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Abstracts

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179. Regulation of Biosynthesis of Intestinal Mucins: Role of Sialic Acid and Fucose. YOUNG S. KIM,* JOSE PERDOMO,* AGUSTIN M. BELLA, JR.,* AND MARVIN H. SLEISENGER,** San Francisco, Calif.

A multiglycosyltransferase system catalyzing the transfer of monosaccharide components in the biosynthesis of intestinal mucin is present in the rat small intestinal mucosa but little is known about its regulatory mechanisms. Sialic acid and fucose are components of the oligosaccharide side chains of intestinal mucin. We have investigated their effect on the ability of *N*-acetylgalactosaminyltransferase to catalyze the transfer of *N*-acetylgalactosamine to the terminal galactose. To determine the role of sialic acid, purified rat small intestinal mucin with and without sialidase treatment containing 0.8 and 12.0% sialic acid, respectively, were compared with regard to their ability to accept labeled *N*-acetylgalactosamine in the presence of small intestinal *N*-acetylgalactosaminyltransferase. Significantly greater (8 ×) incorporation of *N*-acetylgalactosamine was observed with desialized mucin than with untreated mucin. The possible role of fucose in the regulation of mucin biosynthesis was studied by comparing four galactose-containing oligosaccharides with and without fucose, isolated from human milk (2′-fucosyllactose, lacto-*N*-fucopentaose I, lacto-*N*-fucopentaose II, and lacto-*N*-tetraose) for their ability to accept labeled *N*-acetylgalactosamine in the presence of small intestinal *N*-acetylgalactosaminyltransferase. Only those oligosaccharide acceptors with fucose attached to the terminal galactose in α(1-2) linkage incorporated labeled *N*-acetylgalactosamine significantly while those without fucose did not, despite the similarity of the composition and structure. These studies indicate that both sialic acid and fucose may have critical roles in the regulation of the mucin biosynthesis; the addition of sialic acid to the growing oligosaccharide side chains of mucin limits the further addition of *N*-acetylgalactosamine, while a prior addition of fucose to galactose of the oligosaccharide side chains is essential for the addition of *N*-acetylgalactosamine.

180. Dissociation of Renal Gluconeogenesis from Ammonia Production. SAULO KLAHR, St. Louis, Mo.

It is widely held that ammonia formation by the kidney is tightly coupled to gluconeogenesis. The proposed nature of the coupling is as follows: acidosis → increased gluconeogenesis → decreased tissue levels of glutamate → increased glutaminase activity → increased ammonia production from glutamine. The present data suggest that this coupling is neither obligatory nor universal. Indeed either process can occur independently of the other. Studies were performed using rabbit and guinea pig kidney cortical slices. With glutamine as substrate ammonia was produced in vitro but glucose production by the slices was negligible. With glutamate as substrate ammonia formation was minimal, but glucose production was striking. These data contrast with observations using rat kidney cortical slices, in which glucose production from either glutamine or glutamate is identical. In an attempt to dissociate gluconeogenesis from ammonia production in rat kidney slices, quinolinic acid (a metabolic degradation product of tryptophan) and hydrazine, two known inhibitors of hepatic gluconeogenesis in vivo, were used. Both compounds were found to inhibit renal gluconeogenesis in vitro. At pH 7.4 quinolinic acid (1 mM final concentration) decreased glucose production from glutamine by over 50%. The simultaneous rate of ammonia formation from glutamine was unchanged (83.7 μmoles/g wet weight per hr for the control vs. 79.9 μmoles in the presence of quinolinic acid). Hydrazine had similar effects, inhibiting glucose formation from glutamine by 55% while producing no effect on ammoniagenesis. Thus in rat kidney, ammonia production in vitro can be shown to proceed at the same rate even when the rate of gluconeogenesis is decreased by more than 50%. The over-all observations indicate that gluconeogenesis and ammonia production are not tightly coupled in either rabbit or guinea pig kidney cortex. Furthermore, these two processes can be clearly dissociated in rat kidney cortex.

181. Experimental Myeloid Metaplasia. LYNELL KLASSEN,* ELIZABETH ALLEN,* RICHARD RILEY,* AND CLIFFORD GURNEY,** Kansas City, Kans.

Mechanisms producing splenomegaly and extramedullary hematopoiesis in patients with myelofibrosis are unknown. We therefore created a model for study of mechanisms regulating primitive hematopoietic cell replication in extramedullary sites by destroying the marrow of mice with ^{89}Sr, a beta-emitting, bone-seeking isotope. 6μ Ci/g body weight produces marrow aplasia, but splenic red pulp hyperplasia is sufficient to sustain hematopoiesis. Spleen mass increases for 3 wk, then plateaus as animals regain hematopoietic balance. The concentration of megakareocytes in these spleens is particularly impressive. Although microscopic evidence of extramedullary hematopoiesis is observed in lymph nodes, its magnitude is negligible, since more than 90% of these mice will die of pancytopenia after splenectomy. The hematocrits and 72 hr radioiron uptake into newly formed erythrocytes are the same in strontium-treated animals and control mice. However the responsiveness of the erythroblast progenitor pool, measured by hypertransfusing strontium-treated and control animals and determining erythrocyte radioiron incorporation in response to a pulse of exogenous erythropoietin, is quite different. Animals made plethoric 21 days after strontium respond to 1 U of erythropoietin by 4.6% radioiron incorporation, whereas control plethoric mice incorporate 13.3%. We therefore infer one regulatory mechanism of extramedullary hematopoiesis may be the sustained action upon the spleen of increased erythropoietin blood levels. The precision of the mechanisms controlling extramedullary hematopoiesis is demonstrated by the pattern of regeneration after partial splenectomy in the marrowprival animal. 2 days after $\frac{2}{3}$ splenectomy in strontium-treated mice, hypertrophy of the remnant begins. Preoperative weight is reached in only 8 days, after which further growth ceases. The strontium-treated mouse is an excellent model in which to investigate mechanisms governing the production of extramedullary hematopoiesis. (Research supported by NIH Grant AM 14377-02.)

182. Characterization of Myocardium Revascularized by Aorta-to-Coronary Artery Bypass Grafts. FRANCIS J. KLOCKE, GEORGE SCHIMERT,* IVAN L. BUNNELL,* DAVID G. GREENE,* STEPHEN M. WITTENBERG,* AND THOMAS LAJOS,* Buffalo, N. Y.

Blood flow through aorta-to-coronary artery bypass grafts has been measured selectively in 13 patients at or within 6 wk

after operation, using inert gas techniques. Desaturation curves were obtained from coronary sinus blood samples following 7–15 min infusion of dissolved H_2 directly into the graft. Samples were analyzed chromatographically and curves resolved to $< 1\%$ of initial H_2 concentrations. Over-all flow per unit volume (F/V) averaged 70 ±21 (SD) ml/min per 100 g. Semilogarithmic plots showed over-all F/V to be distributed heterogeneously and the perfused segment was approximated by a two-compartment model. The rapidly perfused compartment represented 64 ±7% of the total segment; F/V ranged from 66 to 173 and averaged 91 ml/min per 100 g. F/V for the slowly perfused compartment ranged from 6 to 46 and averaged 15 ml/min per 100 g. In six patients studied at operation, H_2 measurements of over-all F/V were combined with electromagnetic measurements of total flow to determine revascularized tissue mass. Electromagnetic flows ranged from 40 to 170 and averaged 92 ml/min. Tissue mass varied from 55 to 155 and averaged 105 g. We conclude the following. (*a*) Bypass grafts provide nutritive flow to significant amounts of myocardium at and shortly after operation. (*b*) Nutritive flow is not evenly distributed throughout a revascularized segment. The majority of the segment has a F/V within the accepted range of normal but there remain areas in which F/V is significantly reduced. (*c*) The combination of inert gas and electromagnetic techniques allows a revascularized area to be characterized in terms of total flow, over-all and regional F/V, and tissue mass. (Research supported by NIH.)

183. Effect of Hormonal Activity on Human Adipose Tissue Cellularity. JEROME KNITTLE,* LEONARD SUSSMAN,* FREDDA GINSBERG-FELLNER,* AND MELVIN GERTNER,* New York (introduced by Jules Hirsch).

Enlargement of adipose depots in obese adults is characterized by hypercellularity of obscure origin. Indeed, to date only infantile nutritional experiences have effectively modified adipose cell number. The present report demonstrates that hormonal factors also play a role in human adipose tissue development. Adipose cell number and size were determined before and 6 months after therapy in two patients, ages 6 and 8, with isolated growth hormone deficiency, one age 4 with associated thyroid-stimulating hormone (TSH) deficiency and one age 18 with associated gonadotropin deficiency. All but the teenage patient had accelerated growth rates (1.5–2.5 inches). Growth was associated with a decrease in per cent body fat and adipose cell size (0.31, 0.37, 0.39) → (0.20, 0.27, 0.28) μg lipid per cell and with an increase in total body potassium and adipose cell number (7, 5, and 15×10^9) → (9, 8, and 20×10^9) cells. Initial values for the children correlated with their chronological age. The teenage patient displayed values lower than age-matched normals but compatible with her bone age of 8 (12×10^9 and 0.24). In vitro studies of lipolysis revealed no stimulatory effect of added human growth hormone (HGH) in either study period and therefore did not reflect the in vivo decrease in cell lipid content. However, after therapy, one subject with isolated growth hormone deficiency displayed increased in vitro stimulation of glucose-1-^{14}C oxidation when either insulin or HGH was added. Furthermore, oral glucose tolerance was abnormal before therapy and normal after. These results are similar to obese subjects after weight reduction. Thus HGH can effect changes in adipose cell number, size, and metabolism although it does not appear to be necessary for early cellular development. (Research supported by grants from NIH and HRC.)

184. Studies on the Mechanism of Rhabdomyolysis in Potassium Depletion. JAMES P. KNOCHEL* AND EDWARD M. SCHLEIN,* Dallas, Tex. (introduced by Floyd Rector, Jr.).

Rhabdomyolysis may occur in patients with K depletion and is common in young men with environmental heat injury in the course of intensive physical training. Severe K depletion is known to occur during such training. Since release of K from skeletal muscle is thought to mediate increased muscle blood flow (MBF) which normally accompanies exercise, it seemed possible that rhabdomyolysis occurring under these circumstances might represent ischemic necrosis secondary to reduced K release from muscle during exercise. The following study was designed to test this hypothesis by determining the relationship between K release and MBF in normal and K-depleted dogs. MBF and K release were determined in normal and K-depleted dogs during electrically stimulated exercise of the isolated gracilis muscle. In normal dogs (n = 7) whose muscle K (mEq/100 g FFDS) was 42.2, resting MBF (ml/100 g per min) was 6.1 ±0.3 (SEM) and K release (mEq/100 g per min) 0.4. During exercise MBF rose to 23.2 ±2.1 and K release to 35.0 ±2.3. In dogs depleted of K by dietary deprivation and 11-deoxycorticosterone acetate (DOCA) (n = 5) whose muscle K was 22.2, resting MBF was 6.4 ±0.5 and K release 0.3 ±0.1. However, during exercise maximum MBF was only 8.7 ±0.6 and K release only 2.8 ±0.5. In the depleted dogs frank necrosis was present in the exercised gracilis muscle while only minor changes were observed in the contralateral nonexercised muscle. These changes were not observed in the normal muscle after exercise. In summary (*a*) in normal dogs, MBF and K release both rose sharply with exercise, (*b*) after K depletion, both responses were markedly blunted, and (*c*) exercise of K-deficient muscle induced frank rhabdomyolysis. These findings support our hypothesis that ischemia may be the mechanism of rhabdomyolysis with exercise in K depletion. (Supported by designated research funds, Veterans Administration Hospital, Dallas, Tex.)

185. Idiopathic Orthostatic Hypotension. HERMES A. KONTOS, DAVID W. RICHARDSON, AND JOHN E. NORVELL,* Richmond, Va.

Circulatory studies were carried out in 16 patients to identify the nature of the defect in idiopathic orthostatic hypotension. Forearm blood flow did not change significantly in the patients in response to intra-arterial tyramine, while normal subjects displayed the expected vasoconstriction. The patients showed no reflex venoconstriction (occluded limb technique) in response to application of ice to the forehead or deep breathing. In contrast to normal subjects who showed increase in forearm vascular resistance in response to the Valsalva maneuver and in response to application of ice to the forehead, the patients showed decrease in resistance. The decrease in forearm blood flow in response to intra-arterial

norepinephrine was greater in the patients than in the normal subjects indicating supersensitivity to this catecholamine. Transient and steady-state responses of forearm blood flow during tilting were consistent with autoregulation. In three patients the fluorescent technique of Falck and Hillarp showed absence of adrenergic nerve endings in blood vessels of skeletal muscle, while these were present in normal subjects. Neither patients nor normal subjects had acetylcholinesterase-positive nerves in association with blood vessels of skeletal muscle. It is concluded that the abnormal circulatory responses in patients with idiopathic orthostatic hypotension are due to deficiency or absence of norepinephrine in the adrenergic terminals. Abnormal vasodilator responses to Valsalva maneuver or to ice may result from unmasking of autoregulatory manifestations because normal sympathetic vasoconstriction is absent.

186. Complete Structure of the Carbohydrate Moiety of γG Immunoglobulins. ROSALIND KORNFELD,* JAMES KELLER,* JACQUES BAENZIGER,* AND STUART KORNFELD, St. Louis, Mo.

γG immunoglobulins contain on their Fc fragment a carbohydrate moiety. While the complete primary structure of the protein portion of some γG myeloma proteins is known, the structure and function of the carbohydrate moiety remains obscure. We therefore prepared glycopeptides from seven γG myeloma proteins and the γG immunoglobulins of one normal individual. The composition and complete structure of the oligosaccharide portion of the gylcopeptides was determined as follows. Sequential enzymatic degradation of the oligosaccharide chains with glycosidases established the sequences of the sugars. Serial periodate oxidation and methylation of both intact and partially degraded glycopeptides followed by identification of the methylated sugars by gas chromatography and mass spectrometry established the linkages between sugars. The carbohydrate composition of the various glycopeptides (expressed as sugar residues per mole) ranged from: sialic acid 0–2, fucose 0.6–1.2, galactose 0.3–2.3, *N*-acetylglucosamine 3.9–6.0, and mannose 3.0. The variability in the number of sialic acid, fucose, and galactose residues is due to oligosaccharides with incomplete outer chains, whereas the variability in the number of *N*-acetylglucosamine residues represents differences in the sequence of the sugars in the "core" of the molecules. All the glycopeptides contain a branched oligosaccharide with two nonreducing termini with the structure Gal $\xrightarrow{\beta 1 \to 6}$ *N*-acetylglucosamine $\xrightarrow{\beta 1 \to 2}$ Man. Sialic acid, when present, is linked $\alpha 2 \to 6$ to galactose. Both branches are connected to another mannose residue in the core. The core also contains either two or three *N*-acetylglucosamine residues. The sequence of sugars in the core varied from one glycopeptide to another, being either Man → GlcNAc → GlcNAc → Peptide, Man → [GlcNAc →] GlcNAc → Peptide, or Man → GlcNAc → [GlcNAc →] GlcNAc → Peptide. These studies established the complete structure of the carbohydrate moieties of several γG immunoglobulins. They demonstrate that homogeneous γG immunoglobulins of man may have different sequences of sugars in the oligosaccharide chains. (Research supported by NIH and ACS grants.)

187. Antibody to Specific and Cross-Reacting Antigens in Gram-Negative Bacteremia. B. E. KREGER,* S. T. DONTA,* AND WILLIAM R. MCCABE, Boston, Mass.

The role of antibody in protection against human infections caused by Gram-negative bacilli has not been clearly defined. Most enterobacteria possess antigens shared by other Gram-negative bacilli in addition to O-specific antigens. The importance and response of antibody to homologous O-specific antigens and two cross-reacting antigens (Re determinant and common enterobacterial antigen) were evaluated in 150 episodes of Gram-negative bacteremia and in experimental infections. In the absence of a method for relating antibody titers to frequency of development of bacteremia, antibody titers to each of these three antigens in acute serum specimens were correlated with the severity of bacteremia utilizing shock and death as indices of more severe bacteremia. Significant titers of antibody to O-specific antigens of the infecting organism were present in acute serum specimens from most patients but the titer of O antibody bore no relationship to the severity of the bacteremia. Similarly, no relationship was observed between the titer of antibody to common enterobacterial antigen and the frequency of shock or death. In contrast, the frequency of shock and death were significantly less ($X^2 = 7.6$; $P < 0.01$) in patients with antibody titers of ≥ 80 to the rough (Re) determinant than in patients with lower titers. Most (70%) surviving patients demonstrated an increase in antibody titers to both O-specific and Re antigens, but not common antigen, in subsequent serum specimens. Similar protection was observed in experimental animals immunized with Re antigen and challenged with heterologous Gram-negative bacilli. These results suggest that antibody to shared cross-reacting antigens may afford significant protection against lethal Gram-negative bacteremia and raises the possibility of immunization against Gram-negative bacteremia.

188. Growth Hormone and Cortisol Responsiveness in Cushing's Syndrome: Relation to a Possible Central Nervous System Etiology. DOROTHY T. KRIEGER AND SEYMOUR M. GLICK,* New York.

Cortisol and growth horomone responsiveness to insulin hypoglycemia, pitressin, and Piromen administration were determined in six patients with Cushing's syndrome (three with active disease and three in remission after treatment). In addition, the periodicity of the plasma levels of these hormones was studied concomittantly with electroencephalographic (EEG) recording during sleep. The etiology of the Cushing's syndrome in all but one of these patients was nontumorous adrenocortical hyperfunction. (The exception was an untreated active case associated with a suprasellar tumor.) While the plasma cortisol response to insulin hypoglycemia and Piromen was variable irrespective of the activity of the disease, the plasma cortisol response to pitressin administration was noteworthy in that a normal response occurred in all five of the patients whose disease was associated with adrenal hyperplasia. This suggests that this test may be of aid in differentiating this etiology from other causes of Cushing's syndrome. A lack of normal growth hormone responsiveness to insulin hypoglycemia, pitressin, and Piromen administration was noted in all patients, *irrespective of the activity*

of the disease process. None of the patients displayed the normal sleep-associated elevation of plasma growth hormone levels. EEG sleep recording showed a marked diminution in the per cent of sleep time normally spent in stages three and four and in REM periods. These abnormalities in growth hormone secretion in response to stresses presumed to act via central nervous system releasing mechanisms, and the lack of presumably neurally determined growth hormone release during sleep lend additional support to the concept of a neural etiology of nontumorous adrenocortical hyperfunction. (Research supported by Grants NB-02893, FR-71, and AM-09219, NIH.)

189. Glycosphingolipids of Very Low Density Lipoproteins in Hyperlipoproteinemia. TERRENCE T. KUSKE,* New York (introduced by George L. Curran**).

Data obtained on the plasma of two normal subjects and four hyperlipoproteinemic patients (three type IV, and one type III) demonstrated that glycosphingolipids are present in human very low density lipoproteins (VLDL), and that elevated VLDL levels may be associated with increased plasma glycosphingolipid levels. Total lipids were extracted from whole plasma and from VLDL prepared by ultracentrifugation. Glycosphingolipids were isolated from these extracts by silicic acid column chromatography and thin-layer chromatography, and then quantitated by acid hydrolysis and anthrone determination against known standards. Increased quantities of each of the four glycosphingolipids were found in the pretreatment VLDL of all the hyperlipoproteinemic patients as compared to the small quantities of these glycosphingolipids present in the VLDL of the normal controls. In two of the patients, elevations of the plasma glycosphingolipid levels above control values were observed. These increases were due entirely to VLDL glycosphingolipids, which accounted for more than 60% of the plasma levels. Glucosyl ceramide levels in these patients approximated those reported for Gaucher's disease. After treatment with clofibrate or insulin, a 10–72% decrease in VLDL total lipids, and a 4–61% decrease in VLDL glucosyl ceramide, lactosyl ceramide, and trihexosyl ceramide levels were seen in all patients. VLDL globoside content decreased in two of the three patients in whom it was analyzed. (Research supported by a grant from the Health Research Council of the City of New York.)

190. Increased Platelet Aggregation in Diabetes Mellitus. HAU C. KWAAN, JOHN A. COLWELL,* SIDNEY CRUZ,* AND N. SUWANWELA,* Chicago, Ill.

The known relationship between serum lipids and the hemostatic mechanism focused our attention to the hypercoagulable state in diabetes mellitus. We previously reported spontaneous platelet aggregation in a diabetic with hyperlipemia and ketoacidosis. His plasma increased the aggregation of normal platelets. This study included 69 other diabetic subjects. The degree of platelet aggregation induced by 1.05×10^{-6} mole of adenosine diphosphate (ADP) per ml was studied in mixtures of 1:8 v/v of diabetic plasma and normal platelet-rich plasma. A significant increase (mean 33.6%, SEM 2.2%) of aggregation over controls 4 min after addition of ADP was observed in 46 patients, while equivocal effect (mean increase 0.7%, SEM 1.1%) was seen in 23 diabetics and in 5 hyperlipemic nondiabetic nephrotic patients (mean increase 6%, SEM 1.4%). There was no difference between the two groups of diabetics regarding age, height, weight, insulin therapy, coronary artery disease, retinopathy, neuropathy, or mean plasma concentrations of glucose, triglycerides, cholesterol, free fatty acids, urea, or creatinine. They differed significantly, however, regarding presence of diabetic glomerulosclerosis (30% vs. 5%; $P < 0.05$) and duration of diabetes. The aggregation-enhancing effect was not altered by hemodialysis or pancreatic and renal transplantation in one subject, nor by progressive renal failure in three. Six patients with marked enhancing effect (increase of > 50%) had both diabetic retinopathy and glomerulosclerosis. This aggregation-enhancing factor was heat stable, nondialyzable, and inhibited by salicylate and glucagon therapy. Although induced by insulin withdrawal in two ketoacidosis-prone diabetics it was not clearly related to diabetic control. We conclude that in many diabetics a plasma factor is present which enhances ADP-induced platelet aggregation, and suggests that this factor may play a role in the genesis of diabetic glomerulosclerosis.

191. Patterns of Red Blood Cell Antigen-Antibody Thermal Dissociation: a New Diagnostic and Prognostic Test in Autoimmune Disease. PARVIZ LALEZARI* AND NANCY TALLEYRAND,* New York (introduced by Theodore H. Spaet**).

We have previously described a sensitive method for RBC antibody detection in which Polybrene aggregation of cells allows antibodies to produce irreversible agglutination. The original method, performed in a continuous flow system, has been modified to determine the temperature at which Ag-Ab complexes are dispersed, and the temperature at which one-half of the aggregated cells dissociate (T 50%) has proved to be of important diagnostic value. Direct testing of antibodies on patients red cells revealed several patterns of thermal response. Red cells from all of 18 patients with active systemic lupus erythematosus (SLE) produced a positive test with T 50% not exceeding 50°C. In contrast, in 26 patients without demonstrable underlying disease (idiopathic AHA), the T 50% was greater than 50°C, usually being > 60°C. 25 of 45 selected patients with lymphosarcoma had a positive test, in 19 the T 50% was < 50°C, and in 5 > 60°C. Patients with low T 50% values were most readily controlled with steroid therapy, presented fewer complications in management, and the test showed complete reversal during remission. In patients with high T 50% values, steroid therapy was less effective, hemolytic crises were more numerous, and mandatory splenectomy was more frequent. In idiopathic AHA, the T 50% remained high even during complete clinical remission. In lymphosarcoma, even in those with high T 50% values, the test was reversed by treatment. We conclude that (*a*) a positive test with a T 50% < 50°C suggests an identifiable underlying disease; (*b*) a negative direct or a T 50% > 60°C is inconsistent with a diagnosis of SLE; and (*c*) splenectomy is probably the treatment of choice when T 50% is > 60°C. (Research supported by Grant HE-10036-05 from NIH.)

192. Vascular Response in Hypertensive Chronic Renal Failure. J. MICHAEL LAZARUS,* CONSTANTINE L. HAMPERS,* AND JOHN P. MERRILL,** Boston, Mass. (introduced by John P. Merrill**).

The finding of a group of uremic patients on chronic hemodialysis with persistent hypertension after bilateral nephrectomy associated with abnormal postural hypotension suggested an abnormality in the baroreceptor mechanism in uremic hypertension. In a pilot study of 44 hemodiaylsis patients, 8 of 19 prenephrectomy patients and 9 of 25 postnephrectomy patients were hypertensive (> 140/90). The prenephrectomy normotensive group and the postnephrectomy hypertensive group had postural blood pressure changes significantly greater than normal subjects. To further evaluate postural hypotension and a resetting of the baroreceptor mechanism in uremics, the effect of standing and drug-induced blood pressure changes were serially studied in four normotensive and four hypertensive patients before and after nephrectomy. Increased hematocrit, increased total protein, and decreased RISA plasma volume was found with upright position in all patients before and after nephrectomy suggesting that the kidneys have little role in decreased ECV with standing. Postural blood pressure changes were again greater than normal controls in these patients, particularly the hypertensives. Baroreceptor function was evaluated by the pulse response to elevation of blood pressure with angiotensin and lowering of blood pressure with amyl nitrite. Linear regressions of the R-R interval with blood pressure changes were developed and compared to normal responses. The slope of the linear regression for normotensive uremics (b = 1.841) and hypertensive (b = − 0.988) did not change significantly after bilateral nephrectomy (b = 1.762 and − 0.626 respectively). The slopes of all four groups were lower than five normal subjects (b = 6.778) and approached significance. Blunted baroreceptor response was also seen with lowering of blood pressure by amyl nitrite. We conclude that not all renal parenchymal hypertensives are "cured" by bilateral nephrectomy. Persistent hypertension is associated with increased postural hypotension which may be related to an insensitive or "reset" baroreceptor mechanism. Removal of diseased kidneys and moderate control of hypertension does not appear to correct this defect.

193. Identification and Characterization of the Cardiac β-Adrenergic Receptor. ROBERT LEFKOWITZ* AND EDGAR HABER, Boston, Mass.

Cardiac uptake of specific catecholamines is poorly correlated with the effects of these agents on rate and force of cardiac action. Uptake is a complex process which may be fractionated into neural uptake, neural vesicle uptake, and receptor uptake compartments. In these experiments, we have attempted to isolate and examine the β-receptor. Canine ventricles were subjected to homogenization and particles collected which sedimented in 1 hr in 0.25 M sucrose at 78,000 g. Binding of norepinephrine-^{3}H was assessed by counting these particles after either centrifugation or Millipore filtration. Displacement by unlabeled norepinephrine could be detected at 10^{-9} mole/liter. 50% displacement occurred at 1×10^{-7} M norepinephrine, with the other β-active agents, epinephrine, and isoproterenol effective in the same range of concentration. Dopamine effected 50% displacement at 5×10^{-6} mole/liter. 3,4,-Dihydrophenylalanine (DOPA), dihydroxymandelic acid, and α-methyl DOPA displaced in a range one order of magnitude higher. The α-active agents metaraminol, phenylephrine, ephedrine, methoxamine, and mephentermine, as well as the metabolities metanephrine and VMA did not displace until concentrations in excess of 10^{-4} mole/liter were reached. Inhibition of binding by the β-blocking agent propranolol could be detected at 5×10^{-6} mole/liter, yet the α-blocker phentolamine had no effect until concentrations of 10^{-3} mole/liter were reached. The optimal specificity of the binding site studied relates to two unsubstituted hydroxyl groups on the benzene ring, a β-OH group on the aliphatic side chain and an amine group on the α-carbon. The amine may be substituted without impairing binding. This specificity differs from that of uptake by whole heart and neural vesicles. It is consonant with the specificity of β-stimulated inotropic and chronotropic actions of catecholamines on the heart. Applications envisioned include studies on receptor mechanism as well as the development of a competitive radiodisplacement assay. (Supported by NASA Contract 9-10981.)

194. Calcium Crystalluria. J. LEMANN, JR., AND D. W. DONER, JR.,* Milwaukee, Wis., and Boston, Mass.

In an attempt to determine the relevance of urinary calcium concentrations (U_{Ca}) to the nidation and growth of urinary stones, urine specimens voided after overnight thirsting were obtained and sediments examined immediately for crystals at 37°C. No crystals were seen in urine sediments from 15 normal subjects who had no personal or family history of stones. (*a*) Single or clumped calcium oxalate dihydrate crystals, (*b*) an acid-insoluble, highly birefringent crystal, singly, in clumps, or casts, not having the morphology of uric acid, and believed to be calcium oxalate monohydrate, or (*c*) calcium phosphate crystals singly, in clumps, or casts, were seen in sediments from 10 of 13 calcium oxalate stone formers, three of four relatives of such stone formers, three affected of four members in a family with renal tubular acidosis, and five of five patients with primary hyperparathyroidism. Without regard to patient origin, comparison of the composition of 120 urine specimens without crystals (NC) with the composition of 16 urines containing calcium oxalate crystals (CaOxC) and 29 urines containing calcium phosphate crystals (CaPC) showed that U_{Ca} was significantly higher in CaOxC (6.64 ±1.01 SEM mmoles/liter) and CaPC (6.85 ±0.85 mmoles/liter) than NC (4.43 ±0.24 mmoles/liter). In addition U_{Osm} was lower in CaOxC (741 ±38 mOsm/kg) and in CaPC (774 ±36 mOsm/kg) than in NC (946 ±89 mOsm/kg) suggesting higher Ca activity in urines with crystals. Urine pH was higher in CaPC (6.53 ±0.10) than in CaOxC (5.80 ±0.15) or NC (5.76 ±0.05) and hence calculated $U_{HPO_4^-}$ was significantly higher in CaPC. $U_{Oxalate}$, U_{Na}, U_{Mg}, and U_K were comparable in each group. Elevated U_{Ca} and perhaps $\alpha_{Ca^{++}}$ secondary to reduce U_{Osm}, as well as variation in pH, thus appear to be key determinants of crystalluria. (Supported by NIH AM 15089.)

