and Intraventricular Pressure during Isovolumic Left Ventricular Contraction. DEAN T. MASON, JAMES F. SPANN, JR.,* AND ROBERT ZELIS,* Davis, Calif.

Although determination of the maximum intrinsic velocity of the myocardium (V_{max}), utilizing rate of intraventricular pressure rise (dp/dt) and isovolumic systolic pressure (IP), and calculation of the relation between dp/dt and common developed IP have provided two useful approaches to the evaluation of changes in contractility in individual patients, no practical means has been available for comparing basal contractility among different patients. Since velocity of shortening of contractile elements (V_{CE}) and rate of elongation of series elastic are equal in the intact heart during isovolumic contraction, and isovolumic tension is related by a constant to IP, V_{CE} was calculated from instantaneous dp/dt and its corresponding IP as the ratio $dp/dt:(k \cdot IP + c)$, where k (40) and c (80) are constants. Construction of a force-velocity curve relating V_{CE} to IP until aortic valve opening, and extrapolation to zero load, allowed estimation of V_{max}, a measure of contractility free of changes in loading in an individual patient but not independent of large differences in heart size among patients. It was considered that the relation of V_{max} to left ventricular end-diastolic volume (LVEDV) would allow correction for these variations of circumferential fiber lengths. High-fidelity left ventricular pressures, dp/dt, and LVEDV were determined in 18 patients; the ratio of V_{max} to LVEDV index averaged 0.023 $\pm$0.003 (SEM) in normal subjects, 0.010 $\pm$0.002 in compensated cardiac hypertrophy, and 0.006 $\pm$0.002 in heart failure. Separation of the patients into three distinct groups also was obtained when the ratio of instantaneous dp/dt to IP common in each patient (50 mm Hg) was related to LVEDV index to afford an assessment of contractility uninfluenced by differences in preload and afterload; this relation averaged 0.62 $\pm$0.08 in normal subjects, 0.28 $\pm$0.05 in cardiac hypertrophy, and 0.19 $\pm$0.04 in heart failure. Thus, determinations of V_{max} and dp/dt:common IP related to LVEDV provide simple and valid means of assessing contractility in different patients.

179. Manifestations of Overt Secondary Hyperparathyroidism in Patients with Advanced Uremia. SHAUL G. MASSRY,* JACK W. COBURN,* MORDECAI M. POPOVTZER,* JAMES H. SHINABERGER,* MORTON H. MAXWELL, AND CHARLES R. KLEEMAN, Los Angeles, Calif.

The usual biochemical features of primary hyperparathyroidism (hypercalcemia and hypophosphatemia) are uncommon in uremic patients with overt secondary hyperparathyroidism. To evaluate the manifestations of the latter, 15 uremic patients with radiological evidence of hyperparathyroidism (group 1) were studied and compared with 27 patients who had comparable creatinine clearance (<10 ml/min) but no X-ray signs of bone disease (group 2). In addition to clinical and radiological evaluation, the parameters studied included serum calcium, phosphorus, and alkaline phosphatase, effect of phosphate restriction, evidence for extraskeletal calcification, and measurements of calcium content of skin. Results show that in uremic patients with overt secondary hyperparathyroidism, (*a*) serum calcium is normal (10.1 $\pm$0.2 mg/100 ml; mean $\pm$SE) and is significantly higher than that of group 2 (8.3 $\pm$0.3); hypercalcemia is uncommon; (*b*) serum phosphorus is elevated (10.0 $\pm$3.0) and is higher than that of group 2 (6.1 $\pm$2.5 mg/100 ml); (*c*) calcium-phosphorus product is high (75–155) in contrast to lower values (20–75) in group 2; (*d*) phosphate depletion is not infrequently associated with hypercalcemia; (*e*) alkaline phosphatase is usually high; (*f*) vascular and/or soft-tissue calcifications are very common and are seen in 14 of 15 patients; none from group 2 had this abnormality; (*g*) calcium content of skin is high, 547 $\pm$49 mg/kg dry weight as compared with 322 $\pm$18 and 322 $\pm$13 mg/kg dry weight observed in group 2 and in normals respectively; and (*h*) intractable pruritus is very common. Subtotal parathyroidectomy was performed in 11 of 15 patients without mortality or serious morbidity, and was followed by regression of soft-tissue calcification, healing of osteitis fibrosa, reduction in skin calcium, and disappearance of pruritus. (Research supported by contract with the NIH.)

180. Regulation of Erythropoiesis in the Rat Fetus. YEHUDA MATOTH* AND RINA ZAIZOV,* Petah-Tikva, Israel (introduced by Lawrence S. Lilienfield).