195. The Effect of Chronic Volume Expansion on Glucose-Induced Calciuria. E. J. LENNON, J. LEMANN, JR., W. F. PIERING,* AND L. LARSON,* Milwaukee, Wis.

Glucose administration augments urinary calcium and magnesium excretion in man by inhibiting net renal tubular reabsorption of the divalent cations. The relationship between this effect and inhibition of tubular cation reabsorption accompanying ECF volume expansion was examined in eight normal men eating constant diets. Clearances of serum ultrafilterable calcium and magnesium were measured after overnight fasting and during water diuresis before and after administration of 100 g glucose, first in the normal steady state, and again after chronic ECF volume expansion produced by "escape" from the sodium-retaining effects of deoxycorticosterone (DOCA). Before DOCA, control fractional excretion rates (EF) of Na, Ca, and Mg were 0.90 ±0.13 (SEM), 1.92 ±0.40, and 4.60 ±0.67% respectively. After glucose EF_{Na} fell slightly (− 0.27 ±0.13) while peak increments in EF_{Ca} and EF_{Mg} averaged + 1.73 ±0.35 and + 3.85 ±0.69. After "escape," control EF_{Na} averaged 2.39 ±0.32, while EF_{Ca} and EF_{Mg} were proportionally increased averaging 4.34 ±0.41 and 6.32 ±0.36 respectively. Glucose administration after "escape" reduced EF_{Na} strikingly (− 1.17 ±0.40) despite which EF_{Ca} and EF_{Mg} rose, the peak increments being comparable to those induced by glucose before DOCA and averaging + 1.92 ±0.89 and + 3.22 ±1.09 respectively. The fall in EF_{Na} produced by glucose after "escape" was accompanied by a comparable reduction in EF_{Cl} as well as a reduction in V/GFR suggesting augmented proximal reabsorption of Na. We conclude that inhibition of tubular reabsorption of Ca and Mg induced by glucose is independent of the factor(s) accompanying volume expansion that inhibit cation reabsorption. (Supported by NIH FR0058, AM 13316, and AM 15089.)

196. Molecular Intervention in Genetically Determined Cellular Immune Deficiency Disorders. A. S. LEVIN,* L. E. SPITLER,* D. P. STITES,* AND H. H. FUDENBERG, San Francisco, Calif.

1 yr ago we reported the dramatic effect of therapy with dialyzable transfer factor (TF) in a patient with the Wiscott-Aldrich syndrome. Since then we have treated three additional patients with this disease and two patients with familial defects in cellular immunity and mucocutaneous candidiasis-polyendocrinopathy syndromes, and one patient with dysgammaglobulinemia and diminished cellular immunity. Six additional patients were treated with TF prepared by our group and sent to physicians in other hospitals. The original patient suffered a relapse clinically and lost his skin test reactivity and his ability to produce macrophage migration inhibitory factor (MIF) 7 months after the TF therapy. He responded dramatically to retreatment both clinically and by laboratory test and has had no infections for the past 4 months. TF induced cellular immunity in two out of four patients with the Wiscott-Aldrich syndrome, and clinical status of these patients improved. The patients who responded had a defect in the monocyte IgG receptors whereas one patient who did not respond had normal monocyte receptors (the other patient was terminally ill with leukemia and pseudomonas pneumonia). Similar results were obtained by other physicians using TF prepared by us. TF therapy seems to be effective in inducing cellular immunity in certain diseases.

197. Selective Inactivation of an ESF-Generating Factor (EGF) in the Presence of Erythropoietin (ESF). JASPER P. LEWIS,* EMILY T. WELCH,* W. AUBREY NEAL,* RUSSELL R. MOORES,* WILLIAM G. LEWIS,* CLAUDE-STARR WRIGHT,** LINDA L. SMITH,* AND COIT M. DUBOSE, JR.,* Augusta, Ga.

An EGF has been demonstrated in several laboratories. The EGF appeared to be an enzyme that acted on a serum substrate to produce ESF. A deficiency of the substrate could occur in the presence of plenty of EGF. Since both factors bring about the incorporation of ^{59}Fe into heme during the ESF bioassay, it becomes of importance to be able to distinguish between the two activities. Cleland has described a protective reagent for sulhydryl groups, dithiothreitol (DTT), which is capable of maintaining monothiols completely in the reduced state and of reducing disulfides quantitatively. The purpose of this report is to describe the application of DTT to the problem of differentiating between the activities of EGF and ESF. EGF, ESF, and a mixture of both were incubated with DTT. 10 μmoles of DTT inactivated 0.1 mg of an EGF fraction which produced 0.40 ±0.02 IU of ESF without DTT. However, the same amount of DTT did not inactivate ESF. 1 mg of fraction II + III, a urinary concentrate that contained the equivalent of 0.43 ±0.01 IU of a mixture of ESF and EGF, was inactivated 56% by either 0.5 or 10 μmoles of DTT. In a previous report the parallelism of the ESF dose-response and the oxidation of glutathione (GSH) was observed, and it was suggested that a common substance may be responsible for both responses. Possibly GSH is a source of hydrogen ions during the regeneration of EGF, if the two systems are proximate. (Supported in part by Grants HE-10591-07 (HEM), FR-0061, FR-5365, and HE-12958 from NIH.)

198. Modification of Papain-Induced Emphysema by Progesterone and Stilbesterol. JACK LIEBERMAN,* Duarte, Calif. (introduced by D. Comings).

Emphysema was induced in adult hamsters by exposing them to an ultrasonic aerosol of 3% papain for 2 hr. The development of emphysema was dependent upon an adequate rate of aerosol nebulization and an optimum duration of exposure. Nebulization of 1 cc papain per min into a 1 × 1 × 4 ft chamber for 2 hr resulted in maximum emphysema and minimum mortality of the animals. Three groups of 12 hamsters were given daily intraperitoneal injections of (*a*) 0.5 ml saline, (*b*) 0.5 ml (1 mg/ml) diethylstilbesterol, or (*c*) 0.5 ml (1 mg/ml) progesterone. After 10 injections, the hamsters were exposed to the papain aerosol and the injections were continued for 14 days more. All surviving animals were sacrificed 4 wk after the papain aerosol, and the lungs were sectioned and stained. Moderate to severe emphysema was found in 8 of 12 controls, 2 of 12 receiving stilbesterol ($P < 0.01$), and 1 of 12 receiving progesterone ($P < 0.01$). Serial measurements of the serum-trypsin inhibitory capacity in other hamsters receiving identical medication showed no response of the trypsin inhibitor to either stilbesterol or

progesterone (unlike the rise resulting from stilbesterol in humans). Thus, the protective role of the hormones was not related to a rise in the level of protease inhibitor. In any event, the blood of hamsters or man was not capable of inhibiting papain, although it could inhibit bromelain, ficin, subtilisin, and trypsin. Examination of the lungs from hamsters sacrificed 48 hr after exposure to papain showed a reduced inflammatory reaction in animals treated with the hormones. It is concluded that female hormones, particularly progesterone, can prevent the development of emphysema in hamsters by an undefined mechanism unrelated to an increase in protease inhibitor. (Research supported by grant from NIH.)

199. Triiodothyronine Radioimmunoassay. JEFFREY M. LIEBLICH* AND ROBERT D. UTIGER, Philadelphia, Pa.

Highly specific antibody to *l*-triiodothyronine (T_3) has been prepared by immunization of rabbits with T_3 conjugated to bovine serum albumin with carbodiimides. Subsequent analysis by immunoassay of the conjugates indicated T_3: albumin molar ratios of 2.5–8.1:1. Using the double-antibody technique, a radioimmunoassay capable of detecting 0.05 ng unlabeled T_3 has been developed. At an antiserum dose which bound 95% of added T_3-^{125}I (0.2 ng), $< 2\%$ of a similar quantity of thyroxine-^{125}I was found. The ability of various thyroid analogues (on a weight basis) to inhibit the binding of T_3-^{125}I to antibody was compared to that of T_3 (100%) with the following results: *d*-T_3, 85.3%; triiodothyroacetic acid, 31.7%; triiodothyropropionic acid, 52.7%; *l*-thyroxine (five different preparations), 0.14, 0.45, 0.62, 0.66, and 1.34%; *d*-thyroxine, 0.17%; tetraiodothyroacetic acid, 0.26%; desaminiothyroxine, 0.36%; DIT, 0.000025%; MIT, 0.000031%. Chromatography of thyroxine on Sephadex G-25 suggests much of its slight cross-reactivity was due to contaminating T_3. Because of binding of T_3-^{125}I to serum thyroid-binding proteins, direct assay of serum yielded high nondisease related T_3 values. By addition of diphenylhydantoin to inhibit such binding, reliable assay of serum T_3 was possible. In 34 normal subjects, T_3 values were 0.147 $\pm$0.027 μg/100 ml (SD). Mean serum concentrations in patients were as follows: hypothyroid, 0.099 $\pm$0.024 μg/100 ml ($n = 25$); hyperthyroid, 0.448 $\pm$0.149 μg/100 ml ($n = 25$); and pregnancy or estrogen treatment, 0.187 $\pm$0.031 μg/100 ml ($n = 17$). Recovery of T_3 added to serum from all types of patients averaged 96.7%. Addition of T_4 preparations of low cross-reactivity to serum in quantities of 12.5–50 μg/100 ml did not alter measured T_3 values. This simple method for T_3 measurement should greatly facilitate study of the physiology of this hormone in normal subjects and those with various diseases.

200. Renal Hemodynamics and Sodium Handling in the Pregnant Rat. MARSHALL D. LINDHEIMER* AND ADRIAN I. KATZ,* Chicago, Ill. (introduced by Theodore N. Pullman**).

During human pregnancy effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) increase and a moderate, cumulative retention of sodium takes place. Similar changes are thought to occur in experimental animals, but results conflict. Effects of pregnancy on renal hemodynamics, volume of distribution of inulin, and renal sodium reabsorption and excretion were evaluated in anesthetized rats on the 14th and 20th gestational day (pregnancy in the rat lasts 21 days). Simultaneously studied virgin littermates served as controls. 46 animals were included in this part of the study. In 14-day pregnant animals GFR (inulin clearance), ERPF (PAH clearance/extraction), and inulin space per unit nonconceptus weight (IS/100 g) were not different from controls. At term, GFR, IS/100 g, and filtered and reabsorbed sodium were significantly elevated, while ERPF remained unchanged. Contrary to previous reports, both pregnant and control animals were able to reabsorb $> 99\%$ of filtered sodium. Additional experiments (58 rats) were performed to determine the relation of renal microsomal Na^+-K^+-ATPase to sodium reabsorption during pregnancy. No change in enzymatic activity could be detected in 14-day pregnant animals. At term, however, there was a significant increase in the specific activity of Na^+-K^+-ATPase, similar to the per cent increment in net sodium reabsorption, while Mg^{++}-ATPase and glucose-6-phosphatase, two microsomal enzymes not involved in sodium transport, were unchanged. The increment in Na^+-K^+-ATPase was no longer present 10 days postpartum. Results demonstrate a phase during pregnancy in the rat similar to human gestation. The increment in renal Na^+-K^+-ATPase activity in parallel to changes in reabsorptive sodium load suggests that this enzyme may play a role in the alterations of renal sodium reabsorption which accompany normal pregnancy.

201. Quantitative Allotype Deficiencies in Families of Patients with Primary Hypogammaglobulinemia. S. D. LITWIN,* H. H. FUDENBERG, AND R. KAMLIN,* New York and San Francisco, Calif. (introduced by H. Cleve).

Nine families of persons with adult onset primary immune deficiencies were analyzed by means of quantitative determinations of Gm allotype concentrations. The use of antithetical Gm markers for γG1 and γG3 heavy chains facilitated the measurement in heterozygous subjects of allelic γG gene products. The data were correlated with serum Ig levels, and DNA synthesis by stimulated lymphocytes. Over a quarter of first degree relatives had either an absolute deficiency of one Gm allotype or an imbalance between γG1 Gm antigens (in heterozygotes), suggesting a wide difference in activity of allelic Ig genes. In addition, defective DNA synthesis by healthy relatives frequently correlated with the allotype deficiency. Other immune abnormalities noted within these families included deficient or absent γA and hypergammaglobulinemia. Analysis of three families demonstrated the appearance of allelic deficiency in succeeding generations, suggesting genetic segregation. The data supported a genetic basis for the immune deficiency and were interpreted as most consistent with a regulatory gene defect. (Research supported by grants from NIH.)

202. Factors Responsible for the Formation of Atrial Wall Thrombi in the Fat-Fed Mouse. W. R. LOCKWOOD* AND BEN R. CLOWER,* Jackson, Miss. (introduced by Harper K. Hellems**).

Anemia, fatty liver, and atrial mural thrombi followed by death developed by 10 wk in 70–90% of selected strains of mice fed a diet poor in protein, but rich in fat and sucrose, and supplemented with vitamins and lipotrophic substances.

Earlier we showed that pronounced abnormalities developed in the atria several weeks before thrombi formed. The most prominent prethrombotic findings were atrial enlargement, endothelial cell vacuolization with the appearance of numerous microvillus processes on their luminal surfaces, basement membrane thickening, edema, and fibroblast proliferation. No lipid deposits were identified. We have now investigated some of the effects that refeeding a balanced diet has on the changes that precede thrombus formation. Mice fed the thrombogenic diet for 3, 4, 5, or 6 wk before refeeding a balanced chow for 1, 2, or 4 wk were studied by light and electron microscopy. The anemia and fatty liver begin to recover quickly and by 4 wk had returned to normal. The gross heart lesion did not progress, but the micrographs showed that the prethrombotic changes remained essentially unchanged during 4 wk of recovery. Only the edema regressed. Even though the atrial endocardium remained distinctly abnormal, the development of mural thrombi was arrested. Thus the dietary stimulus was shown to be necessary for the induction of the thrombotic process, however, the factors primarily responsible have not been determined.

203. Membrane-Bound Third Component of Complement (C3) and Hemolysis by Cold Agglutinins. GERALD L. LOGUE,* JON P. GOCKERMAN,* AND WENDELL F. ROSSE, Durham, N. C.

In order to relate membrane-bound third component of complement (C3) to hemolysis in vitro and in vivo, we have developed a method for quantitating C3 attached to red cells using anti-C3 and the C1 fixation and transfer test. In vitro, the C3 fixed is directly proportional to the cold agglutinin added. Hemolysis of normal human red cells does not occur until at least 1000 molecules of C3 per red cell are bound (as detected by this method). In vivo, without cold stress, four patients with cold agglutinin syndrome had 350–1300 molecules of C3 per red cell; the hemolytic rate paralleled the quantity of cell-bound C3. In two patients, one arm was cooled (5°C, 2 min) and, in effluent blood, cell-bound C3 and plasma hemoglobin rose and fell abruptly. When a patient was cooled in a refrigerated room (8°C, 23 min), the amount of cell-bound C3 increased 50% and remained elevated for at least 60 min. Hemolysis was accelerated as evidenced by increased plasma hemoglobin and increased endogenous carbon monoxide production. The rate of addition of C3 and its effect on cell survival in the absence of cold stress was determined by transfusion of type O blood into a patient with type B blood. C3 was fixed to the transfused cells gradually and equaled the amount on the patients' own cells within 24–48 hr. The transfused cells were not destroyed rapidly as determined by ^{51}Cr labeling. These studies indicate that both the amount of C3 fixed to red cells and its rate of fixation are important in determining the rate of hemolysis in patients with cold agglutinin disease.

204. Intrarenal Release of Prostaglandins as Determined by the State of Sodium Balance. ANDREW J. LONIGRO,* NORBERTO A. TERRAGNO,* KAFAIT U. MALIK,* AND JOHN C. MCGIFF, St. Louis, Mo.

Prostaglandins may function in a renal feedback control system which regulates renal vascular resistance and sodium excretion and thereby, blood pressure. In chloralose-anesthetized dogs, norepinephrine (50–100 ng/kg per min), infused into the renal artery (IRA), released a substance into renal venous blood in concentrations which varied from 0.05 to 24.17 ng/ml blood above control levels. This substance was indistinguishable from PGE_2 ("PGE") by physicochemical and pharmacological criteria. The threshold dose of angiotensin II, given IRA, which released "PGE" varied between 0.5 and 20 ng/kg per min. Eluates of chromatographed acidic lipid extracts of renal venous blood were assayed for their content of prostaglandins of the E and F series by a parallel pharmacologic procedure. Plasma renin activity (PRA) of thoracic caval blood, an index of the state of sodium balance, was directly related to the threshold dose of angiotensin II which released "PGE" and inversely related to the magnitude of the increase in concentration of "PGE" evoked by norepinephrine ($P < 0.01$). For example, when PRA was elevated (negative sodium balance) the threshold dose of angiotensin II was increased and the amount of "PGE" released by norepinephrine decreased. Furosemide (1 mg/kg; IRA) increased the concentration of "PGE" in renal venous blood by a mean of 0.04 ±0.016 ng/ml ($P < 0.05$). Furosemide and pressor hormones did not increase the concentration of "PGF" in renal venous blood. An inverse relationship obtained between changes in PRA and in concentration of "PGE" in renal venous blood in the face of rapid alterations in sodium balance induced by furosemide ($P < 0.05$). However, the initial increase in renal blood flow and the degree of natriuresis produced by furosemide could not be accounted for by changes in concentration of "PGE." These observations suggest that the release of PGE_2, the dominant renal prostaglandin, is related to the state of sodium balance. (Supported by the USPHS and AHA.)

205. Effect of Elevated Intratubular Pressure (ITP) on Renal Tubular Permeability. W. B. LORENTZ, JR.,* W. E. LASSITER, AND C. W. GOTTSCHALK,** Chapel Hill, N. C.

The effect of elevated ITP on renal tubular permeability was studied with microinjection technics in anesthetized, diuretic rats. ITP was elevated by partial constriction of the renal vein (RVC) or by elevation of the ureteral catheter (EUC). 1.0–4.5 nl of an isotonic saline solution containing mannitol-^{14}C and inulin-^{3}H was microinjected into individual tubules and recovery of the isotopes measured in the urine from injected kidneys. Inulin recovery averaged 100.2 ±2.4 (SD)% at normal and 99.9 ±2.2% at elevated ITP. At normal pressures, mannitol recovery averaged 99.8 ±2.9% after early proximal injection. During elevation of proximal ITP to 30 ±2 mm Hg by RVC, mannitol recovery averaged 83.1 ±7.2% ($P < 0.001$) after early proximal, 90.6 ±3.5% ($P < 0.001$) after late proximal, and 96.2 ±3.9% ($P < 0.01$) after distal injections. Recovery of mannitol after elevation of ITP to 30 ±2 mm Hg by EUC was 85.1 ±6.4 ($P < 0.001$) after early proximal and 92.7 ±2.7% ($P < 0.001$) after distal injections. In proximal reinjection experiments mannitol recovery 5–60 min after release of EUC was 98.1 ±3.3% as compared to 86.4 ±5.5% during EUC ($P < 0.001$). Mannitol recovery was unchanged after EUC of the contralateral kidney. During aortic constriction mannitol recovery was 98.8 ±4.3% and inulin recovery was 99.5 ±2.3%. These studies demon-

strate a change in renal tubular permeability to mannitol but not to inulin after elevation of ITP. The change in permeability was rapidly reversible and was not a function of increased transit time, since no leakage of mannitol was evident after aortic constriction which produced an increase in transit time comparable to that observed during RVC and EUC. Since no effect was demonstrable in the contralateral kidney, the change in permeability appears to result from an intrarenal mechanism.

206. Abnormal Deposition and Clearance of Inhaled Particles during Upper Respiratory Viral Infections. RUY V. LOURENÇO,* EDITH D. STANLEY,* BIENVENIDO GATMAITAN,* AND GEORGE G. JACKSON,** Chicago, Ill.

The fate of inhaled particles in the tracheobronchial tree of patients with respiratory viral infection may determine its course and consequences. Deposition and clearance of particles were studied in 12 persons with acute upper respiratory viral infections without evidence of pneumonia. In three a rhinovirus was isolated, in four a myxovirus, and in three no agent has, as yet, been isolated. In two volunteers infection was induced by nasal instillation of attenuated rhinovirus. All subjects inhaled a monodisperse aerosol (m.m.d., 2 μ; geom. SD 1.10) of iron oxide particles labeled with ^{198}Au. A scintillation camera was used to determine both the initial patterns of deposition and the clearance of particles over a 5 hr period. The results were compared with those of 11 volunteers with no respiratory infection. Abnormal deposition of particles in the larger bronchi was found in four subjects. In the remaining eight, normal diffuse distribution was present. However, in all these subjects altered clearance was evident: photoscintigrams showed regional retention of particles, and radiation counts demonstrated too slow a disappearance of particles from the chest. 6 wk after disappearance of symptoms, four subjects still manifested abnormal, although improved, clearance. The significance of the pattern of deposition demonstrated in this study might be that inhaled particulate matter is deposited in sites of the lungs with decreased resistance capabilities. The pattern of clearance found suggests there are severe and prolonged alterations in bronchial mucociliary activity in subjects with upper respiratory virus infections, even in the absence of bronchopulmonary symptoms. Consequently, the defense mechanisms against inhaled bacteria pollutants may be impaired. (Research supported by NIH Grant HE-13856 and USPHS Grants AI 04059 and AI 00208.)

207. Correction of Metabolic Acidosis by Chronic Antidiuretic Hormone (ADH)-Induced Volume Expansion. DAVID C. LOWANCE,* HOWARD B. GARFINKEL,* WILLIAM D. MATTERN,* AND WILLIAM B. SCHWARTZ,** Boston, Mass.

Balance studies were undertaken to evaluate the influence of chronic ADH-induced volume expansion on metabolic acidosis induced by feeding HCl. Volume expansion was accomplished by the administration of 5 U of vasopressin in oil twice daily for a period of 4–18 days. In each study plasma osmolality was lowered to 240 mOsm/kg or less. ADH-induced volume expansion in acidotic dogs ingesting a normal NaCl diet produced a sharp increase in net acid excretion (mean cumulative increase 126 mEq/liter) and a rise in plasma [HCO_3] to a final value not significantly different from control (mean increase from 13.6 to 20.6 mEq/liter). In acidotic dogs ingesting a low NaCl diet, volume expansion also produced a marked increase in net acid excretion and returned plasma bicarbonate concentration nearly to normal; in these animals K excretion increased significantly during the period of vasopressin administration. In four animals ingesting a low NaCl diet vasopressin was continued for 2–10 days after the point of maximum volume expansion (lowest plasma osmolality); during this period significant expansion persisted and plasma [HCO_3] remained well above the pre-ADH acidotic levels. An additional vasopressin study in four acidotic dogs ingesting a normal NaCl diet demonstrated that when volume expansion was prevented by water restriction, there was no significant change in plasma [HCO_3]. The above findings suggest that hypotonic volume expansion produced a depression of proximal Na reabsorption and a sharp acceleration of distal Na-H exchange which results in an increase in net acid excretion and restoration of plasma [HCO_3] to normal. These data may explain why patients with the syndrome of inappropriate ADH have little or no reduction in plasma [HCO_3] despite severe renal wasting of Na. It seems likely that HCO_3 shunted from the proximal tubule with sodium is conserved distally as the result of accelerated Na-H exchange. (Research supported by grants from NIH HE00759-21 and HE05309-12.)

208. The Proliferating Marrow Cell In Vitro. A. A. MACKINNEY, JR.,* AND DONALD R. KORST,* Madison, Wis. (introduced by Calvin M. Kunin).

The bone marrow is probably the principal source of macrophages throughout the body. In vitro growth of marrow clones resembling macrophages and neutrophils are used to assay stem cells. We investigated the marrow cell capable of forming macrophages by a method of growing marrow in liquid medium. Marrow from calves aged 1–8 wk was grown on coverslips in homologous serum and Eagle's medium in 5% CO_2. Cultures were examined at 12-hr intervals using standard counting, histochemical, radioautographic, and stathmokinetic techniques. At the time of harvest, cells not adhering to glass were studied separately from cells attached to glass. In other experiments, marrow cells were separated on glass bead columns before culturing. Adherent cells in DNA synthesis increased without lag period from 5 to 42% in 4½ days. Mitosis reached a peak of 0.6% per hr on the 6th day. Cells adhering to glass increased 20-fold between the 3rd and 14th days. Cytologic analysis showed that cells of the erythroid and granulocytic series died out rapidly during the first 2 days and were replaced by a typical peroxidase-negative, acid phosphatase–positive macrophage population on the 7th day. These cells were derived from a cell capable of adhering to glass and already in DNA synthesis at the time of starting the culture. Cells in DNA synthesis not adherent to glass did not appear to proliferate under the conditions of the experiment. 85% of the DNA-synthesizing cells attached to glass at the initiation of the culture appeared to be monocytes. Transitions between monocytes and macrophages were frequent. We conclude that differentiated cells, the marrow monocytes, are transformed into an actively dividing macrophage population in vitro. This proliferating system can be

repressed and derepressed by altering serum concentration affording the opportunity to study monocytes in cycle as well as resting macrophages entering DNA synthesis.

209. Effect of Antibiotics on Phagocytized Bacteria. GERALD L. MANDELL,* Charlottesville, Va. (introduced by Edward W. Hook**).

Bacteria are able to survive in abscesses despite intensive antibiotic treatment. To study this phenomenon human polymorphonuclear neutrophils (PMN) were placed in an anaerobic medium maintained by 95% nitrogen–5% CO_2 washout ($P_{O_2} < 5$ mm Hg) to simulate the low P_{O_2} found in pus. PMN under these conditions were unable to kill ingested bacteria in a normal fashion. 5–50 times as many *Staphylococcus aureus* (502A) were able to survive intracellularly in anaerobic PMN as compared with PMN exposed to air. 12 different bactericidal and bacteristatic antibiotics and seven combinations of these antibiotics were studied and it was found that bacteria that had been ingested by anaerobic PMN were protected from the lethal action of antibiotics. Kinetic experiments demonstrated that some protection was evident for as long as 3 days after phagocytosis. Damaging PMN by heating, freezing and thawing, or sonication neutralized their ability to protect ingested bacteria from antibiotics. The addition of dimethyl sulfoxide to enhance intracellular penetration of antibiotics failed to enable antibiotics to kill intracellular bacteria. To determine if intracellular bacteria continued active metabolism, oxygen consumption was studied. PMN that had ingested living bacteria were placed in the chambers of a polarographic oxygen monitor and PMN respiration and bactericidal activity was suppressed with high doses of hydrocortisone (10 mg/ml). Extracellular bacterial oxygen consumption was eliminated by the addition of the enzyme lysostaphin. Under these conditions, it was demonstrated that intracellular bacteria consume oxygen in a normal manner. Thus, intact PMN were able to protect ingested bacteria from the lethal actions of antibiotics despite continued respiration by the intracellular bacteria. This suggests that antibiotics are unable to penetrate the PMN membrane. (Research supported from a grant by The Lilly Research Laboratories.)

210. The Effect of Chloramphenicol on Ferrochelatase Activity and Erythropoiesis in Dogs. DAVID R. MANYAN* AND ADEL A. YUNIS, Miami, Fla.

We have previously provided evidence that reversible bone marrow suppression from chloramphenicol (CAP) results from inhibition by the drug of mitochondrial protein synthesis. The present study was undertaken to examine the question of why the erythroid elements appear to be more vulnerable to CAP. Because CAP inhibits primarily the synthesis of membranous mitochondrial proteins (Firkin and Linnane. 1968. *Biochem. Biophys. Res. Commun.* **32**: 398), and in view of the recent demonstration that ferrochelatase (FC) is associated with the inner mitochondrial membrane (Jones and Jones. 1969. *Biochem. J.* **113**: 507), suppression of FC activity by CAP could be a major determining factor. FC activity was determined in bone marrow mitochondria of dogs receiving 100 mg CAP/kg per day and of control animals. The assay system consisted of measuring the production of radioactive heme from ^{59}Fe and protoporphyrin. FC activity decreased 65–90% in dogs receiving CAP for 3 wk and returned to normal level 8 wk after discontinuation of treatment. Concurrent with the drop in FC activity there was a marked elevation of the level of free erythrocyte protoporphyrin and increase in stainable bone marrow iron. The mechanism of suppression of FC activity by CAP is not clear. It could be due to direct inhibition of synthesis of the enzyme itself or of an FC-binding site on the inner mitochondrial membrane. Regardless of the mechanism, this biochemical effect of CAP provides a likely explanation for the vulnerability of the erythroid cells to the drug. (This work was supported by NIH Grant 1 RO1 AM 13087-03.)

211. Properties of Human Platelet Gangliosides. AARON J. MARCUS, LENORE B. SAFIER,* HARRIS L. ULLMAN,* ROBERT A. GRANT,* AND MARJORIE B. ZUCKER,* New York.

Lipids constitute 16% of the dry weight of human platelets. The principal lipid classes are the glycerophosphatides and "neutral" lipids which comprise 78 and 21% of the total respectively. Gangliosides, which are glycosphingolipids containing one or more residues of acylated neuraminic acid per molecule, have recently been isolated from platelets in our laboratory. They represent 0.5% of the platelet lipids. By means of several biochemical techniques, including gas-liquid and thin-layer chromatography, the major platelet ganglioside has been identified as hematoside (G_6). Characteristically, it contained glucose, galactose, and neuraminic acid in a molar ratio of 1:1:1, and no hexosamine. Fatty acid analyses revealed the presence of mainly stearate (47%), oleate (6%), behenate (15%), lignocerate (7%), and nervonate (8%). The predominant long-chain base appeared to be sphingosine. Another platelet ganglioside fraction, identified as G_3A, was examined for its ability to take up serotonin-^{14}C in an equilibrium dialysis system, using G_6 as a control. Under the experimental conditions employed 10% of the radioactive serotonin was bound to G_3A in its acid form whereas 2% was bound by G_6. The binding persisted for at least 7 days despite removal of unbound serotonin by dialysis against fresh buffer. G_3A has been implicated as a membrane receptor for serotonin. Gangliosides appear to play an important role in cell membrane function and may account at least in part for the affinity of platelet membranes for serotonin and other plasma constituents. (Supported by grants from the VA, NIH, and New York Heart Association.)

212. Decrease in the Size of Experimental Myocardial Infarctions after Acute Coronary Occlusion by Glucose-Insulin-Potassium (GIK). PETER R. MAROKO,* PETER LIBBY,* BURTON E. SOBEL,* COLIN M. BLOOR,* WILLIAM E. SHELL,* JAMES W. COVELL,* AND EUGENE BRAUNWALD, San Diego, Calif.

Although it has been suggested that GIK may be beneficial in acute myocardial infarction, its effect on infarct size is unknown. In 25 open-chest dogs epicardial electrocardiographic maps were taken in 10–14 sites before and after the occlusion of the left anterior descending coronary artery. 24 hr later myocardial creatine phosphokinase (CPK) activity in IU/mg

protein and histologic changes were determined in transmural specimens from the sites previously examined electrocardiographically. In 10 controls (73 biopsies) the inverse relationship between epicardial ST segment elevation (mv) 15 min after occlusion and myocardial CPK 24 hr later was highly significant (Log CPK $= -0.0636$ ST $+ 1.2397$, $r = -0.81$). In 13 dogs (113 biopsies) GIK was infused for 24 hr starting 30 min after occlusion. CPK in normal myocardium (0–2 mv ST elevation) was similar in both groups (18.5 ± 0.5 and 18.9 ± 0.5). At all levels of ST segment elevation there was less CPK depletion after GIK infusion. For example, CPK in sites with ST segment elevations of 3–6 mv (small infarcts and border zones) was always depressed in controls (8.0 ± 0.8) but was nearly normal in the GIK group (14.4 ± 0.7, $P < 0.001$). Histologic examination (13 dogs, 150 biopsies) showed that in sites with ST segment elevation immediately after occlusion changes of early necrosis did not occur in 36% of samples from the GIK group as compared to only 3% of the control group. Thus, the histologic and enzymatic evidence indicates that there is less myocardial damage associated with the coronary occlusion when GIK was infused.

213. Diabetes Mellitus, Optic Atrophy, Neurogenic Bladder, and Neurosensory Hearing Loss with Hyposmia, Hypogeusia, and Familial Dysautonomia. JOHN L. MARQUARDT,* D. LYNN LORIAUX,* AND ROBERT I. HENKIN, Bethesda, Md.

A syndrome consisting of diabetes mellitus, optic atrophy, neurogenic bladder, and neurosensory hearing loss has been previously described. We have studied two sibs, aged 16 and 18, with this combination plus hyposmia, hypogeusia, hyperalanineuria, abnormal heat intolerance, and other autonomic dysfunctions. The diabetes was insulin dependent, but resistant to ketosis. Plasma growth hormone and cortisol were normal, but neither responded to intravenous piromen. Arginine produced markedly elevated and prolonged increases in immunoreactive plasma growth hormone. Metapyrone and adrenocorticotrophic hormone (ACTH) produced normal responses. Oral papillae and taste buds were present. Nerve conduction velocities were normal. Both showed normal responses to intradermal histamine and pilocarpine, but abnormal cold pressor tests. Both had miosis after conjunctival placement of 2.5% methacholine. In a constant environment at 120°F with 12% relative humidity for 45 min their weight losses were significantly less than normal (80 g), demonstrating the absence of sweating, while their body temperatures rose to 40°C with an abnormal temperature curve. Nevertheless, pilocarpine iontophoresis of the skin of the forearm produced normal sweat volumes. Abnormal glucose tolerance curves were demonstrated in the father and paternal uncle. The mother demonstrated miosis after conjunctival placement of 2.5% methacholine. Both parents showed hypogeusia and abnormal cold pressor tests but normal responses to intradermal pilocarpine. These findings suggest that this syndrome belongs in the general category of familial dysautonomia. In contrast to types I and II familial dysautonomia, this syndrome appears to have little peripheral component and to be due primarily to central autonomic dysfunction as opposed to the predominant peripheral autonomic dysfunction in type II, and the central and peripheral autonomic dysfunction in type I familial dysautonomia.

214. Hypothalamic Origin of Idiopathic Hypopituitarism. LUIS G. MARTIN,* PEDRO MARTUL,* THOMAS B. CONNOR,* AND JOHN G. WISWELL,* Baltimore, Md. (introduced by Sheldon E. Greisman).

The possible role of the hypothalamus in the pathogenesis of idiopathic hypopituitarism (IH) was studied in two male patients, ages 20 and 24 yr. Heights of these patients were 58 and 73 inches, respectively, and sexual development was infantile. Each showed unequivocal clinical and biochemical findings of gonadotropin, adrenocorticotrophic hormone (ACTH), and thyroid-stimulating hormone (TSH) deficiency without evidence of pituitary tumor or diabetes insipidus. Plasma growth hormone (PGH) and serum thyroid-stimulating hormone (STSH) were found to be undetectable by radioimmunoassay during hypoglycemic and arginine stimuli in both subjects. The response of each patient to aqueous vasopressin (AV) and synthetic thyrotropin-releasing hormone (TRH-Abbott) was then tested. After intramuscular injection of 10 U of AV, plasma 17-hydroxycorticoids rose from control levels of 0.2 and 4.2 μg/100 ml to peak values of 9.0 and 9.6 μg/100 ml within 30 min after injection. During this same interval PGH rose from <1.0 ng/ml to 7 mμg/ml in the tall patient, but remained undetectable in the subject with short stature. After a rapid injection of 400 μg of TRH intravenously STSH rose from control values of <2 μU/ml to peak levels of 14 and 20 μU/ml respectively within 30 min. The results indicate that in these patients with IH, the pituitary could respond when challenged with hormonal stimuli of hypothalamic origin. It is concluded that the anterior pituitary failure was caused by defective hypothalamic function. (Supported in part by NIH Grants TI-AM5058 and RR-33.)

215. Frequency Dependence of Intrapulmonary Distribution of ^{133}Xe. R. R. MARTIN* AND N. R. ANTHONISEN,* Montreal, Canada (introduced by D. V. Bates**).

The peripheral airways of the lung are the primary site of chronic obstructive disease; lesions of such airways are not readily detectable by standard pulmonary function tests. However, uneven distribution of peripheral airway resistance causes changes in ventilation distribution for which we have devised a test. Erect subjects starting at 50% vital capacity (VC) inhaled boli (4 cc) of ^{133}Xe either very slowly or with maximum speed. The regional intrapulmonary distribution of each bolus was noted and comparison of slow and fast inspirations allowed calculation of relative regional time constants (T). After each inspiration the subject fully expired and ^{133}Xe was measured continuously at the mouth (alveolar plateaux). A subsequent VC inspiration of room air allowed measurement of regional residual volumes (RRV). 28 normal subjects were separated into four groups of seven according to age or smoking habits. A significant difference was demonstrated between young subjects and older subjects and also between young cigarette smokers and young nonsmokers. These differences consisted of abnormal progression of T from apex to base as well as aberrent T values in single lung regions. RRV also showed differences according to age, smoking habits and in smokers RRV differed according to the inspired flow rate, indicating intraregional differences in function. Finally, the alveolar plateau after slow and fast bolus inhalations differed little in young nonsmokers. This difference was signifi-

cantly greater in older nonsmokers, and in cigarette smokers. In a larger group of smokers, frequency dependence of the alveolar plateau was found to correlate very closely with frequency dependence of compliance. This test is simple, quick, and could be adapted to nonradioactive gases. (Research supported by grants from MRC of Quebec and Canada.)

216. The Effect of the Infusion of Strontium Chloride on Parathyroid Gland Activity: Comparison with Hypercalcemia, Hypermagnesemia, and Parathyroidectomy. SHAUL G. MASSRY,* LARRY STERN,* CLAIRE TARGOFF,* AND CHARLES R. KLEEMAN,** Los Angeles, Calif.

Parathyroid activity is suppressed by hypercalcemia and hypermagnesemia. This study was undertaken to evaluate whether this effect may not be entirely specific but may be shared by other divalent cations. The effect of elevated serum levels of strontium on parathyroid function, assessed by changes in (*a*) phosphate/creatinine clearance ratios (Cp/C_{Cr}) and (*b*) serum calcium levels, was studied in nine dogs given strontium chloride ($SrCl_2$) infusion. The influence of diurnal variation on Cp/C_{Cr} was excluded since each study was paired with a control infusion without $SrCl_2$. During $SrCl_2$ infusion, serum phosphate rose. Despite this rise, Cp/C_{Cr} fell in all studies, reaching 1–20% of the control values. A significant fall in Cp/C_{Cr} occurred with serum strontium of 5–8 mg/100 ml. The pattern of Cp/C_{Cr} during $SrCl_2$ infusion resembled that seen during calcium chloride infusion (seven dogs), magnesium chloride infusion (11 dogs), and that after thyroparathyroidectomy (seven dogs). Serum calcium fell by 1–2 mg/100 ml during $SrCl_2$ infusion, and this change was similar to that noted after magnesium chloride infusion or thyroparathyroidectomy. When parathyroid extract was injected into three dogs receiving $SrCl_2$ infusion, both Cp/C_{Cr} and serum calcium rose. In four thyroparathyroidectomized dogs, $SrCl_2$ infusion did not suppress Cp/C_{Cr} or serum calcium. The results demonstrate that strontium, like calcium and magnesium, suppresses parathyroid activity by probability inhibiting either production and/or release of the hormone without interfering with end-organ response. The data indicate that the suppressive effect of calcium and magnesium on parathyroid activity is not specific but may be achieved by a divalent cation not present normally in the body.

217. Computer-Simulated Model of Leukemic Cell Kinetics. ALVIN MAUER, CARL EVERT,* AND BEATRICE LAMPKIN,* Cincinnati, Ohio.

To test current hypotheses of leukemic cell kinetics and mechanisms of drug action, a computer-simulated model for leukemic cells was developed with available GPSS computer language based on known leukemic cell characteristics. Cells were considered individually and moved through phases of the cell cycle with appropriate time delays. After mitosis, cells doubled and variable proportions were assigned to a dormant phase. For most studies, 40% of the cells were begun in this dormant phase from which they entered G_1 of the cell cycle after a variable time period. Results were displayed as line graphs indicating changes in number of cells in the parts of the cell cycle with time. Validity of the model was tested by introducing a "labeled" cohort in the S phase. Results of pulse labeling in vivo with thymidine-^3H were reproduced. One interesting finding was the amount of cell death consistent with clinical observations. Some cells entering the dormant phase were terminated. With usual proliferative characteristics and a 50% "death" rate of dormant cells, population growth continued with a doubling time of 18 days. Only at 62.5% "death" rate did growth stop. Two aspects of drug response simulation were interesting. Vincristine effect in vivo was not reproduced until a block in cell delivery from the dormant phase was introduced in addition to metaphase arrest. This potentially detrimental effect of keeping cells in a drug-resistant dormant phase is consistent with known vincristine depression of RNA synthesis. On the other hand, increased delivery of cells from the dormant phase was necessary to reproduce some in vivo cytosine arabinoside effects. Useful proposals for further studies can thus be derived. (Research supported by NIH Grants CA04826 and CA05196.)

218. Platelet-Binding Antibodies Produced in Vitro by Idiopathic Thrombocytopenic Purpura (ITP) Spleens. ROBERT MCMILLAN,* ROBERT LONGMIRE,* ROBERT YELENOSKY,* AND CHARLES G. CRADDOCK,** La Jolla, Calif.

Although the spleen is the prime platelet destruction site in most cases of idiopathic thrombocytopenic purpura (ITP), its contribution to synthesis of the humoral antiplatelet factor(s) (APF) is unclear. Experimental and clinical evidence suggest that APF is probably an immunoglobulin (Ig) directed against a platelet-associated antigen. Using a sensitive Fab–anti Fab inhibition system for Ig quantitation, we have measured the in vitro synthesis of Ig by splenic leukocytes from seven normal subjects and five patients with ITP. Splenic leukocyte suspensions were prepared from surgical specimens and cultured for 10 days at 37°C in 20% fetal calf serum in Dulbecco's medium. Mean Ig synthesis for normal splenic cells was 625 ng $N/20 \times 10^6$ leukocytes (range 325–800); ITP splenic cells produced an average of 3300 ng $N/20 \times 10^6$ leukocytes (range 1200–4400). These ITP values are similar to the Ig synthesis of normal splenic cells after in vitro exposure to secondary antigens (e.g. small pox). Several cultures from one ITP spleen were pooled, concentrated, and aliquots incubated with homologous human platelets of the same blood type. After incubation, platelet-associated Ig increased significantly (>40%) and the Ig in the incubation supernate decreased proportionally. No such effect was seen with similarly handled control cultures. These data show that (*a*) ITP spleens produce about 5 times more Ig than normal spleens; and (*b*) a major portion of this Ig binds to human platelets and may represent APF. It is suggested that in some cases of ITP, the spleen is the major production site of APF and may be the only area where high enough antibody concentrations exist for platelet destruction. (Research supported by NIH Grant CA-11800.)

219. Hypophosphatemia and Hypouricemia during Parenteral Hyperalimentation with an Amino Acid–Glucose Preparation. R. METZGER,* P. BURKE,* A. THOMPSON,* R. LORDON,* AND G. W. FRIMPTER, San Antonio, Tex.

Striking hypophosphatemia and hypouricemia were observed in all of eight patients receiving a mixture of synthetic essential and nonessential amino acids in 20% glucose solu-

tion. Preinfusion mean serum phosphate (P) and uric acid (UA) concentrations were 3.0 ±0.5 mg/100 ml and 6.0 ±2.4 mg/100 ml respectively. After 5 or more days of hyperalimentation mean P was 0.8 ±0.4 mg/100 ml and UA was 2.4 ±1.9 mg/100 ml. Both abnormalities are statistically significant (P: $P < 0.001$; UA: $P < 0.01$). Mean serum creatinine concentrations before and after initiation of the infusions were unchanged (1.3 ±0.9 mg/100 ml and 1.0±0.9 mg/100 ml respectively, $P > 0.5$). Aminoaciduria including not only those amino acids administered but some intermediary compounds such as ornithine and cystathionine was noted. Plasma concentrations of amino acids were generally increased to 2–3 times normal. Comparison of amino acid clearances with inulin clearance in three patients showed normal tubular reabsorption of amino acids. There was an elevated uric acid clearance (mean 23.0 ±1.8 ml/min) and increased tubular reabsorption of phosphate (mean 98.2 ±1.8%). The data suggest that extravascular deposition of phosphate and a selective inhibition of tubular reabsorption of urate are induced by this amino acid–glucose preparation.

220. Infectious Mononucleosis: Appearance of Epstein-Barr Virus Neutralizing Activity during the Course of Disease. GEORGE MILLER* AND JAMES C. NIEDERMAN,* New Haven, Conn. (introduced by Dorothy M. Horstmann**).

One in vitro assay for the biologic activity of Epstein-Barr virus (EBV) measures the capacity of EBV to induce continuous growth of peripheral blood leukocytes (WBC). In the absence of added EBV small numbers of WBC do not form cell lines. The effect of pre-illness and convalescent phase sera from infectious mononucleosis (IM) cases on the capacity of EBV to stimulate lines from WBC was studied. In the small number of matched samples tested to date neutralizing activity was absent from pre-illness specimens and appeared after clinical disease. All sera were tested at a 1:4 dilution. Neutralizing activity was usually present as soon as clinical disease was apparent and was demonstrable in sera obtained years after illness. Since viral neutralization probably reflects interaction of antibody with the lipoprotein viral envelope, the results suggest that in IM production of complete enveloped viral particles occurs. The results also support the concept derived from previous serologic studies with immunofluorescence and complement fixation that in IM there is a primary infection with EBV. (Supported by Grant JCC257 from the Jane Coffin Childs Memorial Fund for Medical Research, by Grant IN-31-K from the American Cancer Society, and NIH Grants AI-05577, AI-08731, and FR-05358.)

221. Effect of Induced Glucose Intolerance on the Fetal Insulin-Releasing Mechanism. D. H. MINTZ,* R. A. CHEZ,* AND D. L. HUTCHINSON,* Miami, Fla., and Pittsburgh, Pa. (introduced by Solomon Papper**).

Progressive deterioration throughout gestation in maternal glucose tolerance and plasma insulin responses to intravenous glucose were documented after the administration of streptozotocin to 16 pregnant monkeys (*Macaca mulatta*) in the first trimester. During late gestation, fetal interplacental vessels were cannulated and the fetal and maternal plasma glucose and insulin responses to direct intravascular injections of glucose to the fetus were examined. There was a prompt 2- to 5-fold increase in fetal plasma insulin levels in response to the glucose. These findings contrast with the unresponsiveness of the fetal insulin-releasing mechanism in normal subhuman primate pregnancy. To identify a mechanism for this alteration, fetuses from normal pregnancies were infused with theophylline combined with glucose or with dibutyryl cyclic AMP (DBC). Glucose combined with theophylline elicited a prompt rise in fetal plasma insulin, whereas theophylline, alone, did not. DBC also resulted in a prompt fetal insulin response, before any alteration in fetal plasma glucose. It appears that if sufficient cyclic AMP accumulates in the fetal pancreatic beta cell, glucose-mediated insulin release will occur. The data suggest that there is an enhancement of adenyl cyclase activity and/or a reduction in phosphodiesterase activity in the fetal islet as a consequence of maternal induction of fetal hyperglycemia. Thus, the insulin-release mechanism is altered so that fetal hyperglycemia becomes a sufficient stimulus for insulin release. These data also confirm the hypothesis that *in utero* fetal hyperinsulinemia exists in pregnancies complicated by maternal hyperglycemia, as in diabetes mellitus.

222. Studies on the Interaction of the Micellar Aggregate with the Surface of the Hamster Small Intestine. S. MISHKIN,* M. YALOVSKY,* AND J. I. KESSLER, Montreal, Canada.

It is accepted that the end products of lipid digestion undergo micellar solubilization before absorption. The manner in which the micellar aggregate interacts with the epithelial surface and allows the preferential absorption of its fatty acid–monoglyceride component is not known. Everted sacs from proximal and distal small intestine were incubated for 10 min at 37°C in 10 ml of a micellar solution containing labeled palmitic acid (PA), taurodeoxycholate (NaTDC), and inulin, and efflux of these compounds was measured by sequential 1 min rinsings in separate 20-ml volumes of ice-cold Krebs-Ringer phosphate (KRP) buffer or 2.5% bovine albumin (BA) for a total of 25 min. The radioactivity in each rinsing volume, tissue homogenates, and serosal fluids was assayed. A considerable fraction of the labeled substances taken up during the initial incubation could be released by rinsing. Rinsing in BA resulted in the release of PA in excess of that released in KRP buffer. The release of PA was inversely related to the esterifying capacity of the intestine and irrespective of the rinsing solution, the distal small intestine released a greater fraction of PA. Greater amounts of NaTDC and inulin than that of PA were released from both proximal and distal intestine. Analysis of the kinetics of efflux of each substance into KRP indicated that efflux occurred from two compartments, one rapidly and one slowly turning over. The $t_{\frac{1}{2}}$ of efflux of PA and NaTDC from the rapidly turning over compartment were similar to those of inulin, suggesting that superficial binding may be involved in the reversible uptake of these substances. The efflux of PA in BA could be resolved into a single exponential curve with a $t_{\frac{1}{2}}$ of efflux different from that in KRP. A large excess of NaTDC relative to PA was found in each of these compartments. The results indicate that the uptake of the micellar aggregate involves the reversible binding of its components to the epithelial surface and provides an explanation for the preferential uptake of fatty acids by the proximal small intestine.

223. Brainstem Nuclei Mediating Baro- and Chemoreceptor Vasomotor Reflexes. M. MIURA* AND D. J. REIS, New York.

Myelinated (A) fibers of the carotid sinus nerve (CSN) terminate in two nuclei of cat brainstem: the nucleus tractus solitarii (NTS) and paramedian reticular nucleus (PRN). The PRN also serves to integrate control by cerebellum of baroreceptor reflexes. It is not known if baro- and chemoreceptor A-fibers of CSN project to both NTS and PRN, if unmyelinated (C) fibers project to either, nor whether one or both nuclei mediate the blood pressure responses to baro- or chemoreceptor stimulation. We have studied the effects of bilateral electrolytic lesions of NTS or PRN on the depressor response to electrical stimulation of CSN (A baro- and chemoreceptors), the depressor response to sinus stretch (A and C baroreceptors), and the pressor response to close carotid injection of lobeline (A and C chemoreceptors) in cat. Each nucleus was explored with microelectrodes for pulse synchronized or modulated units silenced by carotid occlusion (baroreceptor units) or excited by lobeline (chemoreceptor units). *Lesions of PRN* abolished or reversed depressor responses to CSN stimulation, attenuated depressor responses to sinus stretch, and augmented pressor responses to lobeline. *NTS lesions* (at obex) abolished all reflex responses. Baroreceptor and chemoreceptor units were found in both nuclei. We conclude that (*a*) PRN primarily mediates depressor responses from A-baroreceptors; (*b*) NTS mediates reflex responses to C-baroreceptors and all chemoreceptors; (*c*) the chemoreceptor pressor reflex is buffered by baroreceptors; (*d*) baro- and chemoreceptor afferents of CSN project into both NTS and PRN; and (*e*) vasomotor reflex responses from baro- and chemoreceptors are subserved by different specific nuclei in the medulla. (Supported by NIH grant.)

224. The Pathogenesis of Canine Brucellosis: an Immunologic Deficiency Disease. RICHARD MORISSET,* CRAIG W. HOWE,* AND JOSEPH J. SOCKALOSKY,* Minneapolis, Minn. (introduced by Wesley W. Spink**).

Widespread epidemics of brucellosis first occurred in beagles in 1966 caused by a mucoid strain of brucella, *Brucella canis*. A remarkable feature is prolonged bacteremia observed up to 3 yr. Human cases have been reported. As part of an immunological study the bactericidal activity of beagle serum was investigated. The in vitro antibacterial activity of sera from normal and infected humans, beagles, and rabbits was compared with *Br. canis* and *Brucella abortus*. The sera of normal poodle, mongrel, horse, and cow also were compared. Serial, tenfold brucella suspensions were added to equal quantities of fresh serum. Results reveal the following. (*a*) A heat-labile factor is essential for antibrucella activity. (*b*) Normal canine serum lacks bactericidal activity for *Br. canis* and had less killing activity than the human, cow, and horse sera for *Br. abortus*. However, bactericidal activity for smooth and rough strains of *Escherichia coli* is not decreased. (*c*) Chronically infected beagles demonstrate absent or slight killing effect against both *Br. canis* and *Br. abortus*. (*d*) An inhibitor of the lethal activity of normal beagle serum for *Br. canis* can be removed by immunoadsorption. (*e*) There is no cross-reaction between *Br. canis* and *Br. abortus* bactericidal antibodies of humans and rabbits. (*f*) There was no inhibition of the lethal activity for *Br. canis* with hyperimmune rabbit serum against *Br. canis* such as occurs with human and hyperimmune rabbit serum against *Br. abortus*. These investigations revealed a deficient antibrucella activity in the sera of canine species, and immunogenic properties of *Br. canis* that differ from *Br. abortus*. These features may contribute to the chronicity of the canine disease. (Research supported by AI-04415-09 and 5-TO1-AI-00194-09 from NIH.)

225. The Effect of Mannitol on Glomerular Filtration during Hypoperfusion. C. RICHARD MORRIS,* FRANK J. BRUNS,* EDWARD A. ALEXANDER,* AND NORMAN G. LEVINSKY, Boston, Mass.

In recent studies we found that in renal hypoperfusion 76% of nephrons filtered during mannitol infusion, 12% during saline, and <1% during hydropenia. To extend these observations, we have used a quantitative modification of the Hanssen technique. The minimum detectable nephron glomerular filtration rate (NGFR) in hydropenic rats is 0.5 nliter/min or less. NGFR was not detected in 26 superficial or 26 deep nephrons in three hydropenic animals when perfusion pressure was 40 mm Hg. During mannitol infusion, GFR was 4.7 ±0.9 (SE) nliters/min in 52 superficial and 5.7 ±0.9 nliters/min in 49 deep nephrons. C_{In} was proportionately reduced to 0.13 ±0.02 ml/min. (In one additional rat, NGFR was present but less than 2 nliters/min.) In the above studies mannitol was started before hypoperfusion was induced. The following experiments were performed to determine whether GFR could be reestablished if mannitol were infused after a period of hypoperfusion anuria. Hydropenic rats were given either 5% mannitol in saline (n = 3), 0.9% or 1.7% saline (n = 6), or furosemide (100 mg/kg) (n = 3) after 15 min of anuria at 40 mm Hg. GFR was reestablished in every animal given mannitol (C_{In} 0.29 ±0.08 ml/min). Animals given furosemide remained anuric. Saline controls were anuric except for transient urine flow in one animal. We conclude that during renal hypoperfusion NGFR ceases in hydropenic rats. Mannitol given before or after induction of hypoperfusion maintains significant filtration in nephrons which otherwise would not filter. Effects of hypoperfusion and of mannitol in superficial and deep nephrons are equivalent. The mechanism by which mannitol acts is uncertain. It is not due solely to decreased plasma oncotic pressure. Moreover, mannitol need not be present in the tubular lumen when the effect is initiated. (This work has been supported by USPHS Grants AM 11793, AM 14004, and 5T01-AM5209.)

226. Parathyroid Hormone Modulation of Renal Acidification. R. CURTIS MORRIS, JR., ELISABETH MCSHERRY,* AND ANTHONY SEBASTIAN,* San Francisco, Calif.

In a patient with hereditary fructose intolerance (HFI), hypoparathyroidism, and no evidence of renal disease, we investigated the possibility that circulating parathyroid hormone (PTH) modulates the fructose-induced renal acidification defect of the proximal tubule (RAD). At experimentally sustained blood fructose concentrations $[fructose]_b$ of 20 mg/100 ml the RAD remained strikingly attenuated until parathyroid extract was initiated (intravenously, 1 U/kg per hr), whereupon there occurred almost immediately a RAD of

the severity anticipated with euparathyroidism. Glomerular filtration rate did not change significantly. Whenever fructose and PTH were administered in sustained combination, irrespective of sequence, the full-blown experimental renal dysfunction occurred almost immediately but never when PTH was withheld, despite $[fructose]_b$ >25 mg/100 ml. At $[fructose]_b$ of 7 mg/100 ml, the RAD occurred only after PTH was initiated. In euparathyroid patients with HFI, the hypercalciuria induced by fructose could entrain a positive feedback loop comprised of hypocalcemia, → increased concentration of circulating PTH, *further* amplification of the renal dysfunction → further hypercalciuria. In the euparathyroid monozygotic twin of the patient with HFI and hypoparathyroidism, medullary nephrocalcinosis is striking, and apparently "classic" renal tubular acidosis persists. In patients with HFI who continue ingesting fructose, euparathyroidism may compel not only the expression of renal dysfunction but the operation of an amplifying feedback loop capable of causing, and critical to, "natural progression" of the renal disorder. In normal subjects and animals: (*a*) the experimental PTH-induced renal acidification defect is like that of "proximal" RTA; (*b*) experimentally induced hypercalcemia significantly increases T_m HCO_3^- and renal acid excretion (during NH_4Cl-induced acidosis); and (*c*) metabolic alkalosis attends surgical hypoparathyroidism. These findings strongly suggest that normal concentrations of circulating PTH dampen the H^+ secretory process of the normal proximal tubule.

227. Gluconeogenesis in Toad Urinary Bladder. ANTHONY D. MORRISON,* DAVID B. P. GOODMAN,* ALBERT I. WINEGRAD, AND HOWARD RASMUSSEN, Philadelphia, Pa.