Rats were exposed during the 3rd week of pregnancy to stimuli known to affect erythropoiesis in the adult animal. The effect of these stimuli on the incorporation of ^{59}Fe into red cells of the fetuses was studied. One group of mothers was made polycythemic by hypertransfusion, another group anemic by induced bleeding. A third group was exposed to intermittent hypoxia in a low-pressure chamber at 0.5 atm for 1 wk. A fifth group received 75 units of a human urinary erythropoietin preparation. ^{59}Fe was injected on day 18. On day 20 uptake of ^{59}Fe by red cells of mothers and fetuses was determined. In the fetus, red cell ^{59}Fe incorporation was expressed as a percentage of the amount of isotope available to the fetus, determined by whole-body counting. As expected, ^{59}Fe incorporation was doubled or trebled in anemic and hypoxic mothers and in mothers that had received erythropoietin. Conversely, incorporation was halved in the polycythemic and hyperbaric groups. ^{59}Fe incorporation in the fetuses remained constant and not significantly different from that in the control group. The demonstrated failure of maternal polycythemia to suppress erythropoiesis in the fetus confirms previous work, which showed the regulation of erythropoiesis in the fetus to be independent of the mother. The lack of response to maternal anemia or injection of erythropoietin into the mother may be due either to failure of erythropoietin to cross the placenta or to fetal unresponsiveness. In the hyperbaric and hypoxic groups, however, O_2 tension in the fetus is likely to have increased and decreased respectively. Failure of the fetus to respond to these stimuli suggests that, at least in the rat, mechanisms other than hypoxia control erythropoiesis in the fetal period.

181. A Single Chromosome Region in the Mouse Controlling the Major Histocompatibility Antigens and the Ability to Produce Antibody to Synthetic Polypeptides. HUGH O. MCDEVITT,* DONALD C. SHREFFLER,* AND JACK H. STIMPFLING,* Stanford, Calif., Ann Arbor, Mich., and Great Falls, Mont. (introduced by Halsted R. Holman).

The ability of mice to respond to multichain, synthetic polypeptides containing tyrosine and glutamic acid [(T,G)-A—L] or histidine and glutamic acid [(H,G)-A—L] is a genetically controlled trait, designated Ir-1, which is linked to the major histocompatibility (H-2) locus in the IXth mouse linkage group. H-2^b animals respond well to (T,G)-A—L and poorly to (H,G)-A—L. H-2^a and H-2^k animals respond poorly to (T,G)-A—L and well to (H,G)-A—L. H-2^d animals give a low, variable response to these antigens. Nine H-2 recombinant strains (10 animals each) were tested for their ability to respond to these antigens. Five recombinant strains were nearly reciprocal crossovers between H-2^a and H-2^b alleles. Four strains were crossovers between H-2^d and H-2^k alleles, lying just to the right or to the left of the Ss (serum substance) locus which lies in the middle of the large and complex H-2 locus, and determines the level of a serum β-globulin. In eight of the nine crossover strains, the Ir-1 phenotype (ability to respond well to either (T,G)-A—L or (H,G)-A—L) was that of the donor of the right-hand part of the recombinant H-2 allele. However, in one of the nine crossover strains, the Ir-1 phenotype was that of the donor of the left-hand part of the recombinant H-2 allele. Barring a double crossover, these results indicate precise localization of Ir-1 in the middle of the H-2 locus, lying to the right of Ss and between the Ss gene and the genes coding for H-2 histocompatibility specificities 11 and 31. Thus a single chromosome region includes genes affecting the formation of a cell surface antigen, specific antibodies, and another serum protein; this association may have implications for antigenic recognition and initiation of antibody formation. (Research supported by grants from the NIH and the Arthritis Foundation.)

182. Patterns of Release and Identification of Renal Antihypertensive Substances Produced by Renal Ischemia. J. C. MCGIFF,* N. A. TERRAGNO,* A. J. LONIGRO,* AND K. K. F. NG,* St. Louis, Mo. (introduced by René Wégria**).

The kidney contralateral to an ischemic kidney is known to inhibit the development of hypertension. The superfused blood-bathed organ technique of Vane was used to detect substances in renal venous blood and aortic blood released from kidneys in response to renal ischemia. Three organ banks, each containing three assay organs (rat stomach and colon, chick rectum) arranged in series and selected for specificity and sensitivity, were superfused in parallel by blood obtained from renal veins and aorta. Induction of unilateral renal ischemia in 11 chloralose-anesthetized dogs reduced renal blood flows from mean values of 266 and 259 ml/min to 132 (ischemic) and 216 (contralateral) ml/min, respectively. Concomitantly, mean aortic blood pressure increased from a control of 107 to 117 mm Hg. Substances released from kidneys were tentatively identified and quantitated by matching responses of organ banks to infusions of vasoactive hormones into the extracorporeal circuit with responses produced by ischemia. Simultaneous observations of activities of renal venous and aortic organ banks permitted estimation of removal or activation of substances by lungs. Several patterns of response to renal ischemia occurred: (1) immediate release of prostaglandin-like substance(s) (PLS) from ischemic kidneys upon renal arterial constriction; (2) continuous release of angiotensin-like substance(s) (ALS) and PLS from ischemic kidneys, and of PLS from contralateral kidneys. On release of constriction, a pulse of PLS appeared in the ischemic renal effluent. PGEs and, less frequently, $PGF_{2\alpha}$ were tentatively identified only in renal venous blood. The release of PLS from contralateral kidneys was probably mediated by angiotensin I derived from ischemic kidneys and subsequent formation of angiotensin II in lungs. The effects of renal ischemia on release of PLS were reproduced by renal arterial infusion of angiotensin II. The only failure to identify PLS during renal ischemia occurred in a hypertensive dog (165 mm Hg, mean blood pressure). These results suggest interactions of angiotensins and prostaglandins which determine the development of renal hypertension.