Although the toad bladder has been extensively used as an in vitro system for the study of hormonal effects, gluconeogenesis in this tissue has gone undetected. In the course of studies that demonstrated the presence of the polyol pathway (glucose --> sorbitol --> fructose) in toad bladder, small, but significant concentrations of free glucose were consistently found in tissue incubated for 18 hr without substrate. During a subsequent 3 hr incubation, without added substrate, free glucose was released into the medium at the rate of 12.0 ±1.3 nmoles/g wet weight per hr. The addition of lactate (10 mmoles/liter) resulted in a doubling of the rate of glucose release (mean Δ + 13.1 ±2.1 nmoles/g wet weight per hr) and an increase in tissue glycogen (mean Δ + 92 ±22 nmoles glucose equivalents/g wet weight). Gluconeogenesis from lactate was significantly greater at pH 6.8 than at pH 7.4. Preincubation with aldosterone (10^{-7} mole/liter) increased the rate of glucose release by tissue incubated with lactate (mean Δ + 21.1 ±4.5 nmoles/g wet weight per hr), and also increased the glycogen content. Aldosterone, however, did not alter the rate of glucose release or the glycogen content of toad bladders incubated without substrate. Glucose release by toad bladders incubated with lactate was unaltered by preincubation with cortisol (10^{-7} mole/liter), or by the addition of vasopressin (100 mU/ml). The toad bladder which subserves some of the functions of the mammalian kidney has a capacity for gluconeogenesis; this process is subject to regulation by pH and by aldosterone.

228. A Study of the Androgens and Other Substances in Human Serum Determined by Competitive Protein Binding to the 17β-Hydroxy Steroid-Binding Globulin. BEVERLEY E. P. MURPHY, V. K. GANJAM,* P. A. CURRIE,* AND T. H. CHAN,* Montreal, Canada.

The human sex hormone–binding globulin (SHBG) has been shown to bind strongly only unconjugated steroids having a 17β-hydroxy configuration including 5α-dihydrotestosterone, 5α-androstanediol, testosterone, androstenediol, estradiol, and, very recently, estr-5-enediol which has an affinity more than twice that of testosterone. Since binding affinity seems to be closely related to biological activity it seemed pertinent to investigate the nature of the SHBG-bindable activity of serum which was previously shown to exceed that expected on the basis of the known gonadal hormones. A method was developed whereby the various steroids (excluding estrogens removed by alkaline washing) could be separated and measured individually by competitive protein binding (CPB) using testosterone-^3H as a tracer. Peripheral serum samples, 10 ml or less, from 40 subjects were extracted into diethyl ether, evaporated, and passed through a column of Sephadex LH20. From each column 100 eluate samples were collected, evaporated, and assayed by CPB to give a pattern for each serum. The identity of the eluted steroids was confirmed (for all but the terminal fractions tentatively identified as androstenediol and estrenediol, which are still being processed) by treating 1300 ml of pooled plasma in a similar fashion and analyzing the eluted fractions by mass spectrometry. The major peaks observed in normal adults (with mean concentrations in nanograms per milliliter) were in men, testosterone 5.0, dihydrotestosterone 0.48, estrenediol 0.82, and androstanediol 0.12; in women, estrenediol 0.41, androstanediol 0.32, testosterone 0.30, and dihydrotestosterone 0.12. While the biological role of the steroid tentatively identified as estrenediol remains to be clarified, these results indicate that there are at least three potent circulating androgens and suggest that testosterone, while of prime importance in men, may be of no greater importance than dihydrotestosterone and androstanediol as an index of androgenicity in women. (Supported by the Medical Research Council of Canada.)

229. The Estimation of Platelet Survival from Population Curves. EDMOND A. MURPHY AND MILDRED E. FRANCIS,* Baltimore, Md.

The following developments are reported of the multiple hit model for platelet population curves previously described (1967. *J. Clin. Invest.* **46**: 1099).

$$H(t) = \frac{a}{n}\int_t^\infty \frac{a^n}{(n-1)!}\int_x^\infty e^{-ay}y^{n-1}dydx$$

where t = time for labelling, n = number of hits to destruction, 1/a = mean waiting time between sibs, and c = extent of the initial labelling. (*a*) The model is robust, i.e., (compared with the usual experimental error) insensitive to time-heterogeneity of risk, variation in the size of environmental insults, and the existence of refractory periods (which are departures from the underlying assumptions of the multiple-hit model). (*b*) Experimental error determined by blind replication is acceptably close to Gaussian and with homogeneous

variance. Thus least-squares is also maximum-likelihood estimation and fully efficient. (*c*) Computer simulation with the multiple hit model and independent, normally-distributed errors has been used to determine the mean and bias of the estimates of *n*, *a*, and *c* and their variance-covariance matrix; and also the estimate of mean survival (n/a). (*d*) It is shown that (probably because of the robustness of the model) the estimates of *n* and *a* may be badly biased and have large variances, but that of the mean, n/a, is satisfactory. (*e*) These considerations make it possible to evaluate mean survival for a variety of kinds of platelet survival curve, regardless of the duration of the period of observations. This method has wide applications to study of survival of any element subject to fatigue by repeated trauma (senescence, tooth decay, etc). (Supported by grants from NIH and TIRC.)

230. Control of Parathyroid Secretion in Primary Hyperparathyroidism. TIMOTHY MURRAY,* MUNRO PEACOCK,* DAVID POWELL,* ROBERT NEER,* LEONARD DEFTOS,* AND JOHN POTTS, JR., Boston, Mass.

Parathyroid hormone (PTH) was measured by radioimmunoassay under basal conditions and during EDTA and calcium infusions in 19 patients with primary hyperparathyroidism due to parathyroid adenoma (12 patients), chief-cell hyperplasia (six patients), and parathyroid carcinoma (one patient). Basal plasma PTH ranged from 0.14 to 5.3 mμg/ml. These values showed a significant overlap with the values obtained in 30 normal adults. However, the two groups were more easily separated by plotting PTH as a function of the basal serum calcium. PTH was undetectable in plasma from six patients with hypercalcemia of etiology other than hyperparathyroidism. In 16 of the patients with primary hyperparathyroidism, EDTA-induced hypocalcemia was associated with an increase in plasma PTH ($P < 0.001$) from 1.5- to 8-fold. The degree of response correlated with the weight of parathyroid tissue found at surgery; there was no difference in response between patients with adenomas and those with hyperplasia. In 11 hyperparathyroid patients given calcium infusions, the induced hypercalcemia produced a fall in plasma PTH ($P < 0.001$). In eight patients with adenomas, the mean fall in PTH was to 45% of the basal concentration. Plasma PTH rose and fell significantly in all patients with primary hyperparathyroidism that were tested. Autonomy is not the mechanism of excessive PTH secretion in these patients, regardless of histological type. The defect must be an inappropriately high threshold for suppression of PTH secretion by blood calcium. (Research supported by grants from NIH NASA, and the John A. Hartford Foundation.)

231. Functional Properties of High Affinity Hemoglobin Variants: Hb J Capetown and Hb Hiroshima. RONALD L. NAGEL* AND QUENTIN H. GIBSON,* New York and Ithaca, N. Y. (introduced by Helen M. Ranney**).

Hemoglobin J Capetown ($\alpha_2^{92gln}\beta_2$) exhibits a twofold increase of oxygen affinity and probably a normal Bohr effect. The combination velocity constant for CO is less than two times greater than that for A, while the dithionite reaction with oxy Hb J Capetown shows a decreased rate as compared with Hb A. These data suggest that the kinetic basis of the high affinity of this hemoglobin variant is a combination of an increased combination velocity and a decreased dissociation velocity for ligands. The most striking finding is the presence of considerable cooperativity (n in Hill's equation = 2.2) in the reaction of the hemes with ligands. The difference between the rate of the replacement reaction of O_2 by CO and the rate observed with the dissociation of O_2 by dithionite was comparable to Hb A, confirming the existance of cooperativity. Hemoglobin Hiroshima ($\beta^{143\ His \rightarrow Asp}$) has a fourfold increase in oxygen equilibria and a decreased Bohr effect. Based on a decreased rate of dissociation of O_2 by dithionite and an increased rate of binding of CO by the deoxy form, we have concluded that the kinetic basis of the high affinity exhibited by Hb Hiroshima is also the concurrence of a faster combination rate and a slower dissociation rate for ligands. Mutants capable of altering the ligand affinity and cooperativity of hemoglobin are substitutions that affect the FG corner and the H helix near its C-terminal portion. These changes could be brought about by interference with H23-FG5 hydrogen bond formation. (Supported by NIH and N. Y. Heart.)

232. Acute Variations of Serum Luteinizing Hormone (LH) in Men and Women. HOWARD NANKIN* AND PHILIP TROEN, Pittsburgh, Pa.

To investigate the constancy of circulating LH, venous blood was obtained by indwelling catheters from resting normals at 10-min intervals for 1.5–4 hr. LH was measured by radioimmunoassay (in part NIH-NPA reagents). Nine men (22–36 yr) were studied 13 times. Four women (22–24 yr), not at mid-cycle, participated in seven studies. In 19 of 20 studies at least one trough of two or more values was followed by a sharp rise and peak level of hormone (means +46% in men and +62% in women) over the next 10–20 min. In six studies, the time for LH to fall to a value one-half the peak value averaged 69 min (58–85). The decreases appeared to be linear. In men, 15 peaks were measured during 28.5 hr of observation; 11 peaks during 18.5 hr in the women. In males the increases ranged from 19 to 123%, while 28–278% was the range of increases in women. The mean increase in females of 43.6 mμg/ml (range 23.1–120.8) was almost twice the mean in males—24.3 mμg/ml (15.6–53.7). In six of seven subjects studied twice, the repeat patterns were similar. The sharp rise followed by a slower fall of LH values is consistent with a sudden increased discharge of LH followed by a slower reduction of secretion. The cause, or role, of this is unknown. The demonstration of a pattern of wide changes in a relatively short time period provides a basis for the observed wide range of LH values reported in normals. The pattern may also be important in assessing the significance of individual values in the evaluation of patients.

233. Defective Cellular Immunity in Liver Disease: Depression of Reactivity of Lymphocytes to Phytohemagglutinin by Liver Disease Sera. W. M. NEWBERRY,* J. W. SHOREY,* AND B. COMBES, Dallas, Tex.

Defects in leukocyte function have been demonstrated in patients with acute hepatitis and primary biliary cirrhosis. The pathogenetic mechanism responsible for these defects has not been established, although serum factors have been implicated in hepatitis. The present studies were designed to appraise the effect of serum from patients with various types of

liver disease on normal lymphocyte responses. Lymphocyte function was assessed in vitro by thymidine uptake after stimulation with phytohemagglutinin M. The lymphocytes were obtained from healthy donors and incubated with sera from patients with liver disease or from normal controls different from the cell donors. The liver diseases were Australia antigen–positive (4) and –negative (1) hepatitis, extrahepatic obstruction (3), primary biliary cirrhosis (1), and massive necrosis due to halothane (1). Sera from all 10 patients depressed cell response by 41–93%. Suppression cannot be correlated to levels of serum bilirubin, transaminases, or alkaline phosphatase. Sera from two jaundiced patients with common duct stones initially demonstrated 71 and 85% suppression, whereas 1 wk after surgical correction the sera of these patients supported normal lymphocyte responses. Moreover, the presurgical serum from one of these patients when mixed with normal serum resulted in greater suppression than corresponding mixtures of normal serum with culture medium. This indicates the presence of an inhibitory factor rather than the lack of an essential supporting factor. The addition of bile acids to lymphocyte cultures in normal serum resulted in suppression, suggesting that elevated bile acids may be a contributing inhibitory factor. (Research supported by grants from NIH.)

234. Differential Sensitivity of Epithelial and Fibroblast Cell Types to Virus-Induced Chromosome Breaks. WARREN W. NICHOLS, ALBERT LEVAN,* LARS KJELLÉN,* AND SUE SHELDON,* Camden, N. J.

Epithelial and fibroblast cell lines derived from the same fetus were exposed to adenovirus type 5. After this the chromosomes of the cells were examined in anaphase at various times to relate the time of virus infection to the cell cycle. The epithelial cells exhibited much higher levels of chromosomal damage than the fibroblasts (a maximum of 74% vs. a maximum of 32%), and also a difference in susceptibility in relation to the cell cycle. The epithelial cells revealed maximum chromosome damage after only 2 hr exposure, indicating that the G2 and mitotic portions of the cell cycle were susceptible. Fibroblasts exhibited no chromosomal damage after 2 hr, little damage after 6 hr, and regular chromosomal damage only after 24 hr. This difference in susceptibility may be related to the differentiation of the two cell types and may be important in a differential susceptibility of cells, tissues, or organs to a carcinogenic or teratogenic action of specific viruses. The observations on the epithelial cells indicate that cellular DNA synthesis is not required for induction of chromosomal abnormalities in this system. The materials offer interesting systems for the comparative study of the biochemical mechanisms of virus-induced chromosome defects. (This research was supported by Research Career Award 5-K3-16,749 and General Research Support Grant FR-5582 from the NIH; State of N. J. Grant-In-Aid Contract M-43; and by grants from the Swedish Cancer Society and the John and Augusta Persson Foundation.)

235. Observations on Monocytes, Pulmonary Macrophages, and Large Alveolar Cells. ALBERT H. NIDEN,* Philadelphia, Pa. (introduced by Sol Sherry**).

The relationship between the intravascular monocyte, the pulmonary macrophage, and the large alveolar cell remains controversial. In order to study the interrelationship, if any, between these cells, Thorotrast (colloidal thorium dioxide) was used as an electron-dense marker for the monocyte. Thorotrast was injected intravenously into unanesthetized mice and rabbits. Animals were sacrificed at 24-hr intervals from 1 to 9 days after injection. Lung tissue was prepared for both light and electron microscopic examination. In addition, lung washings were obtained from rabbits after injection. Pellets of cells from these washings were prepared for electron microscopy. 24 hr after injection, the plasma was clear of free Thorotrast. Light microscopic findings revealed many Thorotrast-laden mononuclear cells in the lung of all animals. The labeled cells were indistinguishable from large alveolar cells at this magnification. Alveolar macrophages containing Thorotrast were seen as early as 24 hr after injection. Electron microscopic findings confirmed the presence of Thorotrast in alveolar macrophages both in lung tissue as well as the pellet from the alveolar wash. However, the remainder of the Thorotrast-containing mononuclear cells were intravascular monocytes filling many of the pulmonary capillaries. In contrast, the large alveolar cells contained no Thorotrast. From the above observations it is concluded that (*a*) the lung is a reservoir for intravascular monocytes; (*b*) the monocyte is a precursor of the alveolar macrophage; (*c*) the large alveolar cell does not appear to be related to the monocyte-macrophage system; and (*d*) the intracapillary pulmonary monocyte is indistinguishable from the large alveolar cell at the resolution of the light microscope. This observation questions the validity of previous light microscopy studies whose results were dependent on the proper identification of the large alveolar cell. (Research supported by NIH, Council Tobacco Res.)

236. Cell-Free Hemoglobin Synthesis by β-Thalassemia Ribosomes. A. W. NIENHUIS,* J. M. GILBERT,* A. G. THORNTON,* D. G. LAYCOCK,* R. G. CRYSTAL,* AND W. F. ANDERSON,* Bethesda, Md. (introduced by Theodore Cooper).

Reticulocyte ribosomes obtained from patients with β-thalassemia have been utilized in developing a highly active cell-free protein-synthesizing system in order to investigate the nature of the defect in this disease. The system actively initiates new globin chains and produces in vitro an α/β–chain discrepancy similar to that demonstrable in intact thalassemia cells. Besides supernatant proteins, tRNA, amino acids, and an energy source, the system requires a 0.5 M KCl ribosomal wash fraction. This wash fraction, containing the initiation factors M_1, M_2, and M_3, is required for the formation of the initial peptide bond of rabbit globin chains. By interchanging components between the thalassemic and human nonthalassemic (or rabbit) cell-free systems, it has been shown that the thalassemic initiation factors, supernatant proteins, and tRNA appear to be normal. An RNA fraction containing 8-9S rabbit reticulocyte mRNA was isolated from a sucrose gradient of SDS-treated rabbit reticulocyte polysomes. When this RNA fraction was added to thalassemic ribosomes in the above described cell-free system, an 8-fold stimulation of protein synthesis was produced. Other RNA fractions (28S, 18S, or 4S) produced no stimulation. Carboxymethyl-cellulose chromatography demonstrated that rabbit alpha and beta globin chains were synthesized on thalassemic ribosomes in

response to rabbit globin mRNA. Nonthalassemic human ribosomes were stimulated by rabbit mRNA to produce rabbit globin chains in a similar manner. Thus, the response of thalassemic ribosomes to exogenous heterologous globin mRNA appears similar to the response of nonthalassemic human ribosomes. It appears likely therefore, that the β-thalassemic defect is in the human β-globin mRNA itself, either as a decrease in the amount of message or as an alteration in its nucleotide sequence.

237. Lack of Immunologic Response to Bacteriophage ØX 174 in X-Linked Agammaglobulinemia (X-A). HANS D. OCHS,* STARKEY D. DAVIS,* JOAN D. CRAIN,* FRED S. ROSEN, AND RALPH J. WEDGWOOD,** Seattle, Wash., and Boston, Mass.

Without a positive family history, X-A may be indistinguishable from many other immunodeficiency diseases. We examined the immunologic responses to bacteriophage ØX 174 in 12 patients with X-A, 21 patients with other various immunodeficiency diseases, five normal adults, six immunologically normal children, and one immunologically normal newborn infant. ØX 174 is a potent antigen and the antibody assay is sensitive. Phage was given intravenously, and blood samples were tested at intervals for phage titer or antibody. In normals, phage remained in the blood in high concentration (10^7 plaque-forming units or pfu/ml) for 48 hr, then rapidly cleared from the circulation by the 4th day with the onset of antibody synthesis. No normal had phage in the blood after 4 days. In contrast, 12 patients with X-A had a high titer of phage present at 7 days (mean 2×10^6 pfu/ml) and a mean phage circulation time of 25.6 days (range 11–42 days). Four patients with X-A were given phage a second time and all again had prolonged circulation of phage. Such prolonged circulation of phage has previously been seen only in thymic dysplasia. 20 of the other 21 patients studied cleared the phage by the 4th day. One had 40 pfu/ml at 7 days and no phage at 14 days. All normals made brisk antibody responses. No X-A patient made detectable antibody. 20 of the 21 immunologically deficient patients made low but detectable antibody responses. If thymic dysplasia is excluded, persistence of phage in the circulation for 11 days or more and absence of a detectable antibody response appear to be specific criteria for the diagnosis of X-A from birth onward. (Research supported by Grant AI 07073 from NIH.)

238. Unsaturated and Saturated Long-Chain Fatty Acids: Differences in Intestinal Absorption. R. K. OCKNER,* J. P. PITTMAN,* AND J. L. YAGER,* San Francisco, Calif. (introduced by L. H. Smith, Jr.**).

Previous studies showed that during absorption of unsaturated fat, chylomicrons (>800 A, $S_f > 400$) predominate in intestinal lymph, whereas with saturated fat very low density lipoproteins (300–800 A, S_f 20–400) are more abundant. To explain these observations, possible differences in absorption of linoleic acid (LA unsaturated) and palmitic acid (PA saturated) were explored. Rats received 5 ml intraduodenal micelles containing 28.8 mM ^{14}C-labeled LA or PA, 14.4 mM monoolein, and 20 mM taurocholate (TC) over 30 min. 15 minutes later, the intestine was removed and divided into five segments after recovering lumenal contents; lipids were assayed for radioactivity. Total recoveries of LA and PA were similar, but only 2.8% of LA remained in the lumen, compared to 22.2% of PA. The less efficient absorption of PA was reflected by greater uptake in distal 3/5 of intestine (49.2% of absorbed PA vs. 3.1% of LA). The basis for these differences was examined with everted jejunal sacs, incubated in micelles containing 10 mM TC, 0.9–7.2 mM ^{14}C-labeled LA or PA, and 0.45–3.6 mM monoolein. PA uptake always equaled or exceeded LA, and 1-min uptake of both showed saturation kinetics (V_{max} 1.5 μmole/min per g sac; K_m PA 5.3 mmoles/liter, LA 6.6 mmoles/liter). In contrast, relative and absolute esterification of LA was consistently greater, and at 7.2 mmoles/liter was at least twice PA. Moreover, esterification was inhibited by high PA, but not by LA. Finally, decreasing TC further reduced PA esterification without changing uptake, whereas LA was little affected. These studies show saturation kinetics for initial jejunal fatty acid uptake, with PA ≧ LA. In contrast, LA esterification exceeds PA, which is further reduced by high PA and low TC. Over-all jejunal absorption of LA exceeds PA; PA therefore utilizes distal intestine. These findings may account for greater absorption of unsaturated as compared to saturated fats reported in patients with steatorrhea, and for the observed differences in intestinal lipoprotein production.

239. Effects of Transfer Factor in Cancer Patients. HERBERT OETTGEN,* LLOYD OLD,* JOSEPH FARROW,* FRED VALENTINE,* SHERWOOD LAWRENCE,** AND LEWIS THOMAS,** New York.

Delayed hypersensitivity reactions are often impaired in cancer patients. We investigated the effects of dialysable transfer factor (TF_d), an agent known to reconstitute cellular immunity. We postulated that (*a*) malignant transformation is much commoner than clinical cancer, (*b*) incipient cancers are frequently eliminated by a cell-mediated immune reaction, (*c*) clinical cancer reflects a deficiency of this mechanism, and (*d*) sensitivity to cancer antigens (like normal alloantigens) can be transferred with TF_d. Healthy adults were therefore selected as TF_d donors. Initial trials were conducted in five patients with advanced breast cancer of the "inflammatory" type. Pooled TF_d, 1–4 ml, was injected subcutaneously at sites distant from the cancer, daily or three times weekly, over periods ranging from 21 to 310 days. The total volume given to individual patients was 20–257 ml, equivalent to the same volume of packed blood leukocytes. TF_d did not elicit inflammatory or hypersensitivity reactions or detectable formation of antibody to itself, nor any hematological or biochemical abnormalities or other side effects. Delayed sensitivity to tuberculin and/or streptococcal antigens was transferred to three patients, one of whom also experienced partial regression of her tumor which lasted 6 months. We conclude that (*a*) large amounts of TF_d can be given safely, (*b*) delayed hypersensitivity can be transferred to cancer patients with TF_d, and (*c*) treatment with TF_d may inhibit the growth of advanced cancer in some patients. Extension of therapeutic trials to patients with minimal residual cancer seems justified and timely now that specific TF_d donors can be selected by tests for sensitization to distinct antigens that have been identified in several types of human cancer. (Supported by grants from NCI, Fleischmann Foundation, and USPHS.)

240. Arteriolar Resistances and Effective Filtration Pressure in Glycerol-Induced Acute Renal Failure (ARF) in Rats. DONALD E. OKEN AND MARIE-FRANCE CHEDRU,* Boston, Mass.

Many of the definitive studies of the pathophysiology of ARF stem from micropuncture experiments in rats. Such studies have strongly suggested that increased preglomerular resistance and low filtration pressure are the essential cause of ARF. This study has measured renal cortical blood flow (RCBF) serially in rats with glycerol-induced ARF and others protected from this syndrome by 3 wk of salt loading. Where possible, RCBF and GFR values were used to calculate glomerular filtration pressure. RCBF was measured by the hydrogen washout method of Aukland, and GFR by inulin-^{14}C clearance. Control RCBF was 5.8 ±0.3 (SE) and 6.0 ±0.3 ml/g kidney per min in eight H_2O and six NaCl rats, respectively ($P > 0.5$); GFR was 0.72 ±0.01 ml/100 g body weight in both. RCBF of all animals fell to one-third of control within 1 hr after glycerol, during which time inulin clearances were too low for accurate measurement. Blood pressures were unchanged. RCBF of H_2O rats fell continuously in the succeeding 12 hr to 19% of control, while that of NaCl rats instead rose to 85% of control. GFR remained unmeasurable in H_2O rats but reached 84% of control in NaCl rats. H_2O rats studied in recovery from ARF showed low but markedly improved RCBF. Those with BUN's > 50 mg/100 ml had RCBF of 2.2 ±0.1 ml/g per min and GFR of 0.09 ±0.02 ml/100 g body weight per min; RCBF was 3.4 ±0.2 ml/g per min and GFR 0.34 ±0.03 mg/100 g body weight per min in rats with BUN < 50 mg/100 ml. Calculations of preglomerular resistances and effective filtration pressure showed the latter to be too low to permit significant filtration in glycerol-induced ARF in the rat. Recovery was associated with a progressive decrease in afferent arteriolar constriction, measured GFR values correlating well with calculated glomerular capillary pressures. (Supported by AHA Grant 68 773.)

241. In Vitro Formation of Nondissociable Thyroid Hormone-Protein Complexes. JACK H. OPPENHEIMER, MARTIN I. SURKS,* VLADIMIR KOZYREFF,* ARTHUR RIBA,* AND DIONA KOERNER,* Bronx, N. Y.

A small proportion of injected radioactive thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) enters into nondissociable complexes with tissue and plasma proteins. The correspondence between the slow fractional disappearance rate of these complexes and the slow rate of dissipation of thyroid hormone tissue effects raises the possibility that some of these complexes may initiate and maintain hormonal action. In order to elucidate the mechanism of complex formation experiments were carried out in two in vitro systems. (*a*)Hepatic microsomes from male Sprague-Dawley rats were incubated with T_4-^{125}I or T_3-^{125}I in phosphate buffer (pH 5.8) for 30 min. In addition to iodide-^{125}I liberated into the medium, up to 20% of added radioactivity was incorporated into the microsomes and could not be extracted with ethanol. After chromatography of hydrolysates of this material, more than 20% of the nonextractable ^{125}I was recovered in the form of either T_3 or T_4. Control experiments with ^{131}I-labeled hormones showed that the ^{125}I-labeled T_3 and T_4 did not simply represent incomplete extraction of nonreacted iodothyronine. (*b*) Prolonged dialysis of T_4-^{125}I or T_3-^{125}I in diluted human serum against an ion-exchange resin showed progressive slowing of the fractional exit rate from the dialysis bag. About 20% of the residual radioactivity within the bag at 17 hr could not be extracted into ethanol, whereas less than 1% of freshly added hormone-^{131}I escaped extraction. Pronase hydrolysis of this material again yielded T_3 and T_4 in amounts which could not be attributed to inefficient extraction of unincorporated hormone. Thus, in both systems irreversible complex formation was demonstrated. The capacity of iodothyronines to form nondissociable complexes with proteins may be an important initiating event in the biologic action of these hormones. (NIH; HRC of N.Y.C.; Dept. Army.)

242. Erythrocyte Purine Nucleoside Phosphorylase (PNP); Its Role in the In Vivo and In Vitro Alteration in the Oxygen-Hemoglobin Equilibrium Curve Produced by Inosine. FRANK OSKI, HARVEY SUGERMAN,* THOMAS POLLOCK,* MARIA DELIVORIA-PAPADOPOULOS,* AND LEONARD MILLER,* Philadelphia, Pa.

The 2,3-diphosphoglycerate (DPG)–depleted erythrocytes of humans, monkeys, rabbits, cats, and dogs were incubated in a solution containing pyruvate, inosine, and inorganic phosphate (PIP), all in a final concentration of 0.01 mole/liter. After 4 hr of incubation the erythrocyte DPG levels of the humans and monkeys rose to approximately 140% of normal and the rabbits to normal. DPG levels in the cats and dogs were unchanged. Red cell PNP assays were performed. PNP activity was found to parallel the in vitro response to PIP. No measurable activity was found in the erythrocytes of the dogs and cats, indicating that the initial phosphorylysis of inosine to ribose-1-phosphate and hypoxanthine is essential for ultimate 2,3-DPG synthesis. When infusions of PIP were administered to rhesus monkeys, a species with normal erythrocyte PNP activity, a raise in DPG and a shift to the right in the position of the oxygen-hemoglobin equilibrium curve were observed within 6 hr. The average DPG rose from 4.8 to 7.4 μmoles/ml RBC's and the P_{50} rose from 32.0 to 35.2 mm Hg. The DPG and the P_{50} remained elevated for 24 hr and then gradually declined. These studies illustrate that intravenous infusions of PIP can alter the oxygen dissociation curve when red cell PNP is present. This experimentally produced alteration in the position of the curve should prove a useful model for the further study of oxygen transport and may have applications in clinical states associated with poor oxygen delivery to the tissues.

243. New Methods for the Study of Myocardial Hypertrophy. ERNEST PAGE, Chicago, Ill.

Myocardial ultrastructures have been measured in electron micrographs of rat hearts using a combination of point counting and line integration, and myofibrillar mass has been estimated from the Mg content of glycerinated ventricles after extraction of nonmyofibrillar Mg. Quantitative electron microscopy shows that for normal 200-g female Sprague-Dawley rats the fractions of left ventricular cell volume made up of myofibrils, mitochondria, T-system, and sarcotubules are, respectively 0.481 ±0.009, 0.34 ±0.01, 0.013 ±0.002, and 0.035 ±0.002; the membrane area/unit cell volume for

(T-system + external sarcolemma) and for total sarcotubular system (μ^2/μ^3) are, respectively 0.67 ±0.02 and 2.5 ±0.2; diadic complexes make up 0.08 ±0.02 of sarcotubular volume and 0.12 ±0.02 of sarcotubular membrane area. A fraction of myofibrillar Mg (presumably representing Mg bound to thin filaments during the polymerization of actin) was measured (*a*) as the ^{28}Mg-inexchangeable fraction of Mg in glycerinated ventricle equilibrated at 3°C with 150 mM KCl and 0.1 mM $MgCl_2$, pH 7.2, and (*b*) as the residual Mg in glycerinated ventricles equilibrated with 5 mM EDTA, 150 mM KCl, 1% Triton. For left ventricles of 200-g females this amounts to 2.6% of cell Mg or 1.07 ±0.02 mmoles/kg dry weight. Using this method it was shown that 5 days after aortic constriction (hypertrophy + 23%) myofibrillar mass increases in exact proportion to the increase in total dry mass; and that 25 days after aortic constriction the increase in myofibrillar mass significantly exceeds the increase in total dry mass. (Supported by NIH HE 10503, MIRU Contract 43681334, and a grant from the Chicago and Illinois Heart Associations.)

244. Hypoalaninemia: the Cause of Ketotic Hypoglycemia of Childhood. ANTHONY PAGLIARA,* IRENE KARL,* DARRYL DEVIVO,* RALPH FEIGIN,* AND DAVID KIPNIS, St. Louis, Mo.

The cause of ketotic hypoglycemia (K-H), the commonest form of hypoglycemia in childhood, is not known. The present study was undertaken to determine whether the primary defect in this condition is a deficiency of gluconeogenic precursor(s) or an abnormality in the hepatic gluconeogenic enzyme system. Plasma glucose, alanine, and insulin and blood β-hydroxybutyrate (β-OHB), pyruvate, and lactate levels were determined in seven K-H children and 11 age-matched controls maintained on a normal diet and after fed a provocative hypocaloric low CHO diet (1200 cal/1.73 m², 15% CHO, 17% protein, and 68% fat). On a normal diet, overnight fasting plasma alanine (211 ±10 μmoles/liter) and glucose (68 ±4 mg/100 ml) were significantly lower and blood β-OHB (1.22 ±0.27 mmole/liter) significantly higher in K-H children than in controls (alanine, 315 ±15 μmoles/liter; glucose, 81 ±3 mg/100 ml; β-OHB, 0.18 ±0.08 mmole/liter). All K-H children developed symptomatic hypoglycemia (33 ±3 mg/100 ml) and ketosis (β-OHB, 3.70 ±0.32 mmole/liter) 12–15 hr after starting the provocative diet and a further decline in plasma alanine (155 ±17 μmoles/liter). Normal children, even after 36 hr on this diet, maintained higher plasma glucose (48 ±2 mg/100 ml) and alanine (207 ±10 μmoles/liter) and lower β-OHB levels (2.56 ±0.44 mmoles/liter). Intravenous infusions of alanine (250 mg/kg) uniformly restored the hypoglycemic plasma glucose levels of K-H children to normal. Cortisone acetate (300 mg/m²), given orally in six divided doses 24 hr before or during feeding of the provocative diet, produced a 3- to 4-fold increase in plasma alanine 4–6 hr after beginning steroid therapy and completely prevented the development of hypoglycemia and ketosis. Plasma insulin and blood lactate and pyruvate levels did not differ significantly between groups under all conditions examined. These results support the hypothesis that a deficiency in gluconeogenic precursor (i.e. alanine) rather than a defect in hepatic gluconeogenesis is the primary factor in the pathogenesis of ketotic hypoglycemia. (Research supported by Grants AM 1921 and RR-36 from the National Institutes of Health.)

245. Permeability of the Alveolo-Capillary Membrane to Proteins. GIUSEPPE G. PIETRA,* JAN P. SZIDON,* EILEEN J. CALLAHAN,* AND ALFRED P. FISHMAN,** Philadelphia, Pa.

The permeability of the alveolo-capillary membrane to proteins of small molecular weight was investigated by means of ultrastructural cytochemistry. For this purpose the left lower lobe of dogs was perfused in vivo under controlled capillary and colloid osmotic pressures using solutions of myoglobin (mol wt 17,000), horseradish peroxidase (mol wt 40,000), and hemoglobin (mol wt 64,500). These molecules were identified by electron microscopy, from the electron-dense product formed with 3-3′-diaminobenzidine and osmium tetroxide, by the method of Graham and Karnovsky. At perfusion conditions which approximated the normal intravascular hydrostatic and colloid osmotic pressures of pulmonary capillaries, the different protein molecules remained within the confines of the intravascular space, in spite of the range in molecular sizes. Increasing capillary hydrostatic pressure, decreasing colloid osmotic pressure and overinflation of the lung promoted the passage of protein molecules from the capillary lumen to the interstitium through the inter-endothelial junctions; the alveolar epithelium acted as a barrier for the subsequent movement of proteins. Only at very high hydrostatic pressures (70 mm Hg) did proteins gain access to the alveolar space. In lungs made severely edematous by perfusion with Ringer's solution and subsequently tested, hemoglobin molecules still remained within the capillary lumen when perfused at normal pressures. Our experiments indicate that, ordinarily, the alveolo-capillary membrane of dogs offers severe restriction to the passage of protein molecules of the molecular sizes studied. However, the permeability characteristics are quickly altered by mechanical influences which either stretch capillaries or tend to promote bulk movement of fluid out of capillaries. The accumulation of fluid in the interstitium, per se, does not appear to be primarily involved in the observed changes in permeability. (Supported by USPHS Grant HE-08805.)

246. The Identification of Thyroacetic Acid as a Major Urinary Metabolite after the Administration of Thyroxine-^{14}C. CONSTANCE S. PITTMAN,* JOSEPH B. CHAMBERS, JR.,* MELVIN W. BUCK, AND VIRGINIA H. READ,* Birmingham, Ala. (introduced by Thomas N. James).

Recent studies showed that the ratio of phenolic ring to nonphenolic ring in the thyroxine metabolites remained 1:1 in human urine after the administration of radiothyroxines (Pittman et al., 1970). These studies suggested the possibility that the diphenyl ether nucleus is left intact during thyroxine metabolism, contrary to the conclusions of earlier in vitro experiments. The present study was carried out to identify the deiodinated metabolites at the end of thyroxine degradation in vivo. Eight normal human subjects were given intravenous injections of a thyroxine labeled with ^{14}C on the nonphenolic ring and the alanyl side chain, 8–11 μg/day for 10 days. A total of six ^{14}C-labeled metabolites were separated

from untreated urine by chromatography. The metabolites were isolated by passing urine over Amberlite, X AD-2, 20–50 mesh. After washings, 72% of the total urinary radioactivity was recovered in the methanol eluent and 41% was recovered in one fraction which showed the chromatographic characteristics of thyroacetic acid, or 4-(4′-hydroxyphenoxy)phenyl acetic acid. After acid hydrolysis of the isolated metabolites, greater than 48% of the total urinary radioactivity was contained in the thyroacetic acid fraction. Because of the presence of small amounts of thyronine-^{14}C in urine, the ^{14}C-labeled thyroacetic acid was enriched with a reference sample of thyroacetic acid and dissolved in hot 0.1 N Na_2CO_3. The mixture was recrystallized 7–9 times and throughout the procedure the specific activity of the precipitates remained constant. Our findings constitute the first conclusive evidence that in man the diphenyl ether nucleus of thyroxine remains intact at the end of degradation and complete deiodination. (Research supported by grant from NIH.)

247. Evidence for a Mucosal Iron Transport Protein. SIMEON POLLACK,* THERESA CAMPANA,* AND ANNETTE ARCARIO,* New York (introduced by J. Sandson).

The mechanism of intestinal absorption of iron has never been clarified. A search for an intermediary in ^{59}Fe absorption was conducted by studying in vivo turnover of ^{59}Fe by intestinal mucosa. Substantial turnover of label would characterize a moiety involved in ^{59}Fe transport. Therefore ^{59}Fe turnover of fractions of guinea pig (GP) intestinal mucosa after an in vivo intraluminal pulse of ^{59}Fe was determined in GP either bled (B) or given parenteral iron (PI). B-GP absorbed 6 times as much of an oral dose of ^{59}Fe as PI-GP. Increased ^{59}Fe turnover was demonstrated in 140,000 *g* mucosal supernatants and pellets from B-GP. Three supernatant fractions (F) binding ^{59}Fe were identified using Sephadex G-200 chromatography. The second fraction (F2) (200,000 > mol wt > 40,000) was responsible for most of the supernatant ^{59}Fe turnover. In B-GP: F1 contained 30% of the ^{59}Fe at 0 min and had no significant 15 min turnover; F2 58% ^{59}Fe at 0 min and lost $\frac{1}{2}$ at 15 min; F3 12% ^{59}Fe at 0 min and lost $\frac{1}{2}$ at 15 min; total 0 min supernatant = 113.8 + 36.4 mμg ^{59}Fe. In PI-GP: F1 contained 35% of the ^{59}Fe at 0 min and had no significant 15 min turnover; F2 50% ^{59}Fe and lost $\frac{1}{4}$ at 15 min; F3 15% ^{59}Fe at 0 min and no significant turnover; total 0 min supernatant = 96.9 + 14.8 mμg ^{59}Fe. Polyacrylamide-gel electrophoresis of F2 revealed two bands of radioactivity: the slower with the same mobility as ^{59}Fe bound to GP serum, the faster moving with a Coomasie blue staining band on prolonged electrophoresis indicating it was protein bound. These observations are consistent with the existence of carrier proteins for intramucosal iron transport. (Research supported by NIH grant.)

248. Role of Bence Jones and Other Urinary Proteins in Renal Dysfunction. H. PREUSS,* F. WEISS,* R. IAMMARINO,* W. HAMACK,* AND H. V. MURDAUGH, Pittsburgh, Pa.

Acute and chronic renal failure frequently occur in multiple myeloma. A correlation between Bence Jones proteins (BJ) and renal injury has been made, but whether BJ have direct toxic action on renal epithelium has not been proved. In this study, proteins from several urines were investigated in vitro to define further the role of BJ and other urinary proteins in renal injury. The proteins were divided into two categories: (*a*) those from patients with nephrotic syndrome and (*b*) those from patients with multiple myeloma, ladened with BJ. Cortical slices from rat kidneys were halved, each half preincubated in control media without protein or media containing the urinary proteins from myeloma or nephrotic patients (10 mg/ml). After 1 hr preincubation, slices were studied for their ability to accumulate hippurate and tetraethylammonium (TEA) and to produce ammonia and glucose. In slices preincubated in urinary proteins from myeloma patients, all functions were decreased compared to control: hippurate accumulation −43 ±11% of control ($P < 0.001$), TEA accumulation −57 ±09% of control ($P < 0.001$), ammoniagenesis −46 ±08% of control ($P < 0.001$), and gluconeogenesis −49 ±10% of control ($P < 0.001$). The higher the concentration of protein, the lower the hippurate accumulation. Slices preincubated in urinary proteins from nephrotic patients showed no significant decrease in hippurate accumulation (−08 ±08%), TEA accumulation (−07 ±21%), ammoniagenesis (−06 ±11%), or gluconeogenesis (−09 ±13%) of control. The major proteins in all myeloma urines were BJ (7 L, 1 K); in the nephrotic patients principally albumin. Because of the design of these studies BJ appear to be the variable causing the deleterious effects noted. That the urinary proteins containing BJ are destructive to in vitro slice function suggests that BJ plays a role in the clinical renal dysfunction seen in multiple myeloma. Further, the results verify the clinical impression that heavy proteinuria without BJ in nephrotic syndrome rarely causes proximal tubule dysfunction.

249. Lipid Transport in Biliary Cirrhosis. S. H. QUARFORDT,* Durham, N. C. (introduced by Jerome S. Harris**).

Three primary biliary cirrhotic patients with elevated plasma phospholipid (P) and free cholesterol (C) levels were studied to define plasma lipid transport. Virtually all of the P and C was recovered as S_f 0–20 low density lipoprotein (LDL) with diminished high density lipoprotein (HDL). This LDL had two distinct Schlierin peaks, one between S_f 0–12 (peak I) and the other between S_f 12–20 (peak II). With higher plasma lipid levels peak II predominated. LDL antisera precipitated peak I but did not effect peak II. HDL antisera effected neither peak. Purified peak II was nearly all P and C with a molar ratio of 1:1. The P was mostly palmityl oleyl lecithin. Most LDL-conjugated bile acid was in peak II but its removal did not effect peak II stability. Apo-HDL was found in delipidated peak II. Peak I had much more protein and cholesterol ester than peak II. The delipidated peak I peptide pattern by dodecyl sulfate polyacrylamide electrophoresis did not resemble normal LDL, being more like normal very low density lipoprotein (VLDL). A loss of many of the usual VLDL peptides was noted for biliary cirrhotic VLDL. Biliary cirrhotic LDL revealed two structures in the electron microscope: (*a*) a 230 A spherical particle resembling normal LDL, and (*b*) lamellae whorls and sheets similar to "neat phase" structure. "Neat phase" was also suggested by observations with crossed nicols. Only lamellated structures were seen in peak II. These data suggest that some plasma lipid in these patients exists as lamellated colloidal dispersions,

possibly binding other plasma peptides and producing an altered protein composition of LDL. (Research supported by American Heart Association Grant No. 69-1011.)

250. Stem Cell Response to Erythropoietin (EP). PETER QUESENBERRY,* NICHOLAS J. RENCRICCA,* KEVIN RICKARD,* DONALD HOWARD,* MARIANNE GARRITY,* AND FREDERICK STOHLMAN, JR., Boston, Mass.

Migration of pluripotent (CFU) and committed (CFC) stem cells results from erythroid stimulation. Such migration might be due to a direct effect of EP on the stem cell or changes within the stem cell compartment due to differentiation of precursor cells into the erythroid compartment. To distinguish between these alternatives, the numbers of CFU and CFC (using the soft agar technique) in marrow, spleen, and peripheral blood in mice given EP q 8 hr for 4 days were compared with those in mice treated similarly but in which erythroid differentiation had been blocked by actinomycin D (AC). The tibial CFU decreased ~40% but the circulating CFU increased ~5-fold and splenic CFU 15-fold in EP-treated mice. Parallel changes were observed in CFC. In the EP-AC group there was no erythroid differentiation as judged from bone marrow morphology and peripheral reticulocytes. Despite this lack of differentiation, the changes in both CFU and CFC in the EP-AC–treated mice paralleled those seen in the group treated with EP alone. We conclude that the migration of stem cells seen in erythropoietically stressed animals is independent of differentiation of precursor cells out of the stem cell compartment.

251. Renal Manifestations of Chronic Hepatitis. RUSSELL E. RANDALL, JR.,* ABDUL R. ABUKURAH,* MILLIE Y. TUNG,* ELIZABETH R. VAUGHAN,* AND WILLIAM J. S. STILL,* Richmond, Va. (introduced by David W. Richardson).

Six patients with chronic hepatitis have been studied because of renal manifestations. Three still living have less severity of hepatitis since treatment with prednisone (three) and cyclophosphamide (two of three). Three (untreated) have died, two showing nodular cirrhosis. Hyperimmunoglobulinemia was present in all. Hypocomplementemia was present in five. Serum antibodies were present against nuclear protein in five, against human glomerular basement membrane in two, smooth muscle in two, but negative in all against mitochondria, thyroid, gastric mucosa, Australia antigen, and infective hepatitis–associated antigen. Creatinine clearance was normal in two, moderately reduced (30–40 ml/min) in two, and markedly reduced (10–12 ml/min) in two. All show membrano-proliferative glomerulonephritis of varying severity. Two patients with nephrosis had electron-dense deposits within glomerular basement membranes. Lumpy deposits of immune globulins and complement were detected by fluorescent techniques within glomeruli from the four whose tissue was available for study. A linear pattern was also present in two. Tubular deposits were prominent in four, one of whom presented with renal acidosis, potassium, and water wasting. Serial biopsies in one suggested a linear IgG pattern followed an original lumpy IgM deposition. Virus-like particles were present within basement membranes in one and in tubular lumina in another. In the three surviving patients, deranged renal function is improving during immunosuppressive therapy. These data suggest that chronic hepatitis may result in glomerular and tubular dysfunction, presumably by immune globulin deposition. (Research supported by Grant HE 05684 from NIH.)

252. Studies on the Origin of Iodine-Poor Thyroglobulin of Multinodular Goiter. BASIL RAPOPORT,* HUGO NIEPOMNISZCZE,* MARIO BIGAZZI,* RATHA HATI,* AND LESLIE J. DEGROOT, Chicago, Ill.

Thyroglobulin (TG) is characteristically poorly iodinated in sporadic multinodular goiter. We have studied a euthyroid 26 F with a 280 g goiter. Mean idodine (I) excretion was 346 μg/day. Known metabolic defects were excluded by RAIU of 29%, negative perchlorate discharge test, absence of iodotyrosines in blood, and in vivo deiodination of MIT-^{131}I. Serum NBE-^{125}I was 30% of serum T-^{125}I and was not iodoalbumin. Thyroid tissue I content was 19.03 μg/g tissue (normal = 400). Sephadex G-200 fractionation of thyroid 105,000 *g* supernatant produced a single TG peak, identical with normal TG. I content of TG was low (0.044%). TG could be iodinated nonenzymatically in vitro to 0.6%. TG —S—S— groups were normal in number, with 1.2% in the reduced state commensurate with the low iodination. TG salted out of phosphate at 1.8 moles/liter. Thyroid slice incubation in ^{125}I- and ^{3}H-labeled amino acids showed normal incorporation into TG by starch-gel electrophoresis of products. TG inhibited agglutination of thyroglobulin-coated red cells by Hashimoto serum. Pancreatin digestion of thyroglobulin revealed 65% MIT, 22% DIT, 4.6% T_4, and 0.4% T_3. In vitro studies of iodination showed normal NADPH-cytochrome *c*-reductase (0.0125 μEq/mg protein per min in 39,000 *g* particulate fraction). Iodide peroxidase activity (triiodide assay) was twice normal (2.2 U). Iodide peroxidase-tyrosine-iodinase activity with H_2O_2 generation was normal. Transaminase, dehalogenase, and protease were normal. In this nontoxic multinodular goiter there was defective TG iodination despite an abundant supply of I, a chemically and immunologically normal TG receptor, and normal in vitro iodinating apparatus. We hypothesize (*a*) there may be excessive thyroglobulin synthesis in relation to iodinating activity, processes which normally appear synchronous; or (*b*) TG may continually recirculate between follicle and cell, being progressively iodinated with each passage. Endocytosis or colloid droplet migration involved in this process may be defective. (Supported by Grant AM-13377, AM-13643, and Damon Runyon Fund.)

253. Left Ventricular Function Curves in Patients with Acute Myocardial Infarction. CHARLES E. RACKLEY,* RICHARD O. RUSSELL, JR.,* ROBERT A. RATSHIN,* AND BOLLING J. FEILD,* Birmingham, Ala. (introduced by T. Joseph Reeves**).

Cardiac performance was measured in 38 patients with acute myocardial infarction. Initial left ventricular filling pressure (ILVFP) was recorded as pulmonary artery end-diastolic pressure or left ventricular end-diastolic pressure before any specific intervention. 200–800 ml of low molecular weight dextran was infused in 50-ml increments and serial measurements of cardiac index, stroke index, and stroke work index were related to left ventricular filling pressure to con-

struct ventricular function curves. Seven patients were clinically uncomplicated, 21 were complicated by rales and/or ventricular gallop, and 11 demonstrated the clinical features of cardiogenic shock with a systolic blood pressure less than 90. Function curves were characterized by the initial relationship of cardiac index and left ventricular filling pressure, the slope of the curve, and the break or descending limb. In the two uncomplicated patients with an ILVFP < 15 mm Hg the function curves were steep but in the five with an ILVFP > 15 mm Hg the function curve was depressed. In the complicated patients with ILVFP < 15 mm Hg, five of 12 function curves were depressed, whereas in those with ILVFP > 15 mm Hg, five of eight curves were depressed. In 8 of the 11 patients in shock flat curves were demonstrated. In 37 patients the slope of the function curve was defined by the initial 200 ml of the dextran infusion. Patients with anterior myocardial infarction demonstrated depressed curves at a lower LVFP than those with inferior infarction. In the patients with cardiogenic shock the asymptote of cardiac index was reached at a LVFP ≤ 20 mm Hg. These data demonstrate that (*a*) ventricular function is significantly depressed in man with acute myocardial infarction; (*b*) such depression correlates imperfectly with clinical estimates; and (*c*) in patients with cardiogenic shock, no significant increase in cardiac output or work can be achieved by increasing LVFP to levels greater than 20 mm Hg. (MIRU Contract 43-67-1441.)

254. Cellular Sensitivity Studies in Human Renal Transplantation. STANLEY E. READ,* VINCENT A. FISCHETTI,* ROBERT J. ELLIS,* AND JOHN B. ZABRISKIE, New York.

A number of investigators have now demonstrated that inhibition of migration of peripheral white blood cells from capillary tubes in the presence of specific sensitizing antigen is an accurate reflection of the degree of cellular reactivity to that antigen. In view of the known serological and biological cross-reactivity of group A streptococcal membrane antigens and glomerular membrane antigens, the lymphocytes obtained from 20 renal transplantation patients were studied with this method using both human glomerular basement membrane and streptococcal membrane antigens. The degree of inhibition of leukocytes was measured after the introduction of 10 gamma of either streptococcal or pooled human glomerular basement membrane antigens into tissue culture media surrounding the capillary tubes. The results of these studies indicate that patients with creatinine clearance of <50 ml/min had an average of 25% inhibition while those patients with a creatinine clearance of more than 50 ml/min had an average of 6% inhibition. In a small number of patients the degree of cellular reactivity to either streptococcal or renal membrane antigens appeared to be the same. A control group of nonnephritic patients had essentially no inhibition to both antigens. Four patients were studied both before and after renal transplantation. Three of these patients demonstrated a marked change in the degree of inhibition to streptococcal membranes after the transplant (4.5% before transplant and 28% after transplant). The fourth patient of this group had no evidence of sensitization to streptococcal membranes after transplantation and this patient was an A match. These studies indicate that altered cellular reactivity to streptococcal and glomerular antigens is a sensitive indicator of renal rejection. (Research supported by Grant HE-13919 from NIH.)

255. The Effect of Phenobarbital on Bile Salt Metabolism and Cholesterol Secretion in the Primate. RICHARD N. REDINGER* AND DONALD M. SMALL, Boston, Mass.

Phenobarbital, by inducing liver microsomal enzymes, may effect bile salt (BS) synthesis from cholesterol and thus alter the secretion of biliary lipids and the composition of bile. We, therefore, determined the effects of phenobarbital on biliary lipid secretion, BS synthesis, and pool size. Using an experimental preparation which allows controlled interruption of the enterohepatic circulation (1970. *J. Clin. Invest.* **49**: 232), we administered 5–30 mg/kg per day of phenobarbital to four healthy monkeys for 1–2 wk to achieve steady-state conditions. Each animal also served as its own control and was studied with an intact enterohepatic circulation (EHC) and/or a total bile fistula (eight studies). Total bile flow and secretion of BS, phospholipid, and cholesterol were measured daily. In two animals with intact EHC we determined BS synthesis and pool size. Phenobarbital at doses of 5 mg/kg per day increased bile flow in all animals. Phenobarbital also increased BS and phospholipid secretion but decreased cholesterol secretion ($P < 0.05$–0.005). Consequently, the concentration of cholesterol relative to BS and phospholipid was decreased ($P < 0.005$). Phenobarbital enhanced the maximal rate of BS synthesis in monkeys with total bile fistulae ($P < 0.05$). It also augmented BS synthesis and pool size (25%) in animals with intact EHC despite the fact that the rates of BS returning to the liver in these animals would have inhibited bile salt synthesis in control animals. Thus, phenobarbital not only increases the maximal rate of BS synthesis but impairs the feedback inhibition which ordinarily occurs when BS return to the liver. These findings suggest that phenobarbital may increase conversion of hepatic cholesterol to BS so that BS synthesis increases and biliary secretion of cholesterol decreases. (Research supported by NIH Grants AM 11453 and 12890.)

256. Effects of Atropine on the Degree of Myocardial Ischemia during Coronary Occlusion in the Conscious Dog. DAVID R. REDWOOD,* ELDON R. SMITH,* MARTIN H. MILLER,* AND STEPHEN E. EPSTEIN, Bethesda, Md.

Atropine is frequently employed in patients with acute myocardial ischemia (MI) to treat bradycardia-induced arrhythmias, and we have shown experimentally that atropine is effective in treating arrhythmias occurring during the onset of myocardial infarction. It is not known, however, whether the atropine-induced increase in heart rate has any deleterious effect on the degree of MI. To avoid the tachycardia present in open-chest anesthetized dogs, we studied the ST segment response to repeated 5-min occlusions of the left anterior descending coronary artery (LAD) at hourly intervals in four closed-chest conscious dogs in which we previously had implanted in inflatable balloon around the LAD and 12 myocardial electrodes into the area supplied by the LAD. Occlusions were conducted in random order (*a*) at control heart rate (average 95), (*b*) with atropine pretreatment (0.005–0.05 mg/kg intravenously), and (*c*) during atrial pacing. Repeated

control occlusions caused no change in degree of ST response. When compared to control occlusion there was a significant correlation between the per cent increase in heart rate produced by atropine and per cent increase in total ST elevation ($y = 0.89\ x + 5.00$, $r = 0.95$, $P < 0.001$). There was no significant difference between the increase in ST elevation with occlusion after atropine and that after occlusion during rate-matched atrial pacing. We conclude that if the degree of ST elevation reflects severity or extent of ischemia, increases in rate in experimentally produced acute MI are associated with proportional increases in the degree of MI. Thus, when atropine is administered to control bradycardia-induced arrhythmias during acute MI, the lowest effective dose should be used and excessive increases in rate avoided.

257. In Vivo Quantitation of the Role of Pyruvate Carboxylase Inhibition in Ethanol-Induced Suppression of Hepatic Gluconeogenesis. KLAUS REES* AND LEONARD MADISON,** Dallas, Tex.

In vivo studies from this laboratory demonstrated that ethanol inhibits hepatic gluconeogenesis. Krebs and others attributed part of the decrease in hepatic gluconeogenesis to a suppression of pyruvate carboxylase due to a lack of pyruvate (coming from lactate, alanine, or serine) caused by its reduction to lactate, a result in turn of the increased $NADH_2$:NAD ratio characteristic of hepatic ethanol oxidation. The present studies were designed to quantify the contribution of inhibition of pyruvate carboxylase to the total suppression in hepatic gluconeogenesis by measuring changes in hepatic lactate uptake and hepatic gluconeogenesis during ethanol infusion. Hepatic lactate uptake and hepatic gluconeogenesis were measured in 16 dogs starved for 48 hr by the hepatic venous catheter technique during a 30 min control and 60 min period of ethanol infusion (0.2 mmole/kg per min). Ethanol produced a significant decline in both hepatic gluconeogenesis and hepatic lactate uptake. Hepatic lactate uptake decreased 8 mg/min from control values whereas hepatic gluconeogenesis fell 32 mg/min during ethanol infusion, a fourfold greater decline. Since all precursors of glucose which have pyruvate as an intermediate must be recovered as lactate when diverted from gluconeogenesis and the drop in hepatic lactate uptake could account for only 8 mg of glucose per min, therefore pyruvate carboxylase inhibition could account for no more than 25% of the total fall in hepatic gluconeogenesis. These data indicate that in addition to decreased pyruvate utilization, other steps in hepatic gluconeogenesis are significantly depressed and play the major role in the inhibition of hepatic gluconeogenesis by ethanol. (Research supported by grant from NIH.)

258. Treatment-Resistant Pseudomonas Endocarditis. MILAGROS P. REYES,* AGUSTIN M. ARBULU,* AND A. MARTIN LERNER (with the collaboration of S. Pursel,* W. Palutke,* and R. Wylin*), Detroit, Mich.

During 15 months of 1969–1970 nine patients with pseudomonas endocarditis were seen. Diagnosis required (*a*) persistent *Pseudomonas aeruginosa* bacteremia and (*b*) absence of a focus of infection other than the heart. Supportive findings included (*c*) intravenous use of heroin (8/9), (*d*) cardiac murmurs (tricuspid, 6/9), and (*e*) septic emboli, especially pulmonary (5/9). Histopathologic and bacteriologic studies of the involved value were possible in six, and in each case confirmed the clinical diagnosis. Patients were mostly men (7/9) and were 19–50 yr old. Two patients had had an earlier successfully treated *Staphylococcus aureus* tricuspid endocarditis, and one had preexistent mitral stenosis. Cardiac catheterization in one patient without prior heart disease did not reveal valvular incompetence. Patients received prolonged courses of parenteral gentamicin or polymyxin B along with intravenous carbenicillin. During treatment one patient remained bacteremic while afebrile; his serum was green with a nonverdoglobin pigment, probably pseudomonas fluorescein. All strains were sensitive to gentamicin. The mean MIC to carbenicillin was 166 μg/ml. Serum bactericidal levels ranged from $\frac{1}{2}$–$\frac{1}{8}$. Four of the nine received only medical treatment. Bacteremia cleared in only one who is well. The five remaining patients with unremitting or relapsing bacteremias submitted to surgery. Tricuspid valvulectomies and insertions of prosthetic valves were performed upon two patients; both died of postoperative bacteremia. Three others had tricuspid valvulectomies alone. They all developed massive right ventricular failure, but two suffered postoperative relapses of their pseudomonas bacteremias. Therefore, seven of nine patients with pseudomonas endocarditis were resistant to medical or combined medical and surgical treatments. (Research supported by grants from NIH (AI 09336), Beecham Pharmaceuticals, and Schering Corporation.)

259. Translation of β-Chain mRNA in β-Thalassemia. RONALD F. RIEDER, Brooklyn, N. Y.

Clinical and genetic evidence and in vitro studies of hemoglobin synthesis indicate that in Cooley's anemia there is imparied synthesis of β-globin chains. A possible mechanism for reduced globin synthesis is defective translation of mRNA with decreased rate of ribosomal assembly of the polypeptide chain. Previous studies by others comparing normal reticulocytes and cells from Thai patients with β-thalassemia detected no defect in β-chain assembly in the thalassemic cells. The present experiments were undertaken to examine globin chain assembly in thalassemic cells from other ethnic groups. Reticulocytes from four patients (three Italians, one Negro) with β-thalassemia major were incubated with L-valine-2, 3-^{3}H for 3, 6, and 9 min. The tritiated β-globin was purified and mixed with β-globin uniformly labeled with L-valine-^{14}C. The mixed protein was digested with trypsin and fingerprinted. The ^{3}H:^{14}C ratios were measured in the tryptic peptides and the tritium incorporation gradient was plotted from the NH_2-terminal to COOH-terminal ends of the β-polypeptide chain. In five experiments with thalassemic reticulocytes, the amount of ^{3}H in the NH_2-terminal amino acid relative to the COOH-terminal (extrapolated) was 54% (range 40–69) at 3 min, 72% (range 58–77) at 6 min, and 86% (range 84–88) at 9 min. In reticulocytes from a patient with pyruvate kinase deficiency the results at the three time points were 37%, 59%, and 84%. The gradients appeared to be straight lines. These results are similar to those obtained with the Thai patients and indicate that there is no delay in β-globin translation in β-thalassemia. The experiments suggest that the synthetic defect in thalassemia may lie in defective initiation of globin chain synthesis or in a deficiency of mRNA. (Supported by

NIH Grant AM-12401 and Life Insurance Medical Research Fund.)

260. The Guillain-Barre Syndrome and Multiple Sclerosis: In Vitro Cellular Responses to Nervous Tissue Antigens. ROSS ROCKLIN,* WILLIAM SHEREMATA,* ROBERT FELDMAN,* MARIAN KIES,* AND JOHN DAVID, Boston, Mass.

It has been suggested that cellular hypersensitivity may be involved in the pathogenesis of the Guillain-Barre syndrome and multiple sclerosis. An in vitro assay (the macrophage migration inhibition technique) was used to further study cellular hypersensitivity in these and other neurologic diseases. Blood lymphocytes from 83 subjects (59 patients with neurological disease and 24 normal individuals) were assayed for the production of migration inhibitory factor (MIF) in response to peripheral (human sciatic nerve) and central (human basic protein) nervous tissue antigens. In the group of 25 patients with peripheral neuropathies, including Guillain-Barre (7), alcoholic polyneuropathy (9), diabetic polyneuropathy (4), and miscellaneous polyneuropathies (5), only lymphocytes from patients with the Guillain-Barre syndrome (5/7) produced MIF in response to peripheral nerve antigen. These patients did not produce MIF in response to basic protein antigen. The 34 patients with central nervous system disease studied included multiple sclerosis (15), cerebrovascular accidents (9), amyotrophic lateral sclerosis (5), metastatic carcinoma to brain (1), Wilson's disease (1), Schilder's disease (1), and syringomyelia (2). Lymphocytes from 5/15 patients with multiple sclerosis (4/4 acute and 1/11 chronic) produced MIF when incubated with basic protein antigen. An unexpected finding was that MIF was produced in response to basic protein antigen by lymphocytes from 6/9 patients who had had cerebrovascular accidents. These results further demonstrate that cellular hypersensitivity to components of nervous tissue is present in some neurologic disease states. Although the data suggest that sensitive cells may be involved in the pathogenesis of some neurologic diseases, the results from studies in patients with cerebrovascular accidents demonstrate that cellular hypersensitivity could be secondary to nervous tissue damage. (This work was supported in part by USPHS Grant AI-07685.)

261. Parathyroid Involution after Successful Renal Transplant. BETTY S. ROOF,* C. PIEL,* B. CARPENTER,* G. S. GORDAN,** S. KOUNTZ,* AND F. BELZER,* San Francisco, Calif.

In 69 patients with chronic renal disease parathormone (PTH) and Ca were measured before and after successful renal transplantation. 20 were children; of these, 17 received calcium infusions (10 mg/kg) before transplantation. Suppression was less complete in 10 with osteodystrophy than in 5 without, presumably because bone avidity prevented adequate rise of serum Ca. In those with bone disease, PTH rebounded rapidly; in those without, suppression lasted 9+ hr. In 9 patients with elevated PTH and osteodystrophy, vitamin D therapy corrected elevated PTH and the bone healed. In children, PTH fell rapidly to normal by 1–2 months in 75%, and more slowly in the others. In 11 hypercalcemic sera from 4 patients 1 month after transplant, there was no measureable PTH. In 49 adults, pretransplant PTH levels were similarly high. Posttransplant PTH fell more slowly and < 50% were normal by 60–90 days. All of 14 hypercalcemie sera 1 or more months after transplant contained appreciable PTH. In all children who had hypercalcemia posttransplant, causes other than hyperparathyroidism were found. Our only proved parathyroid adenoma was diagnosed by high PTH and Ca 1 yr after transplant. We conclude that (*a*) the elevated PTH levels of uremia are regularly suppressible when serum calcium is adequately raised by calcium infusion or vitamin D; and (*b*) PTH falls rapidly after transplant in children, more slowly in adults, probably in relation to the mass of hyperplastic parathyroid tissue undergoing involution.

262. Inhibition of Intestinal γ-Glutamyl Carboxypeptidase by Yeast Nucleic Acid: an Explanation of Variability in Utilization of Dietary Polyglutamyl Folate. IRWIN H. ROSENBERG* AND HERMAN A. GODWIN,* Chicago, Ill., and Boston, Mass. (introduced by Joseph B. Kirsner**).

Differing yeast folate preparations, equally potent in chick anemia assay, have been utilized with variable efficiency by man. Lower efficiency of absorption of these polyglutamyl folates vs. monoglutamyl folate (PGA) remains unexplained. To study possible inhibitors of deconjugation or absorption, substrates free of inhibitors were required. Specifically labeled 3′-5′-pteroyl heptaglutamate-^{3}H (^{3}HPtGlu$_7$), synthesized in our laboratory, and yeast polyglutamates were administered to 15 normal volunteers to assess absorption relative to PGA. Folate from crude yeast resulted in serum folate elevations less than 3% of those observed with equimolar PGA, DEAE column–"purified" yeast folate caused elevations (60–70%) of those after PGA, and ^{3}HPtGlu$_7$ was absorbed 80% as well as PGA-^{3}H (mean 60% vs. 75% total urinary recovery of folate-^{3}H.) PGA absorption was uneffected by added yeast extract. In vitro studies of intestinal deconjugating enzyme γ-glutamyl carboxypeptidase employed paper chromatographic separation of substrate ^{3}HPtGlu$_7$ from products. Yeast extract (0.5 mg), added to an incubation system with intestinal homogenate and 2 μmolar ^{3}HPtGlu$_7$, was a potent (50–60%) inhibitor of enzymatic deconjugation. Inhibition by 0.2 mg yeast nucleic acid and 0.1 mg pure yeast RNA was 80% (0.04 mμmole hydrolyzed per hour vs. 0.21 mμmole). Relative concentrations of inhibitor and substrate were similar to those in natural yeast extract; optimal pH was maintained. Avian intestinal and pancreatic γ-glutamyl carboxypeptidase were not inhibited by yeast nucleic acid. High voltage electrophoretograms of radioactive products indicate that the avian enzyme differs from mammalian in final product: diglutamate in chick, monoglutamate in mammals. Thus, study of species-variable folate utilization related to inhibition of intestinal γ-glutamyl carboxypeptidase by food nucleic acid provides insight into mechanisms of control of polyglutamyl folate absorption and utilization. (Research supported in part by NIH Grants FR 76, AM 795, and AM 9115.)

263. The Effect of Teaching the Rationale of Therapy on Cooperation with a Therapeutic Regimen. HAROLD ROTH,* HERBERT CARON,* AND BARTHOLOMEW HSI,* Cleveland, Ohio (introduced by Reginald Shipley**).

To determine whether teaching would improve cooperation, 160 consecutive ulcer patients were randomly divided into

three groups: (1) taught the rationale of therapy; (2) control group taught nonrelevant material; and (3) untaught control group. Knowledge and antacid intake were measured for all patients during a 2 yr follow-up. We used socratic methods to convey basic concepts (e.g. "neutralization") and eliminate misconceptions (e.g. "acid comes from food"). After 3 visits 82% of taught patients answered direct questions correctly, but comprehension, tested separately, vaired with IQ. Physicians' estimates of antacid intake were found inaccurate. Regular deliveries of the medication of patients' homes permitted accurate counts of empty bottles. A trace of sodium bromide in the medication permitted validation of bottle counts via blood bromide levels. The three groups showed no difference in antacid intake. Consumption averaged 0.30 bottles per day (54% of prescription). When the groups were subdivided by social class, education, and IQ, there were still no significant differences but there were some seemingly contradictory trends. With higher IQ there was higher intake (in group 1), whereas with higher social class and educational level there was lower intake (in all groups). Subdivision by race produced a significant difference ($P < 0.01$), most marked in group 1; Negroes took only 0.23, whereas Caucasians took 0.45 bottles/day. Negroes were slightly higher in educational level. Possibly pertinent: only one staff member, a physician, was Negro. His estimates of Negro patients' intakes were more accurate ($r = 0.71$) than those of Caucasian physicians ($r = 0.25$ and 0.28). This suggests the potential importance of race of staff as well as patient in the response to medical programs. (Research supported by grants from NIH.)

264. Evidence for the Simultaneous Accumulation of Intrinsic Factor (IF) and Vitamin B_{12} (B_{12}) in the Mitochondria of Guinea Pig Ileum Epithelial Cells during B_{12} Absorption. SHELDON P. ROTHENBERG,* HERBERT WEISBERG,* AND ANTHONY FICARRA,* New York (introduced by Solomon A. Berson**).

In an attempt to resolve the question of whether IF enters the intestinal epithelial cell during B_{12} absorption, $^{57}CoB_{12}$ bound to human IF was incubated in ileal loops of anesthetized guinea pigs. Electron microscopically controlled subcellular fractions of the mucosa were then analyzed for radioactivity and immunoreactive IF. Radioactivity in the mitochondria and cell debris increased over 120 min to 2200 and 5900 cpm/mg protein, respectively. Microsomal and cytosol fractions accumulated significantly less activity. By coated charcoal assay between 80 and 94% of the $^{57}CoB_{12}$ in sonicated cell debris and 58 and 78% of that in sonicated mitochondria was protein bound. After incubating sonicated mitochondria and cell debris with anti-human IF antibody, 73–90% and 79–87% of the radioactivity of each fraction, respectively, precipitated with the antibody indicating the major binder to be IF or an immunoreactive fragment. Free $^{57}CoB_{12}$ incubated with sonicated mitochondria prepared from a fresh ileum not exposed to human IF did not precipitate with anti-IF antibody. To determine dissociability of absorbed mitochondrial IF-$^{57}CoB_{12}$, radioactivity precipitating with anti-IF antibody in a nonsonicated mitochondria fraction was measured while incubating at 37°C. IF-bound radioactivity decreased by 32–45% in 180 min indicating gradual dissociation of the vitamin from the complex. No dissociation occurred at 4°C. No dissociation of IF-bound $^{57}CoB_{12}$ occurred at 37°C unless sonicated mitochondria (but not intact mitochondria) were added to the mixture. These results suggest that during B_{12} absorption in the guinea pig ileum IF or an immunoreactive fragment accumulates in the mucosal mitochondria complexed with the vitamin. In addition, there is evidence that this mitochondrial IF-B_{12} complex then undergoes gradual dissociation via an intrinsic enzymatic mechanism.

265. Electron Microscopic Radioautographic Identification of Serotonin-Synthesizing Cells in the Mouse Gastric Mucosa. WALTER RUBIN,* MICHAEL D. GERSHON,* AND LEONARD L. ROSS,* Philadelphia, Pa., and New York (introduced by Donald Kaye).

This study correlates the fine structure of mouse gastric endocrine cells with their ability to synthesize serotonin (5-HT) from 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT biosynthesis. Six mice were injected intravenously with 5.0 mCi of 5-HTP-^{3}H and sacrificed 2 hr later. By this time label had disappeared from the plasma and gastric radioactivity had attained levels of 165–320 dpm/g. The stomachs were removed, washed, fixed, and embedded in Epon; sections were processed for light and electron microscopic radioautography. These procedures retained essentially all tissue 5-HT-^{3}H while washing out essentially all labeled 5-HTP and 5-hydroxytryptamine-*o*-glucuronide, the only other labeled compounds of significant concentration. Seven additional mice were injected intravenously with 5-HT-^{3}H, sacrificed at 2 hr, and their stomachs processed similarly. All morphologic types (at least five) of mouse gastric endocrine cells exhibited a similar facility to incorporate exogenous 5-HTP and to convert it to 5-HT which was bound intracellularly. Differences in densities of silver grains observed over endocrine cells indicated that individual cells indeed varied in their ability to synthesize and/or to bind 5-HT; such variations, however, were not reflected by differences in fine structure, with the exception that endocrine cells with few granules always contained little newly synthesized 5-HT. The newly synthesized 5-HT was associated with the intracellular granules. Gastric endocrine cells were not labeled by exogenous 5-HT-^{3}H, whereas mast cells were labeled by either 5-HT-^{3}H or 5-HTP-^{3}H administration. This study supports the position that the gastric endocrine cells represent a single cell type, at least in respect to serotonin metabolism. (Research supported by ACS Grant P423B and USPHS Grants AM-14348, NS-07436, and NS-05539.)

266. Responsiveness of Human Subjects to Human Growth Hormone (HGH). DANIEL RUDMAN, SAMUEL B. CHYATTE,* JOSEPH H. PATTERSON,* IRMA O'BEIRNE,* GLYNDA G. GERRON,* AND ROBERT MOSTELLER,* Atlanta, Ga.

The effect of HGH on N, P, Na, and K balance, and on body weight (BW) was measured in nine cases (age 6–69) with HGH deficiency (group I) and eight cases (age 9–79) with normal endogenous HGH (group II). After 7-day control period, HGH was administered for 7 days. Each subject was tested at doses A, B, and C of HGH: 0.0168, 0.0532, and 0.168 U/kg $BW^{3/4}$ per day respectively. In eight subjects, response to each dose was measured twice. In group I responsiveness to HGH declined with age. Two subjects aged 6 yr

responded to all three doses of HGH with positive balances in N, P, Na, and K and increase in BW. At ages 15–17, responses occurred only to doses B and C (three cases) or to dose C (two cases). Two subjects aged 42 and 69 responded only to dose C. Not only did threshold dose increase with age in group I, but magnitude of responses declined with age as well. Group II cases were less responsive to all doses of HGH than were patients of group I at comparable age. None responded to doses A or B. All responded to dose C, but the increments in balances and in BW stimulated by this dose were only $\frac{1}{3}$ to $\frac{1}{5}$ as great as in HGH-deficient subjects of similar age. Regression analysis showed the following. For group I, increment in N balance [g/day per kg $BW^{3/4}$] (ΔN) = 3.43 + 1.42 (log dose HGH) − 0.080(age) + 0.00088$(age)^2$. For group II, ΔN = 1.08 + 0.63(log dose HGH) + 0.0010(age) − 0.000041 $(age)^2$. Thus responsiveness to exogenous HGH rises with deficiency of endogeneous HGH, and declines with advancing age. (Supported by NIH Grant FR-39.)

267. Vasoactive Intestinal Polypeptide (VIP): Mediator of Hemodynamic and Respiratory Changes in Liver Cirrhosis? SAMI I. SAID, TAKAHITO HIROSE,* SATOSHI KITAMURA,* AND STEPHEN R. SIEGEL,* Richmond, Va.

Hyperventilation, peripheral vasodilation, high cardiac output, and a tendency to arterial hypotension are common but poorly explained features of hepatic failure. We have tested the hypothesis that these abnormalities are mediated by VIP, a polypeptide recently isolated from small intestine. Essential to this hypothesis are that VIP: (*a*) can simulate these physiologic alterations, (*b*) is effectively removed from the portal blood by normal liver, and (*c*) may enter the systemic circulation in the presence of liver disease. Intravenous or intra-arterial infusion of the peptide in anesthetized dogs and rats increased peripheral arterial blood flow and cardiac output, reduced systemic blood pressure, augmented respiration and alveolar ventilation, and raised blood sugar. Infused into the portal vein, VIP was approximately one-fourth as effective on blood pressure and respiration as when given intravenously. The relative concentration of VIP was assayed in arterial blood plasma from 16 patients with hepatic cirrhosis and 18 subjects without liver disease. The assay was based on the direct relaxant effect of the peptide on rat stomach strip, perfused with Krebs' solution. Since plasma contains substances such as serotonin and prostaglandins that contract this tissue, normal plasma contracts rat stomach. On addition of VIP to plasma, however, the contraction becomes progressively weaker, and eventually turns into relaxation. Plasma from cirrhotics gave significantly weaker ($P < 0.001$) contractions than did plasma from controls. The hypothesis is thus supported, though not confirmed, that in the presence of liver damage or shunting of portal blood, VIP might escape inactivation and circulate in the blood, causing hyperventilation, peripheral vasodilation, and other effects. (Supported by NIH Grant HE-04226 and grants from American Heart Association and NTRDA.)

268. Increased Synthesis of Cholestanol in Cerebrotendinous Xanthomatosis (CTX). GERALD SALEN,* E. H. AHRENS, JR.,** AND SCOTT M. GRUNDY,* New York.

The turnover of cholestanol (5α-cholestan-3β-ol) was measured by the isotope kinetic and sterol balance methods in two individuals with CTX (an inherited disease characterized by progressive neurologic dysfunction, xanthomatosis, premature atherosclerosis, normal plasma cholesterol concentrations, and extraordinary levels of cholestanol in all tissues) and in four normolipidemic patients. All were hospitalized on a metabolic ward and fed formula diets that were cholestanol free. Plasma concentrations of cholestanol, measured by gas-liquid chromatography (GLC) after cholesterol was removed by $AgNO_3$-thin-layer chromatography (TLC), were 1.5 and 1.3 mg/100 ml in the two CTX patients and 0.50 ±0.2 mg/100 in the controls. After pulse labeling with cholestanol-1, 2-^{3}H intravenously, specific activity–time curves conformed to a two pool model. This permitted calculation of daily turnover for cholestanol: since cholestanol was absent from the diet, daily turnover was equivalent to daily synthesis. Accordingly, synthesis rates were calculated as 58.3 and 48.5 mg/day in the two CTX patients and 8.3 ±2.1 mg/day in the four controls. The pool of cholestanol turning over more rapidly (pool A) was 211 and 147 mg in the two CTX patients, and 50 mg in one control. The administration of clofibrate to one CTX patient resulted in a 35% reduction in cholestanol synthesis (from 58.3 to 38 mg/day) and a 50% decrease in the plasma cholestanol concentration (from 1.5 to 0.75 mg/day), but the size of pool A remained unchanged (211 vs. 201 mg). These data indicate that excessive accumulation of cholestanol in the tissues of CTX is associated with overproduction of this sterol. Treatment with clofibrate inhibits its synthesis and might be useful therapeutically. (This study was supported by USPHS Grants HE-06222 from NHLI, and FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources.)

269. The Role of Bile Acid Micelles in Absorption of Fatty Acids Across the Intestinal Brush Border. VERNEY L. SALLEE* AND JOHN M. DIETSCHY, Dallas, Tex.

We have shown that absorption of intact micelles by the intestine does not occur; therefore, a critical question is what is the mechanism whereby micelles promote fatty acid (FA) absorption? Using a technique that measures uptake velocities across the brush border, permeation constants (P) for the soluble fatty acid series C_2–C_{10} were determined. P for ionized FA's increased with chain length from 38 (C_2) to 150 (C_{10}) mμmoles/(min·100 mg·mM): P for the corresponding unionized FA's were 20-fold higher. The quantity $\ln(P)$ (mol wt)$^{\frac{1}{3}}$ varies linearly with FA chain length, and this relationship corresponds to an incremental free energy of − 189 cal/mole per CH_2 group. This relationship allows calculation of theoretical P values for the C_{14}–C_{18} FA's. The rates of long-chain FA uptake from micellar solutions saturated with FA were shown to approximate the product of (P) (maximum concentration FA in bulk water). Utilizing the finite solubility of C_{12}-FA, tissue uptake was measured as a function of FA concentration up to saturation in the absence and presence of bile acid. Maximum uptake rates were approximately the same, even though total concentration of FA was 0.7 mmole/liter and 15 mmoles/liter, respectively, in these two solutions. Thus, tissue uptake again equaled the product of (P) (bulk water FA concentration). These data indicate that velocity of FA absorption can be accounted for by passive FA monomer diffusion; the mixed micelle may serve only to transport FA

to the microvillus region where it is crucial for the maintenance of a high FA concentration in the unstirred bulk water phase adjacent to the membrane. (Supported by NIH HE-09610.)

270. Zinc Deficiency: Effect on Nucleic Acid Metabolism in Brain and Liver. HAROLD H. SANDSTEAD,* MARJORIE TERHUNE,* AND ROBERT N. BRADY,* Nashville, Tenn. (introduced by Bert L. Vallee**).

Zinc is essential for man. Deficiency results in retarded growth and sexual maturation. Though zinc is a component of 20-odd enzymes, this aspect of its metabolism does not provide a wholly satisfactory explanation for all aspects of zinc deficiency. Zinc deficiency in microorganisms profoundly affects nucleic acid metabolism. Therefore, nucleic acid metabolism has now been studied in rodents. Newly parturient rats and adult mice were fed a biotin-enriched, 20% sprayed egg white diet (0.5 ppm Zn) and divided into sets of zinc-deficient, pair-fed control, and ad lib. control animals. Controls were given sufficient zinc to maintain normal growth in the ad lib. animals. The following were studied: in vivo incorporation of thymidine-^{3}H into brain and liver DNA and of ^{35}S into protein of the same organs of 11-day-old suckling rat pups nursed by the above dams; lipid composition of brains of the pups; activity of liver nuclear DNA-dependent RNA polymerase of similar pups to 16 days of age; sucrose density gradient liver ribosomal profiles of adult rats and mice and in vivo incorporation of uridine-^{3}H into liver polysomes of the mice. Specific activities of DNA and protein were decreased in brain and liver of zinc-deficient pups as was the concentration of total lipids in brain. Phospholipid concentration and fatty acid pattern in brain were unaffected. RNA polymerase activity progressively decreased from the 10th to 16th day in the zinc-deficient pups. Liver ribosomal profiles were abnormal in the zinc-deficient animals; monomers were increased while dimers and polymers were decreased. Uridine-^{3}H incorporation into polysomes was also decreased. The effects of zinc on enzyme action and nucleic acid metabolism remain to be integrated, to provide a basis for the clinical manifestations of zinc deficiency. (Research supported by Grants 397 and 430 from the Nutrition Foundation.)

271. Reinterpretation of "Electrical Position of the Heart" in Terms of Over-All Scalar ECG Pattern Distribution. JOHN J. SAYEN* AND GEORGE PEIRCE,* Philadelphia, Pa. (introduced by Hugh Montgomery**).

In each of 50 male subjects, normal and abnormal, using an oscilloscopic pattern matching technique, the over-all pattern distribution was determined. All patterns appeared at maximum size on a line or band encircling the torso, the line of maximum amplitude (LMA), then with diminishing size and slowly changing form along a closed line crossing the LMA twice. The decrement lines (DL's) (Wolferth, 1940) met at one or two common points depending on and isopotential with the reference used in tracing them. Unlike the DL's the LMA was independent of the reference. On all DL's paired points occur with equal pattern size hence mutually isopotential (null pairs). In about one-fourth of several large unselected samples of routine ECG'S, normal and abnormal, very small patterns or nulls appeared in one or an adjacent pair of the six limb leads. The DL's represent planes, the nulls lines, both perpendicular to the mean plane of the vector loop. Correspondences between certain "unipolar" limb leads and precordial leads long used to determine "electrical position of the heart" simply represent pairs of points on particular DL's (*not* projections of epicardial patterns in a particular direction). They cannot define all possible shifts of over-all pattern orientation, although such determination is, as long recognized, highly desirable for comparing different ECG's, normal and abnormal. The LMA and the DL's, taken together, provide a coordinate system *on the body surface* which, while requiring no assumptions about actual heart orientation or projection of particular patterns from the heart, completely describes the orientation of all scalar patterns.

272. Implications of Acute Hemodynamic and Pathologic Studies for the Treatment of Acute Myocardial Infarction and Cardiogenic Shock. STEPHEN SCHEIDT,* DANIEL ALONSO,* AND THOMAS KILLIP, New York.

Cardiogenic shock is usually caused by massive myocardial damage. Mean cardiac index in eight patients with shock was 1.4 liters/min per m^2, stroke index 15 ml/beat per m^2, left ventricular end-diastolic pressure (LVEDP) 27 mm Hg, and cardiac work 2.1 kg-m/min. Pathologic findings confirmed massive damage. Mean loss of left ventricular myocardium, recent and old, totalled 50% (range 37–59%). Certain observations suggest myocardial damage may occur in a progressive or stepwise fashion rather than at one time. Only 7% of a group of 72 patients with shock developed shock within 1 hr of infarction. The onset of shock was delayed more than 24 hr after infarction in over half of the group. In order to select patients in whom early and possibly unconventional intervention might limit damage and reduce mortality, hemodynamic studies were performed on 47 patients from 5 to 239 hr after infarction. Mean heart rate was higher, cardiac index, stroke index, and cardiac work lower ($P < 0.005$ in each) in patients who died as compared to those who survived. There was no significant difference in central blood volume or LVEDP between the two groups. Certain physiologic measurements obtained during the initial physiologic study are predictive of death. Thus, cardiac work failed to reach 3.0 kg-m/min in 18 of 19 patients who died, but exceed this value in 27 of 28 patients who survived. Time of death varied from 5 hr to 2 months (median 51 hr) after the initial physiologic measurements. Marked depression of cardiac work identified with $> 90\%$ accuracy patients who may be candidates for unconventional therapy. Limitation of myocardial damage by appropriate early intervention may prove more successful than current therapy of established shock in reducing mortality from myocardial pump failure. (Supported by NIH Contract PH 43-67-1439.)

273. Ectopic Adenyl Cyclase Receptors in Endocrine Tumors. I. SCHORR* AND R. L. NEY, Chapel Hill, N. C.

Adenyl cyclase responses to adrencorticotropin (ACTH) and other hormones were compared in a corticosterone-secret-

ing adrenal carcinoma and normal adrenals of the rat. Whole homogenates or particles sedimenting at 1000 *g* were used. The enzyme assay was based on conversion of alpha-ATP-^{32}P to cyclic AMP, with cyclic AMP-^{3}H added for estimation of recoveries. Tumor cyclase was stimulated not only by ACTH, but unexpectedly also by epinephrine, norepinephrine, thyroid-stimulating hormone (TSH), and luteinizing hormone (LH). The hormones produced dose-related effects and were active at concentrations comparable to those of ACTH. Responses to the pituitary trophin preparations could not be ascribed to contaminating hormones. Vasopressin, angiotensin II, glucagon, insulin, growth hormone, parathyroid hormone, and thyrocalcitonin did not stimulate the tumor cyclase. ACTH was the only hormone that stimulated normal adrenal adenyl cyclase. The possibility that the tumor possesses a single degenerate receptor that responds to many hormones was excluded by the observation that propranalol abolished responses to catecholamines but had no effect on stimulation by other hormones. From experiments with ACTH analogues, it was established that the tumor ACTH receptor requires similar portions of the ACTH molecule for cyclase activation as the normal adrenal receptor. At maximally stimulating concentrations of each, ACTH, TSH, and epinephrine did not have additive effects on tumor cyclase activity, suggesting a common effector or catalytic unit for the different receptors. Human endocrine tumors also have exhibited unexpected cyclase responses. A parathyroid adenoma cyclase was stimulated by glucagon, and a chromophobe adenoma by ACTH. The results suggest the presence of "ectopic" hormone receptors in the adenyl cyclase system of some endocrine tumors. Responses to hormones other than normal regulators of the parent gland could be a factor in the apparently autonomous function of these tumors.

274. The Role of Antibody and Complement in the Immune Clearance and Destruction of Erythrocytes. A. D. SCHREIBER* AND MICHAEL M. FRANK,* Bethesda, Md. (introduced by R. J. Wurtman).

A model for immune clearance and destruction of homologous erythrocytes was established in the guinea pig. IgM and IgG immunoglobulins were isolated from rabbit anti-guinea pig erythrocyte antisera and were used to sensitize ^{51}Cr-labeled guinea pig erythrocytes. The average number of complement-fixing sites per erythrocyte in the various preparations was determined on an absolute molecular basis by "C1a fixation and transfer." The rate of clearance and the organ localization was determined for cells sensitized with IgM and IgG antibody and dose reponse curves were established. At least 60 complement-fixing sites per cell were required for accelerated clearance of IgM-sensitized erythrocytes. The bulk of cells with IgM sites were cleared by the liver within 5 min after injection and were then slowly returned to the circulation where they survived normally. As few as 1.4 IgG complement-fixing sites per cell resulted in decreased erythrocyte survival; there was no evidence of immediate tissue sequestration and release. Trapping and destruction of erythrocytes by the spleen was responsible for most of the clearance of IgG-sensitized cells. The accelerated clearance of IgM- and IgG-sensitized erythrocytes was markedly impaired in a strain of guinea pigs with a genetically controlled, total deficiency of the complement component C4, indicating that complement plays a major role in the clearance of cells sensitized with either immunoglobulin. These data support the concept that IgM and IgG antibodies interact with complement by different mechanisms, and that IgG may produce more membrane damage per complement-fixing site than IgM. They provide a new molecular approach to the study of hemolytic anemia.

275. Protein Degradation in Acute Cardiac Overload. SIDNEY S. SCHREIBER,* MURRAY ORATZ,* AND MARCUS A. ROTHSCHILD,** New York.

Acute cardiac overload leads to early augmented nuclear RNA polymerase activity, mRNA synthesis, microsome protein synthesis, and after 3 hr, increased myosin synthesis. The trigger for this sequence is not known, but later increases in myofibrillar breakdown have been observed and protein degradation inferred. This question was studied in prelabeled hearts perfused in vitro for 3 hr. 300-g guinea pigs were injected with lysine-^{14}C 7 days before preparation of the hearts for perfusion, and each received three or four injections of nonradioactive lysine during the week before sacrifice. Left ventricular fluid load was 35–40 ml/min per g dry weight with aortic pressure 35 mm Hg in controls and 70 mm Hg in overload. After passage through the heart, nonrecirculating perfusate was collected for 30-min periods. Lysine-^{3}H was then added to each collection, the perfusates dried, extracted with acid alcohol, treated with cold trichloroacetic acid (TCA), and the total lysine-^{14}C activity determined from the ^{14}C/^{3}H ratio. Total activity in the hearts varied from 125,000 to 160,000 cpm in the overloaded and controls. The total non-TCA–precipitable activity collected for 3 hr was 2.17 $\pm$0.3% and 1.89 $\pm$0.2% of the original counts in overload and control respectively. More than 95% of the activity recovered moved with lysine on paper chromatography. There was no significant difference between overload and control in any 30-min collections. The findings suggested that cardiac protein synthesis and degradation are not interdependent, and that the early increases in protein synthesis in overload were not triggered by increased protein degradation. (This study was supported in part by Grant 09562 from the NIH.)

276. Variant Postheparin Lipolytic Activity (PHLA) in Familial Exogenous Hypertriglyceridemia (FEH). PAUL H. SCHREIBMAN,* DANIEL L. ARONS,* AND RONALD A. ARKY, Boston, Mass.

A 5-yr-old male proband (P) and his 4-yr-old sister with hypertriglyceridemia and hepatosplenomegaly since birth, had a rapid decrease in serum triglycerides on a 3 g fat diet. Oral glucose tolerance tests were normal. PHLA against chylomicrons was virtually absent (0.14 and 0.22 μEq free fatty acid/ml per hr). Their mother and a normolipemic sister had levels (2.68 and 2.46 respectively) approximately 50% normal (6.94 $\pm$SD 1.96; n = 12). However, PHLA against an emulsified soybean oil triglyceride (Intralipid) was normal in all four family members (3.90, 4.40, 6.30, 4.10 respectively; normal = 6.22 $\pm$2.42). In contrast, two additional children from different kindreds with FEH (type I) and two patients

with an acquired mixed-type (type V) of hypertriglyceridemia all had deficient PHLA against both substrates. No inhibitors of PHLA were demonstrated in P's concentrated plasma. Neither normal plasma nor lipoprotein fraction d > 1.063 enhanced P's low PHLA. Michaelis-Menton kinetics revealed that P's postheparin plasma required almost 20 times the substrate concentration to reach half V_{max} (K_m = 175 mmoles/liter vs. 9.3 mmoles/liter in four normals). P and his affected sister had normal postheparin monoglyceridase and only moderately decreased postheparin phospholipase activities. Both affected siblings showed markedly elevated prebeta lipoproteinemia as well as chylomicronemia on a diet containing 36% of the calories as carbohydrate. When P's postheparin plasma was incubated with chylomicrons, a smaller S_f 20–400 "remnant" with prebeta mobility was produced. Under similar conditions, normal postheparin plasma did not produce these changes. These studies suggest that in FEH, a mutant gene may produce a lipoprotein lipase with unusual substrate specificity and kinetics that may account for the accumulation of chylomicrons and increased prebeta lipoproteinemia. (Research supported by NIH.)

277. Alteration of Smooth Muscle Electrical and Motor Activity by Prostaglandins. MARVIN M. SCHUSTER* AND BOON VANASIN,* Baltimore, Md. (introduced by Douglas G. Carroll**).

Prostaglandins, normally present in the gut, are released by histamine, pentagastrin, 5-hydroxytryptamine, and vagal stimulation, and can cause diarrhea in minute oral doses. The effects of prostaglandins on electrical and motor activity were studied in 60 isolated colon muscle strips from 24 dogs and 10 strips from four humans. In longitudinal muscle PGE_1, E_2, $F_1\alpha$, $F_2\alpha$, and A (10^{-8}–10^{-6} mole/liter) dramatically increased the frequency and amplitude of electrical slow waves which was associated with augmented "tone," and also increased spike potentials associated with strong phasic contractions. Hyoscine and atropine administered before or after prostaglandins decreased both tonic and phasic contraction. Hexamethonium had no effect. In circular muscle $PGF_1\alpha$, $F_2\alpha$, and A produced contraction, while E_1 and E_2 produced relaxation. Prostaglandin antagonist SC 19220 inhibited contractions induced by PGE_1 and E_2. These findings indicate the following. (*a*) Electrical slow waves correlate with muscle tone and spike activity with phasic contractions. (*b*) Prostaglandins augment tone by increasing frequency and amplitude of slow waves. (*c*) Prostaglandins evoke phasic contractions by stimulating spike activity. (*d*) All prostaglandins studied produce contraction of longitudinal muscle. (*e*) $PGF_1\alpha$, $F_2\alpha$, and A contract, while PGE_1 and E_2 relax circular muscle. (*f*) Prostaglandins appear to act on postganglionic cholinergic receptors since hexamethonium, a ganglionic blocker, does not inhibit prostaglandin-induced response, while hyoscine, a postganglionic blocker does. (*g*) Prostaglandins also act directly on smooth muscle, since prostaglandins stimulate electrical activity which is myogenic in origin. (*h*) Prostaglandin antagonist SC 19220 competes at cholinergic-type but not adrenergic-like receptor sites, since SC 19220 prevents the contractile but not the relaxing effects on PGE_1 and E_2. Prostaglandin in physiologic amounts may play a role in colonic motility.

278. Bone Marrow Globin Synthesis in Heterozygous Beta Thalassemia. ELIAS SCHWARTZ,* Philadelphia, Pa. (introduced by Allan J. Erslev**).

I previously described two patients with heterozygous β-thalassemia in whom there was decreased synthesis of β-chain in reticulocytes and equal synthesis of α- and β-chains in marrow cells (1970. *Science.* **167**: 1513). In the present study, samples of peripheral blood and bone marrow from three additional patients were incubated with leucine-^{14}C for 2 hr at 37°C. The α- and β-chains were separated by chromatography on carboxymethyl-cellulose in 8 M urea. Total radioactivity of each of the chains was determined and the β/α ratios calculated. In two patients heterozygous for β-thalassemia, the β/α ratios were 0.64 and 0.68 in peripheral blood and 1.06 and 0.97 in marrow samples, confirming the previous findings. In a patient with Hb S–β-thalassemia who produced decreased amounts of Hb A, similar studies were performed to determine the relative output of nonthalassemic (β^S) and thalassemic ($\beta^{thal(A)}$) loci and to compare the synthesis of each chain during red cell maturation. The $\beta^{thal(A)}/\alpha$ ratios in the marrow and blood were 0.21 and 0.20 respectively, while the β^S/α ratios were 0.84 and 0.59. These results indicate that balanced production of globin chains in the marrow in heterozygous β-thalassemia is due to a marked increase in synthesis of β-chain directed by the nonthalassemic locus in compensation for decreased synthesis by the thalassemic locus. The hypothesis of rapid decay of mRNA produced by the thalassemic locus is not confirmed. On the contrary, it appears that after the loss of the red cell nucleus the rate of synthesis of β-chain reflecting the nonthalassemic locus decreases more rapidly than that reflecting the thalassemic locus. (Supported by NIH Grant AM-12896.)

279. Glycogenolysis and Glycogenesis in a Particulate System from Rat Liver. ROBERT B. SCOTT* AND LAVERNE W. COOPER,* Richmond, Va. (introduced by G. Watson James III**).

The functional unit of glycogen metabolism (the glycosome) consists of the glycogen polymer and attached enzymes of glycogen metabolism. Isolation of a "native" glycogen particle with the ability to synthesize and degrade glycogen would lead to better understanding of true physiologic function of the glycosome. Liver from fed rats was homogenized in 0.01 M Tris buffer pH 8.0 and centrifuged at 8000 *g* to remove nuclei and granules. Glycogen was precipitated from the 8000 *g* supernate with concanavalin A, a bean lectin which specifically precipitates branched polysaccharides such as glycogen. This precipitate was collected at 4000 *g* and washed with saline. The glycogen-concanavalin aggregates were suspended and dialized against a 0.05 M phosphate buffer, pH 6.9, to activate phosphorylase. During glycogenolysis, glycogen-metabolizing enzymes and partially degraded glycogen were released from the concanavalin aggregates. Aliquots were placed in buffer containing 0.1 M uridine diphosphoglucose-^{14}C, and 0.1 M glucose-6-phosphate, pH 7.5. Glycogen resynthesis took place at room temperature during a 20 hr incubation. Glycogen degradation released glucose-1-phosphate and glucose into the dialysis medium, indicating activity of phosphorylase. In overnight incubation, glycogenolysis was

virtually complete. Maltose, the product of amylase activity, was not detected. Unequivocal glycogen synthesis was shown by incorporation of ^{14}C into purified glycogen and by chemical measurements. This glycogen contained a branched structure like native glycogen, as shown by the failure of beta amylase to remove more than 30% of the newly synthesized glycogen radioactivity. These data indicate that the isolated glycogen particles contain activities of both degradative and synthetic enzymes, and that these two functions can be selectively activated in vitro. This system thus provides a working model for quantitative study of the native glycosomes in the liver.

280. Role of Adrenocorticosteroids in Mediation of Therapeutic Effect of Corticotropin in Severe, Generalized Myasthenia Gravis. MENACHEM S. SHAPIRO,* TATSUJI NAMBA,* NORMAN G. BRUNNER,* AND DAVID GROB,** Brooklyn, N. Y.

In patients with severe myasthenia gravis, short, intensive courses of corticotropin (100 U intravenously or 160 U intramuscularly daily for 10 days) produced increased weakness during 97% of 68 courses in 22 patients, followed by improvement of strength above the initial level after 93% of the courses, lasting an average of 78 days. Since most reports have described little effect of glucocorticoids on the course of the disease, it has been postulated that this unique effect of corticotropin is mediated in some other way than by increased glucocorticoid secretion. However, short, intensive courses of methylprednisolone (60 mg intramuscularly daily) or hydrocortisone (300 mg intramuscularly daily) for 10 days produced increased weakness during 73% of 48 courses in nine patients, followed by improvement after 84% of the courses, lasting an average of 70 days. Plasma cortisol levels were 112–178 μg/ml during corticotropin administration and 30–167 μg/ml during hydrocortisone administration. Administration of metyrapone (3.0–4.5 g orally daily) with corticotropin diminished the degree of exacerbation and improvement, concomitant with a reduction in cortisol synthesis, as indicated by urinary excretion of 50–80% of the total urinary 17-hydroxycorticoids as tetrahydrodeoxycortisol. Administration of aldosterone to two patients (total of 11.7 mg intravenously for 14 days and 12.9 mg intravenously for 18 days, respectively) produced expected changes in electrolytes, but no significant changes in muscle strength. These results indicate that glucocorticoids are also effective in the management of severe myasthenia gravis, and the therapeutic effect of short, intensive courses of corticotropin appears to be mediated mainly by increased glucocorticoid secretion. (Supported by USPHS Grant NS 03464 from NIH.)

281. Primary Care for the Patient with Diabetes Mellitus. JOSEPH C. SHIPP AND JUDY JORDAN,* Gainesville, Fla., and Omaha, Nebr.

Primary care for 17 insulin-dependent patients with diabetes mellitus was provided by a nurse specialist in diabetes for 6–16 months. Reduced hospitalizations, improved work-home-economic status, and improved regulation of diabetes resulted from effective patient and family education, individualized and reasonable goals, and continuity of care. Effectiveness of the nurse specialist was also shown in a group of 15 noninsulin-dependent adults with diabetes. This experience suggests that the nurse specialist, or a properly trained physician assistant, can contribute significantly in improving the care of a large number of patients with this chronic disorder. (Supported by NIH Grant AM 5444.)

282. *Mycoplasma hominis*, and Organism That Escapes and Impairs Phagocytosis by Granulocytes. MICHAEL S. SIMBERKOFF,* PETER ELSBACH, PENELOPE PETTIS,* AND PIERLUIGI PATRIARCA,* New York.

Little is known about cellular host defense mechanisms during infection with mycoplasma. We have examined in vitro the interaction between polymorphonuclear leukocytes and *Mycoplasma hominis* (PPLO). Upon incubation of PPLO for 2 hr with rabbit peritoneal exudate granulocytes or leukocytes from human peripheral blood no significant killing was observed either in the presence or absence of type-specific antibody. However, $^{14}CO_2$ production from glucose-1-^{14}C was stimulated about 10-fold in the presence of live or heat-killed PPLO. The extent of stimulation depended upon PPLO number and the presence of type-specific antibody. The stimulation is not due to tight adherence of PPLO to the granulocytes, since PPLO were quantitatively recovered in the medium after sedimenting the granulocytes. Medium of PPLO suspensions from which the organisms had been removed by centrifugation also stimulated $^{14}CO_2$ production by granulocytes. Phospholipids of PPLO labeled with palmitate-1-^{14}C underwent no degradation when the organisms were exposed to intact granulocytes but were rapidly degraded in the presence of granulocyte homogenates. Killing of *E. coli* or *S. marcescens*, organisms that are readily engulfed and killed by granulocytes in vitro, was imparied when PPLO were also present. These findings indicate that *Mycoplasma hominis* is not ingested by granulocytes but that it affects the granulocyte's metabolism and also impairs phagocytosis of other microorganisms. These effects appear mediated, in part, by an agent or agents released by PPLO into the medium. (Supported by USPHS Grant AM 05472.)

283. Inhibition of Mitochondrial Protein Synthesis by Chloramphenicol (CAP) in an Intact Vertebrate. LAWRENCE M. SIMON,* JAMES THEODORE,* AND EUGENE D. ROBIN,** Stanford, Calif.

Evidence in isolated cell preparations suggests that an independent mitochondrial genetic system plays a major role in regulating mitochondrial protein synthesis. This system can be inhibited in vitro by appropriate doses of chloramphenicol (CAP). Data concerning this hypothesis are lacking in intact animals. In the present studies the effect of CAP on cytochrome oxidase (CO), a mitochondrial enzyme, has been compared with the effect on a cytoplasmic enzyme, pyruvate kinase (PK), in the fresh-water turtle. 12 animals were studied, six controls and six receiving 100 mg/kg per day of CAP intramuscularly for 3 days (doses moderately in excess of clinically employed levels). CO and PK activities were measured spectrophotometrically in heart and skeletal muscle homogenates. CAP administration resulted in a striking reduction in CO activity (μmoles reduced cytochrome *c* oxidized $\times$ min^{-1} $\times$ mg $protein^{-1}$) of both heart and skeletal muscle:

heart: control 7.12 ±0.66, $P < 0.001$, CAP 5.33 ±0.70; skeletal: control 1.31 ±0.25, $P < 0.01$, CAP 0.71 ±0.12. PK activity (mmoles phosphenol-pyruvate converted to pyruvate × min^{-1} × mg $protein^{-1}$) was not significantly affected: heart: control 87 ±10, $P > 0.5$, CAP 81 ±19; skeletal: control 230 ±150, $P > 0.6$, CAP 272 ±120. These results suggest: (*a*) independent mitochondrial regulation of CO synthesis in vivo; (*b*) CO turnover times are relatively short (<72 hr); (*c*) CAP selectively inhibits mitochondrial protein synthesis without affecting cytoplasmic protein synthesis in the intact vertebrate as well as isolated cellular systems; and (*d*) some of the toxic effects of CAP noted clinically may be related to inhibition of important enzymes involved in oxygen-dependent energy transduction.

284. Adaptive Changes Produced by Chronic Metabolic Acidosis in Homogenates and Mitochondria from Renal Cortex. DAVID P. SIMPSON,* Seattle, Wash. (introduced by Marvin Turck).

In chronic metabolic acidosis production of NH_3 from glutamine by cells of renal cortex is greatly increased, enabling the kidney to excrete increased amounts of H^+ as NH_4^+. This phenomenon is reflected in vitro by an increase in the rates of glutamine and citrate oxidation by slices of renal cortex from chronically acidotic dogs. Recently we have demonstrated similar metabolic adaptations in subcellular fractions of renal cortex. Cell-free homogenates, prepared from renal cortex from littermate dogs with chronic metabolic acidosis or alkalosis, were incubated with glutamine-^{14}C or citrate-^{14}C. $^{14}CO_2$ production from either labeled substrate was 20–60% greater in homogenates from kidneys of acidotic dogs than in identically prepared tissue from alkalotic animals. Mitochondria were isolated from these tissue preparations and incubated under similar conditions. In some cases glutamine oxidation was greater in mitochondria from acidotic dogs; in other cases mitochondria from acidotic and alkalotic animals oxidized glutamine at the same rate. Mitochondrial citrate oxidation usually occurred at the same rate regardless of the previous acid-base state of the dog. However when mitochondria were suspended in dialyzed, 105,000 *g* supernatant of renal cortex from either acidotic or alkalotic animals, the rates of glutamine and citrate oxidation were enhanced and mitochondria from acidotic dogs consistently oxidized these substrates at higher rates than were obtained with mitochondria from alkalotic animals. The magnitude of this difference was similar to that observed in homogenates and slices. Thus chronic metabolic acidosis induces a change in renal cortical mitochondria which permits increased oxidation of glutamine and citrate. A high molecular weight substance in the cytosol is necessary to demonstrate this adaptation. (Research supported by NIH Grant AM09822.)

285. Secreted Molecular Species of Human Parathyroid Hormone (hPTH): Purification, Immunologic and Chemical Characterization. GLEN W. SIZEMORE,* SUSAN B. OLDHAM,* JAN A. FISCHER,* AND CLAUDE D. ARNAUD,* Rochester, Minn. (introduced by A. Albert**).

In a previous report we showed that the hPTH in the medium of parathyroid adenoma cultures was immunochemically indistinguishable from the hPTH in hyperparathyroid serum but both differed markedly from the hPTH purified from urea extracts of parathyroid adenomata. We have purified the hPTH secreted into the defined medium (no serum) of large-scale cultures of parathyroid adenomata slices on the assumption that it is representative of the native "secreted" molecular species of hPTH. When crude, desalted, lyophilized culture medium is fractionated on 2 × 200 cm columns of Bio-Gel P-10 polyacrylamide beads, at least three immunologically distinguishable species are obtained. The first (mol wt > 14,000) is greatest in quantity (protein) but has the lowest immunoreactivity (< 1% of total recovered). The second (mol wt approximately 9000) cannot be distinguished immunologically from hPTH purified from urea extracts of parathyroid adenomata and comprises 20% of the total recoverable immunoreactivity. The third (mol wt approximately 6000) has the immunologic characteristics of the hPTH in hyperparathyroid serum and represents the major immunoreactive fraction recovered (80%). The third is approximately 80% homogeneous on disc-gel electrophoresis and has an amino acid composition which closely resembles that of the *N*-terminal 55 residues of bovine PTH. These data suggest that "secreted" hPTH is immunoheterogeneous but that the major immunoreactive species of PTH in serum has a smaller molecular weight than the predominant glandular species. We believe that the best of several alternative explanations of this phenomenon is that "secreted" PTH is derived from a structurally different, precursor, glandular species of PTH. (Research supported by Grant AM 12302 from NIH.)

286. Immunologic Reversal of the Inotropic Effects of Digoxin. C. LYNN SKELTON,* VINCENT P. BUTLER, DONALD H. SCHMIDT,* AND EDMUND H. SONNENBLICK, Boston, Mass., and New York.

Although antibodies specific for digoxin have been shown to rapidly reverse severe digoxin toxicity in dogs, the effects of these antibodies on the inotropic action of digoxin has not been studied. Accordingly, this problem was examined in 22 isolated cat papillary muscles contracting isometrically at 37°C. Digoxin antisera were obtained from rabbits immunized by injections of digoxin conjugated to bovine serum albumin. Prior exposure of the muscles to nondepressant amounts of digoxin antisera completely prevented the inotropic effects of digoxin in concentrations below 10^{-6} mole/liter. The antisera inhibition could be overcome by 5×10^{-6} M digoxin, a toxic concentration in the absence of digoxin antibodies. Likewise digoxin antisera completely reversed the increases in active tension and rate of tension development produced by 5×10^{-7} M digoxin, the return to control levels of contractile function requiring approximately 90 min. In contrast, no reversal of the inotropic effects of digoxin occurred during a 90 min observation period after replacing the digoxin-containing bathing solution with a digoxin-free medium. The subsequent addition of antisera to these muscles also resulted in a prompt return toward control levels of contractile function. Serum from nonimmunized rabbits did not prevent or reverse the usual inotropic response to 5×10^{-7} M digoxin. These findings suggest that the reversal of the inotropic effects of digoxin by specific antisera occurs via an interaction of the antibody with digoxin bound to the cell. The reduction in the concentration of free

digoxin in the bathing solution which results from antibody binding appears to be of less importance in reversing digoxin-induced inotropy. Furthermore, our results provide indirect evidence that the inotropic effects of digoxin are mediated at the cell membrane. (Supported by NIH Grants HE 11306 and HE 5890-01.)

287. Further Observations on the RNA- and DNA-Dependent DNA Polymerases of Normal and Leukemic Cells and Selective Inhibition by Rifamycin Derivatives. G. SMITH,* S. YANG,* F. HERRERA,* J. WHANG-PENG,* AND R. GALLO,* Bethesda, Md. (introduced by C. Gordon Zubrod**).

An earlier report from this laboratory detailed detection and partial purification of RNA-dependent DNA polymerase (RDP) in human leukemic blasts (Gallo, Yang, and Ting. 1970. *Nature (London).* **228**: 927). Similar activity was not found in proliferating normal cells. This enzyme is analogous but not necessarily identical with RDP regularly found in RNA tumor viruses (Temin. 1970. *Nature (London).* **22**: 1211; and Baltimore. 1970. *Nature (London).* **22**: 1209). Subsequently we have found RDP activity in human myeloblastic leukemic tissue. A modified technique including high-speed centrifugation and DEAE-column chromatography has yielded RDP activity in leukemic thymus from AKR mouse; no activity was seen in L1210 or P388 murine leukemic tissue. This difference may reflect varying etiologies of these tumors. Human RDP is active with a variety of "physiologic" RNA's: *E. coli* $tRNA^{Glu}$ (purified isoaccepting species), 23S *E. coli* rRNA, homologous leukemic tRNA, homologous leukemic rRNA. This opens the possibility that RDP may function in gene amplification and information transfer. The rifamycin derivative *N*-demethylrifampicin inhibits human RDP to 60 and 100% of control activity at concentrations of 200 and 400 μg/ml respectively; corresponding levels of rifampicin produce only 20 and 35% inhibition. Green observed similar results with virion RDP (Green, in press). Two species of DNA-dependent DNA polymerase purified from a normal human lymphoblastic cell line were inhibited minimally by either rifamycin derivative. When grown with 250 μg/ml of *N*-demethylrifampicin, fresh human leukemic blasts progressively disappear, with preservation of more mature cells. Although *N*-demethylrifampicin slightly inhibits transformation of PHA-stimulated normal lymphocytes (rifampicin is without effect), lymphoblast viability appears morphologically unaltered.

288. Turnover and Transport of Vitamin D in Human Plasma. JOHN E. SMITH* AND DEWITT S. GOODMAN, New York.

Four healthy men were injected intravenously with physiological doses (6 μg) of vitamin D_3-1, 2-^{3}H. Serial samples of plasma were collected for 50 days. Total lipid extracts were chromatographed on silicic acid columns or thin-layer plates in order to characterize the radioactive components. Labeled vitamin D_3 (D_3) disappeared rapidly from plasma (initial half-life approximately 12 hr); after 7 days D_3 represented less than 1% of circulating radioactivity. Coincident with D_3 disappearance a more polar component appeared with chromatographic properties identical with those of 25-hydroxy vitamin D_3 (OH-D_3). The disappearance of OH-D_3 was relatively slow, with half-life 19.6 ±0.8 days. Most (approximately 92%) of the plasma total radioactivity was represented by this component throughout the study. Plasma samples collected at various times were adjusted to density (d) 1.21, and were ultracentrifuged to separate plasma lipoproteins from proteins with $d > 1.21$. In all samples almost all (mean 94%) of the radioactivity was found in association with proteins of $d > 1.21$. This observation was confirmed by bioassay, measuring uptake of ^{45}Ca by intestinal slices. All plasma bioassayable vitamin D was found in association with proteins of $d > 1.21$; 55% of bioactivity was found in chromatographic fractions corresponding to OH-D_3, and 44% in those representing D_3. Since both D_3 and OH-D_3 are lipid-soluble sterol derivatives, the finding that these compounds do not circulate in association with the known plasma lipoproteins provides presumptive evidence for the existence of a specific transport protein of $d > 1.21$. This protein has been partly characterized by gel filtration on Sephadex G-200 and by electrophoresis on polyacrylamide gel. The protein has an apparent size slightly smaller than serum albumin (approximate mol wt 50,000–60,000) and an electrophoretic mobility very slightly greater than that of albumin. Studies are in progress to further fractionate and characterize the transport protein. (Supported by NIH Grant AM-05968.)

289. Myocardial Blood Flow in Man: Effect of Collaterals and Acute Revascularization. S. C. SMITH,* R. GORLIN, M. V. HERMAN,* W. J. TAYLOR,* AND J. J. COLLINS,* Boston, Mass.

Coronary artery surgery, using saphenous vein bypass (SVB), provides a unique opportunity to study myocardial blood flow and hemodynamics. In 16 patients flow reconstituted via SVB was compared to preexisting distal coronary artery (DCA) flow by intraoperatively injecting ^{133}Xe into the distal SVB with proximal SVB both open and occluded. Pressure gradients across bypassed obstructions were measured. Results were correlated with preoperative coronary arteriography to determine the effect of collateral supply on myocardial clearance curves and DCA perfusion pressures. Clearance was monoexponential with proximal SVB occluded, and averaged 17 ±2 cc/min per 100 g. This preexisting DCA flow was 21% higher in patients with collaterals than in those without collaterals. When the proximal SVB was opened, triple exponential clearance curves were obtained: phase I, 15–20 sec duration, 309 ±32 cc/min per 100 g; phase II, 104 ±14 cc/min per 100 g; and terminal phase III, slow flow, 23 ±3 cc/min per 100 g. Phase I represents either reactive hyperemia after release of clamped SVB, or indicator shunting. Phases II and III demonstrate the persistence of heterogeneous myocardial flow after SVB. Phase II represents increased antegrade flow to formerly obstructed DCA myocardium. Phase III correlated with, but was 25% ($P < 0.05$) higher than monoexponential flows obtained with SVB occluded and possibly represents improved perfusion of myocardial compartment formerly supplied only by collaterals or restricted antegrade flow. Pressure gradients did not correlate with arteriographic per cent stenosis. However, as with DCA flow, DCA pressures were higher (187% $P < 0.05$) in patients having demonstrable collaterals. Lowest DCA pressures were obtained in patients without collaterals and poor filling of the

DCA. Intra-operative assessment of SVB and DCA flow and pressure relationships using ^{133}Xe clearance demonstrates heterogeneity of myocardial flow, reveals the variable functioning capacity of collateral pathways, and establishes the efficacy of coronary artery bypass surgery to acutely improve nutrient myocardial blood flow.

290. Lipid Composition Changes in Human Polymorphonuclear Cell Fractions after Phagocytosis. JAMES E. SMOLEN,* STEPHEN B. SHOHET,* PHILIP COHEN,* ROBERT L. BAEHNER,* AND MORRIS J. KARNOVSKY,* Boston, Mass. (introduced by Louis K. Diamond**).

During phagocytosis new phospholipid is synthesized from triglyceride fatty acid (FA) and may be utilized to form the membrane walls of phagocytic vacuoles. In additoin H_2O_2, which can peroxidize unsaturated membrane FA, is generated. Because both of these processes could change membrane FA composition during the transformation of lysosomes into phagosomes, the lipid compositions of lysosomes and phagosomes were examined. Since phagosomes may arise from plasma membranes they were also examined. Phagosomes were prepared by density gradient centrifugation of dense polystyrene particles after phagocytosis. The phagosomes had intact membranes by electron microscopy, contained myeloperoxidase and hydrolytic enzymes, and demonstrated latency. Plasma membranes and lysosomes were prepared by differential centrifugation of cell homogenates made before phagocytosis by nitrogen cavitation. The percentages of various FA's were determined by thin-layer chromatography and gas-liquid chromatography in the presence of antioxidants. An unsaturation index (UI) was determined as the sum of the unsaturated bonds. Phagosomes showed a major reduction in unsaturated FA in comparison to prephagocytic lysosomes (UI 66 $\pm$6 vs. UI 110 $\pm$5). These changes were most marked in phosphatidylethanolamine but were also present in phosphatidylcholine. They were primarily due to reduced oleic and arachadonic acids and increased palmitic and stearic acids. Plasma membranes were also saturated in comparison to lysosomes (UI 94 $\pm$8). However, this difference was not sufficient to explain the marked comparative saturation of the phagosomes. The observed increase in FA saturation in phagosomes may be induced by new phospholipid synthesis from predominantly saturated triglyceride FA or by peroxidative destruction of unsaturated FA during phagocytosis. In either case the resultant change in FA composition may reduce phagosome permeability and control cell autodigestion after phagocytosis. (Supported by grants from NIH, the John A. Hartford Foundation, and the Medical Foundation, Inc.)

291. Direct Lysis of Lymphocytes in Systemic Lupus Erythematosus on Exposure to Complement. PETER STASTNY* AND MORRIS ZIFF,** Dallas, Tex.

It has been observed that circulating lymphocytes of patients with active systemic lupus erythematosus (SLE) show an increased susceptibility to lysis upon addition of rabbit complement. When lymphocytes from SLE patients were separated and incubated with complement at room temperature, up to 70% of the cells were lysed. This was maximal after 40–60 min. Lymphocytes from 12 SLE patients studied serially lost their abnormal susceptibility to rabbit complement after steroid treatment of the patients and concomitant clinical improvement. Direct lymphocyte lysis (DLL) was not observed when human, instead of rabbit complement was used. Mean DLL (expressed as per cent lymphocytes surviving after incubation with complement) of 25 SLE patients was 74.5 $\pm$20.1, of 25 patient controls 94.8 $\pm$7.9 ($P < 0.001$), and of 25 normal individuals 96.5 $\pm$5.2 ($P < 0.001$). Seven eluates from SLE lymphocytes obtained by either heat or acid elution were found to contain chiefly IgG and to be cytotoxic for selected homologous lymphocytes. Two eluates from normal lymphocytes had no cytotoxic effect. Eluates from one active SLE patient demonstrated autologous cytotoxicity. These observations suggest that circulating lymphocytes of patients with active SLE are coated with an autoantibody which renders them susceptible to lysis by added complement; they offer a simple technique for the detection of autologous sensitization in patients with active SLE and in patients with immunologically induced leukopenia. (Supported by USPHS Grants AM-09989, AM-05154, and AM-01235.)

292. Genomic Recognition of Hormone-Receptor Complexes: a General Concept for Steroid Hormones. ALAN W. STEGGLES,* THOMAS C. SPELSBERG,* AND BERT W. O'MALLEY, Nashville, Tenn.

Current theories have suggested that steriod hormones act on target tissues by regulating gene activity and controlling nuclear RNA synthesis. At least part of this regulation has been thought to involve alterations in transcription of chromatin. Since we have recently demonstrated the presence of a specific progesterone receptor in chick oviduct cytoplasm which binds the hormone and transfers it to the nucleus, we initiated a search for "acceptor" sites on the target cell chromatin. Crude cytoplasmic receptor was prelabeled with progesterone-^3H and incubated (under exacting conditions of pH, temperature, and ionic strength) directly with purified chick tissue chromatins. The progesterone-^3H–oviduct receptor complex displayed a much greater affinity for oviduct chromatin (1 μg progesterone per g DNA) than for chromatins of chick spleen, heart, or erythrocytes. Similar results were obtained using purified (2500-fold) receptor. The tissue-specific binding of receptor to chromatin is maintained in chromatins which have been dissociated and reconstituted in high salt and urea. Reconstituted "hybrid" chromatins, composed of histones from chromatin of one tissue, and the DNA-acidic proteins from the chromatin of another tissue, were also prepared. The association of oviduct progesterone–receptor complex with these "hybrid" chromatins demonstrated that the chromatin acidic proteins, but not histones, were responsible for specific association of progesterone with the oviduct chromatin. The generality of this concept for mammals was verified by demonstrating specific binding of dihydrotestosterone-^3H receptor to rat prostate chromatin and rat uterine estradiol-^3H–receptor to rat uterine chromatin, but not to their respective nontarget chromatins. Our evidence is thus compatible with the general concept that the genome of target cells is preprogrammed to accept the hormone-receptor complex upon its entrance into the nucleus, an event immediately preceding stimulation of RNA synthesis.

293. 6-Aminonicotinamide (6 AN) As a Diabetogenic Agent; In Vitro and In Vivo Studies in the Rat. JURGEN STEINKE AND H. P. T. AMMON,* Boston, Mass.

In the brain 6 AN interferes with NADP synthesis and function and thus decreases activity of the pentose phosphate shunt. Controversy exists if in the pancreatic beta cell it is the glycolytic or the shunt pathway which is necessary for insulin release. Therefore we examined the effect of 6 AN on glucose-induced insulin release with collagenase-isolated pancreatic islets in vitro and in the living rat. For both studies the animals were pretreated with 6 AN (35 mg/kg). In the in vitro experiments the islets were incubated for 90 min in buffer or 3 mg/ml glucose. Whereas control islets increased their insulin output from 20 ±6 to 460 ±62 μU/ml of immunoreactive insulin (IRI), 6 AN islets released significantly less IRI (167 ±48 μU/ml). This lower response did not result from decreased insulin content as acid ethanol extraction of respective islets showed no difference (1.6 ±0.2 vs. 1.5 ±0.2 mU/islet). Furthermore addition of tolbutamide (0.1 mg/ml) fully restored insulin release. For the in vivo studies intraperitoneal glucose tolerance tests were performed after either a 6 or 24 hr fast. Glucose dose was 0.5 mg/kg; samples were collected at time 0, 10, 20, and 60 min. Parameters measured were urinary glucose, blood sugar, and serum insulin. All 6 AN pretreated animals (n = 12) exhibited massive glycosuria in the fasting state and at time zero the mean blood sugar was 322 mg/100 ml after 6 hr, and 212 mg/100 ml after 24 hr fast. Respective blood sugars in controls were 120 and 86 mg/100 ml. After a 24 hr fast and after intraperitoneal glucose 6 AN animals had the following mean blood sugars: 382, 332, 318 mg/100 ml and serum IRI 23, 22, 17 μU/ml. Control rats exhibited significantly lower blood glucose values of: 176, 179, 111 mg/100 ml and a normal insulin response. Conclusion: 6 AN possesses a potent diabetogenic action, manifest already in the fasting state. There is decreased release of insulin both in vitro and in vivo presumably due to decreased activity of the pentose shunt. 6 AN by interfering with nucleotide synthesis produces a new variant of experimental diabetes. (Research supported by NIH Grant HD 02171 and the Martini Foundation.)

294. Interaction of Macrophages with Soluble and Immune Complexes of Peroxidase. RALPH M. STEINMAN* AND ZANVIL A. COHN, New York.

The uptake, distribution, and fate of horseradish peroxidase (HRP) in homogeneous cultures of mouse macrophages have been studied using sensitive biochemical (±1 ng/cc) and cytochemical procedures. The exposure of 10^7 cells to soluble HRP results in the pinocytosis of 0.01–0.02% in 3 hr. The HRP can be localized to membrane-bound organelles of the vacuolar apparatus, and none is detectable on the surface membrane by electron microscopy. Trypsinization of macrophages does not release demonstrable enzyme. After endocytosis, the enzyme is inactivated in an exponential fashion ($t_{\frac{1}{2}}$ 6–8 hr) and none remains at 3 days. Similar studies examined enzymatically active immune complexes formed between HRP and purified rabbit anti-HRP at equivalence. In contrast to the soluble HRP, 30–40% of the added complexed HRP was ingested and segregated in phagolysosomes. Small but detectable amounts (< 10%) remained on the cell surface. In addition, the intralysosomal degradation of HRP-IgG complexes exhibited a delay of 36–48 hr before the onset of inactivation. Therefore, exponential inactivation occurred and reached completion at 7–9 days. Experiments now in progress, employing physically aggregated HRP and immune complexes formed in both antigen and antibody excess, suggest an important role for immunoglobulins in the attachment and ingestion phases of endocytosis, as well as in the intracellular fate of antigens. The use of peroxidase as an antigen now allows a more detailed examination of the macrophage as a mediator of the immune response. (Research supported by grants from the Leukemia Society of America and Grant AI-07012 from NIH.)

295. The Effect of Experimentally Induced Variations in Plasma Insulin Secretion on Plasma Triglyceride Concentration and Transport in Man. MICHAEL P. STERN,* GERALD M. REAVEN, JERROLD M. OLEFSKY,* AND JOHN W. FARQUHAR, Stanford, Calif.

We previously postulated an important role for excessive postprandial insulin response in causing fasting hypertriglyceridemia in man. Evidence for and against this hypothesis has since been brought forth by others. We have compared insulin response to diet and fasting triglyceride levels in the steady state before and after a series of dietary and pharmacologic perturbations. The perturbations were: a high carbohydrate diet vs. a low carbohydrate diet (n = 10 pairs), a high carbohydrate diet before and after an approximately 10 kg weight loss (n = 7 pairs), before and after sulfonylurea therapy during a high carbohydrate diet (n = 8 pairs), and an ad lib. diet before and after an approximately 10 kg weight reduction (n = 12 pairs). In all instances the groups' insulin responses and fasting triglycerides changed in the same direction. These results are consistent with our original hypothesis and with a more speculative view that phasic elevations of plasma insulin stimulate the liver's transport of very low density lipoprotins into plasma. The rate of this transport (VLDL-tr) was measured by a technique of endogenous labeling of plasma VLDL. VLDL-tr in these studies correlated very highly with the $\log_{10}$ of fasting triglyceride ($r = 0.88$, $P < 0.001$) and with insulin response ($r = 0.62$, $P < 0.001$). Therefore, these perturbation studies furnish further evidence for the hypothesis that excessive concentration of postprandial plasma insulin is an important determinant of fasting plasma triglycerides in man. (Research supported by grants from NIH and Nutrition Foundation.)

296. Host Factors in Dissemination of Herpes Zoster. DAVID A. STEVENS* AND THOMAS C. MERIGAN, Stanford, Calif.

89 cases of herpes zoster infection were seen in 18 months, including patients with no underlying disease (47), Hodgkin's disease (18), and other diagnoses (24). Dissemination occurred in these groups with an incidence of 13%, 72%, and 33%. Dissemination was longer and total pox counts were higher in the latter two groups. Therapy with cytotoxic drugs, steroids, and radiation, and stage of disease were important influences on the clincial course. Circulating lymphocyte counts, quantitative immunoglobulin levels, delayed hyper-

sensitivity to multiple skin test antigens, and lymphocyte transformation by phytohemagglutinin were investigated. Within each patient group there was no correlation between results of these tests and occurrence of dissemination. Development of virus-specific complement-fixing antibody (CFA) was delayed in disseminated zoster. Our earlier studies suggested localized and disseminated varicella zoster infection could be distinguished by vesicular interferon (V-IF) levels; patients continuing to disseminate having levels < 100 U/ml and lymphopenia. Therefore kinetics of V-IF in these two clinical presentations were studied. We found that (*a*) dissemination could occur with intermediate interferon levels (~ 1000 U/ml); (*b*) low levels and dissemination could occur without lymphopenia; (*c*) V-IF in patients with dissemination eventually rose to high titers (mean of 14 patients, 10,290; range 1270–28,900 U/ml), and (*d*) dissemination ceased within 2 days from development of peak interferon levels. In four patients with dissemination studied with serial V-IF and serum CFA levels, dissemination ceased after interferon levels peaked and before CFA was detectable. In patients with localized zoster, significantly higher V-IF levels were found in early stages of the disease. V-IF declined as pustulation and crusting occurred. Hence, it appears that V-IF correlates closely with the course of infection.

297. Significance of Precise Determination of Basal Plasma Renin in Primary Aldosteronism. J. R. STOCKIGT,* R. D. COLLINS,* C. A. NOAKES,* AND E. G. BIGLIERI, San Francisco, Calif.

Prediction of adrenal pathology in primary aldosteronism has been unreliable but is important because bilateral adrenalectomy rarely alleviates hypertension when no distinct adenoma is found. Bioassays have demonstrated subnormal renin in both aldosterone-producing adenoma (APA) and bilateral adrenal hyperplasia, but lack of sensitivity has made precise measurement difficult. The degree of preoperative renin suppression in 20 patients with surgically proven APA was compared with that of six patients (10 studies) with hyperaldosteronism due to proven bilateral adrenal hyperplasia using a highly sensitive assay for plasma renin concentration (PRC). Angiotensin I generation rate was determined by radioimmunoassay after 6–14 hr incubation of plasma under zero-order conditions with an excess of heterologous renin substrate from the plasma of anephric sheep. This technique gave precise measurement of renin at markedly subnormal levels. PRC responded to salt restriction and upright posture in both groups, but basal PRC, defined as the level after overnight recumbency while on 100–120 mEq sodium intake, showed least variation within each group and was the best parameter for discriminating between APA and hyperplasia. Mean basal PRC was 0.71 ± 0.14 (SEM) ng/ml per hr in APA and 2.83 ± 0.46 ng/ml per hr in hyperplasia ($P < 0.001$). Aldosterone excretion after 3 days of deoxycorticosterone acetate (DOCA) was less elevated in hyperplasia, and correlation of this parameter with basal PRC effectively separated the two groups. It is suggested that the prediction of a distinct APA can best be made in patients with hyperaldosteronism when the exact degree of renin suppression is taken into account, and when DOCA is used to assess suppressibility of aldosterone. The less complete suppression of renin in hyperplasia may be significant in the pathogenesis of this disorder.

298. Isolation of Phagocytic Vesicles from Granulocytes and Macrophages. T. P. STOSSEL,* T. D. POLLARD,* R. J. MASON,* AND M. VAUGHAN, Bethesda, Md.

Phagocytic vesicles (PV) were isolated from guinea pig and human granulocytes (PMN) and rabbit alveolar macrophages (AM) that had ingested paraffin oil containing Oil Red O emulsified with albumin. Homogenates of washed cells were placed in the center of a three-step sucrose gradient and centrifuged, thereby washing and separating PV which floated and other particles which sedimented from the soluble fraction. Oil Red O was extracted from cells or subcellular fractions into dioxane and colorimetrically assayed. Purity of isolated PV from guinea pig PMN was documented by electron microscopy and chemical analysis. The PV contained 97% of cell-associated Oil Red O, were essentially free of DNA, RNA, or glycogen, and had a cholesterol/phospholipid molar ratio of 0.69. The integrity of the PV was established by their retention of albumin-^{125}I. The PV acquired protein, phospholipid, acid phosphatase, alkaline phosphatase, peroxidase, NADH oxidase, and β-glucuronidase activities in parallel with particle uptake. The activities of these enzymes decreased concomitantly in the pellet and remained unchanged in the soluble fraction. Enyzme transfer was essentially limited to the period of phagocytosis. The various enzymes were translocated at reproducibly differing rates. Catalase and succinic dehydrogenase were not found in the PV. PV isolated from human PMN or AM were similar to guinea pig PV with certain exceptions. Human PMN ingested fewer particles and hence fewer PV were recovered. AM had no peroxidase or alkaline phosphatase, and 8% of the recovered catalase was found in the PV. Our findings provide biochemical evidence that enzymes are indeed transferred from granules to PV (not the soluble fraction) during phagocytosis, and that this process is coupled temporally to particle engulfment.

299. Intermittent Acute Porphyria (IAP): New Evidence for a Basic Defect in Uroporphyrinogen Synthetase (URO-S). L. J. STRAND,* B. F. FELSHER,* A. G. REDEKER,** AND H. S. MARVER, Dallas, Tex., and Los Angeles, Calif.

Utilizing sensitive microassays developed in this laboratory, we have demonstrated two enzymatic abnormalities of hepatic heme biosynthesis in four patients with IAP as compared to 15 controls: (*a*) a previously undescribed $\geq 50\%$ decrease in URO-S and (*b*) a 5–12 $\times$ increase in δ-aminolevulinic acid synthetase (ALA-S), the first and rate-limiting enzyme in the pathway. To explain the latter unusual finding of enzyme (ALA-S) induction in a genetic disease, a primary mutation in genetic control of ALA-S has been widely proposed. However, our data favor an alternative mechanism in which increased ALA-S results from a primary defect in heme synthesis at URO-S, thereby derepressing ALA-S. This alternative is suggested because (*a*) heme participates in repression of ALA-S; (*b*) patients with IAP predominantly excrete excess porphyrin precursors; and (*c*) our assays indicate that the decrease in URO-S in IAP could render this enzyme rate limiting. Critical to this mechanism is demonstration that experi-

mentally produced defects in heme synthesis induce ALA-S. This has been achieved by partially blocking prophyrin and heme synthesis in cultured hepatocytes with lead, aminotriazole, iron chelators, or an inhibitor of URO-S. When porphyrin formation was reduced 50–75%. ALA-S was induced 3–6 ×. Furthermore, significant decreases in heme synthesis increased responsiveness to drug-mediated induction of ALA-S, a characteristic of IAP. To determine whether patients with IAP exhibit a relative decrease in URO-S in tissues other than liver, we assayed this enzyme microfluorometrically in erythrocytes and cultured fibroblasts. Erythrocyte URO-S in four IAP patients was 19 pmoles porphyrin/hr per mg protein (range 16–22) vs. 40 (SD ±8) in 20 controls. Preliminary results suggest a similar defect in IAP fibroblasts. These data provide evidence for a primary, generalized deficiency of URO-S in IAP. The secondary induction of ALA-S may be relevant to other genetic disorders.

300. Studies of Plasma Thrombopoietin in Man. LOUIS W. SULLIVAN,* WILLIAM H. ADAMS,* AND YONG K. LIU,* Boston, Mass. (introduced by Charles P. Emerson**).

Our previous studies demonstrated thrombopoietin in plasma of thrombocytopenic alcoholics, which was absent after recovery from thrombocytopenia. Alcohol ingestion impaired the response to thrombopoietin. Present studies were to elucidate the biology of thrombopoietin production and utilization. Thrombocytopenia was induced in two healthy well nourished alcoholic volunteers by rapid thrombopheresis, reducing platelets to 28% of original. Plasma was obtained before (plasma A), immediately after (plasma B), and 12 hr after (plasma C) thrombopheresis and frozen at − 70°C. After platelets returned to normal each plasma was reinfused, and platelets were counted for 10–14 days. Plasmas A and C did not affect the platelet count. Plasma B caused a 40 and 50% rise in platelets sustained for 8 and 9 days, respectively. Thrombocytopenia was again induced by thrombopheresis during 18 days of alcohol ingestion, and plasmas were obtained immediately after (plasma D), and 12 hr (plasma E), 4 and 5 days (plasma F), and 15 days (plasma G) after thrombopheresis. When reinfused, plasmas D, E, F, and G caused 40–80% increases in platelets, sustained for 9–16 days. These results suggest that induction of acute thrombocytopenia in man results in rapid production or release of thrombopoietin which is no longer detectable 12 hr later. Increased plasma thrombopoietin with drinking and its persistence for 15 days during alcohol add further evidence that alcohol impairs utilization (or stimulates production and/or release) of thrombopoietin in thrombocytopenic man. Human plasmas with increased thrombopoietic activity caused a 2-fold stimulation of incorporation of selenomethionine 75 into mouse platelets; normal plasmas did not. These correlations suggest the potential usefulness of the mouse system for assaying human thrombopoietin. (Research supported by grants from NIH.)

301. Isolated Neutrophil Dysfunction in Adults: a New Entity. JAMES S. TAN,* JOHN J. AKABUTU,* ALVIN M. MAUER, AND JOHN P. PHAIR,* Cincinnati, Ohio (introduced by Virginia Donaldson**).

Defective phagocytosis can be differentiated from impaired intracellular killing by a modified neutrophil bactericidal assay which uses *Staphylococcus aureus* 502A as the test organism and lysostaphin to eliminate extracellular bacteria. Lysostaphin, a rapidly acting muralytic enzyme specific for *Staphylococcus aureus*, does not enter either resting neutrophils or neutrophils which have completed phagocytosis. The enzyme eliminates nonphagocytized bacteria after incubation of the neutrophil-bacteria suspension. Two adults, age 33 and 65, with serious infections, have been found to have normal immunoglobulin levels, normal monocyte bactericidal capacity, and a persistent isolated neutrophil dysfunction. After 2 hr of incubation of the neutrophil-bacteria suspension, extracellular bacteria were eliminated by lysostaphin. Approximately 1 × $\log_{10}$ more bacteria survived within the patients' neutrophils than in control cells. To evaluate phagocytosis, the total and surviving intracellular bacteria were enumerated after 10, 20, 30, 60, and 120 min of incubation of the leukocyte-bacteria suspension. Neutrophils from both patients had slower rates of phagocytosis than control cells. The patients' neutrophils demonstrated a positive Nitro Blue Tetrazolium test, good candidacidal ability, and an inability to kill streptococci. Production of $^{14}CO_2$ from glucose-1-^{14}C by the patients' neutrophils which were phagocytizing latex particles indicated normal hexose monophosphate shunt activity. This defect differs from that found in chronic granulomatous disease and other known defects of neutrophil function. (Research supported in part by grants from NIH and ATS.)

302. Solubility of Cholesterol Esters in Aqueous Media: X-ray Diffraction Studies and Relevance to the Structural Problem of Human Serum High Density Lipoproteins (HDL). A. TARDIEU* AND A. SCANU,** Gif-sur-Yvette, France and Chicago, Ill.

Serum lipoproteins are exceptional among lipoprotein systems in that they contain a high percentage of cholesterol esters (CE), particularly oleate, linoleate, and palmitate. The mechanism by which CE, water-insoluble compounds, are kept in solution by circulating lipoproteins is not known. In the present studies this problem was analyzed by X-ray diffraction techniques using as a model the human serum HDL of d 1.063–1.125 g/ml. CE isolated from HDL lipids or from corresponding synthetic products had definite crystalline → liquid crystalline temperature transitions varying from 82°C (cholesterol palmitate) to 39°C (cholesterol linoleate). When studied in the presence of HDL phospholipids or mixtures of phospholipids + free cholesterol, CE exhibited the same temperature transitions as when they were studied alone: the X-ray diffraction patterns indicated that the CE did not mix with the lamellar phases of phospholipids and free cholesterol, regardless of the final water content of the lipid mixture analyzed. Solubilization of CE was only observed at the melting temperatures of their alkyl chains in the presence of both HDL apoprotein and phospholipids. Once incorporated into such a complex, the CE retained the liquid crystalline state even at low temperatures. This was also observed in native HDL_2. The studies suggest that the solubilization of CE in HDL depends on a cooperative process involving at least HDL protein and phospholipids, and appropriate physical conditions of the components. The molecular mechanisms of such interactions are under investigation.

303. Phenotypic Expression of Thymidine Kinase during Human Fetal Development. ANDREW T. TAYLOR,* MARY A. STAFFORD,* AND OLIVER W. JONES,* La Jolla, Calif. (introduced by Leonard D. Garren).

The precise role of thymidine kinase in DNA replication remains unknown. However, thymidylate monophosphate (TMP) is the unique pyrimidine in DNA, and conceivably the availability of TMP may exert some control in DNA replication. Thymidine kinase has been studied in human fetal tissue at various stages of gestation and compared with the enzyme in human adult tissue. The enzyme was partially purified by homogenization, high speed centrifugation, and ammonium sulfate fractionation. Thymidine kinase in fetal spleen and liver have similar electrophoretic properties. However, the electrophoretic mobility of thymidine kinase is different in fetal skin. Compared with thymidine kinase in human adult liver, certain variations were noted. Beyond the 28 cm crown-rump size, fetal thymidine kinase migrates rapidly in starch-block or polyacrylamide-gel similar to the adult form. However, between 3 and 10 cm size, all detectable thymidine kinase activity migrates at approximately one-half that of the adult form. Between 12 and 28 cm fetal size, another unique form of thymidine kinase appears. This enzyme has very slow electrophoretic migration. Certain properties suggest that this fetal enzyme fraction is in the form of a large aggregate with a molecular weight in excess of 660,000. Additional variations from adult enzyme include increased lability at 45°C, inhibition at high [+ H] concentrations, and different affinity for thymidine and thymidine analogs. The electrophoretic properties are not altered by RNAase or DNAase. The role of this fetal enzyme is unknown at present. However, it appears during a period of rapid fetal growth and may be directly related to DNA replication or possibly a brief period of amplification of DNA synthesis during fetal development.

304. Kinetics of Sodium Efflux in Controls, Uremics, and Those with an Inheritable Disorder of Red Cell Transport. R. C. TAYLOR,* L. R. DUFRESNE,* AND L. G. WELT,** Chapel Hill, N. C.

Previous reports have described the observations that the concentration of Na in erythrocytes (Na_c) is high in some patients with advanced uremia. This is associated with diminished pump (P) activity and a decrease in ouabain-sensitive ATPase activity. In addition, we have reported an inheritable disorder unassociated with disease in which the Na_c is elevated and the P and ATPase are also diminished. These observations have been extended in terms of the influence of Na_c on the kinetics of P with respect to its maximal rate (V_{max}) and the concentration of Na_c necessary to reach one-half V_{max} ($S_{0.5}$). The Na_c of erythrocytes was altered to cover a wide range of values using *p*-chloromercuriphenyl sulfonic acid (PCMBS) and solutions with varying sodium concentrations. Studies were done on controls, uremics with normal and high Na_c, and on members of the family with the inheritable disorder. The V_{max} was not different among the groups of controls and the two groups of uremics, but was distinctly lower in the family. In contrast, the $S_{0.5}$ was lower in the control group than in the two groups of uremics. The $S_{0.5}$ of the family, in contrast, was not different than controls. These data suggest that uremics are capable of pumping normally when the Na_c is high and may imply that the number of active pump sites may be modified by the level of Na_c. In contrast, the family defect can be interpreted to mean that the kinetics of activation are normal, but the ability to pump maximally may be constrained by a diminished number of pump sites. (Research supported by AM-08458 and AM-05054.)

305. Reduction of Thyroid Hormone Release by Lithium in Thyrotoxicosis. R. TEMPLE,* M. BERMAN,* AND J. WOLFF, Bethesda, Md.

Lithium ion blocks the release of iodine from the rat thyroid in a manner similar to iodides. Since iodides obscure diagnostic thyroid function tests and serve as a substrate for hormone synthesis, we investigate Li^+ as an alternative therapeutic agent when a rapid fall in hormone level is desired. Four hyperthyroid patients were studied during a control period, a 2–3 wk period of lithium carbonate therapy (serum Li^+ = 0.5–1.2 mEq/liter), and a second control period. Conventional parameters of thyroid function were measured and the distribution of a tracer dose of ^{131}I into the thyroid, serum, and urine was followed. In every patient, Li^+ treatment produced a prompt decrease in the rate of ^{131}I loss from the thyroid gland, a fall in serum PBI-^{131}I and urinary ^{131}I excretion, and a fall in serum T_4 associated with decreased clinical evidence of hyperthyroidism. The mean fall in serum T_4, achieved within 6 days, was 28%. Multicompartment analysis revealed that the principal kinetic parameter affected was the fractional hormone secretion rate, which was reduced by ~50%. We conclude that Li^+ at nontoxic serum levels, can promptly lower circulating thyroid hormone concentrations in thyrotoxic patients by blocking hormone release. Since the thyroidal iodide clearance was not decreased, the calculated glandular iodine content was increased, corresponding to the measured increase in glandular iodine found in rats. Thus, even with persisting inhibition of the fractional hormone release rate, the absolute amount of hormone released will gradually rise. This is, in fact, observed. Hence, Li^+ alone is beneficial principally for short-term treatment of hyperthyroidism: since Li^+ is effective in the presence of mercaptoimidazole, combined therapy appears to be promising.

306. Plasma Zinc Determinations in Normal Subjects and Hypertensive Patients. GURDARSHAN S. THIND,* GRACE M. FISCHER,* AND HARRY F. ZINSSER,** Philadelphia, Pa.

Low plasma zinc levels have been reported in patients with chronic uremia, hepatic, and other diseases; however, alterations in plasma zinc concentrations in different forms of human hypertension have not been investigated. Plasma zinc (μg/100 ml) was determined by atomic absorption spectrophotometry in 95 selective renal venous, inferior vena caval, or peripheral venous specimens obtained from 15 normal subjects and 32 hypertensive patients without significant renal failure. Hypertensive work-ups including renal arteriography showed renal artery stenosis in 17, renal parenchymal disease in 5, and essential hypertension in 10 patients. Plasma zinc concentration (mean ±SEM) in the inferior vena caval or peripheral venous samples of normal subjects was 113.03 ±4.64

and in essential hypertension patients was 120.44 ±10.55, a difference not significant (P value > 0.50). Significantly lower plasma zinc concentrations were found in the inferior vena caval or peripheral venous specimens of patients with renal artery stenosis (90.87 ±4.48) and renal parenchymal disease (96.38 ±4.31) with P values of 0.005 and 0.025 respectively. Plasma zinc determinations in right and left renal venous specimens of 24 of the 32 hypertensive patients and 2 of the normal subjects were not significantly different from the corresponding zinc values in inferior vena caval or peripheral venous samples. It is concluded that plasma zinc was decreased in patients with renal artery stenosis and renal parenchymal disease in the absence of significant renal failure. The decreased plasma zinc in renal forms of hypertension may be due to redistribution or total body deficiency of zinc or both. (This investigation was supported in part by USPHS Grants HE-07762 and N ONR-551 (54).)

307. Intrinsic Factor X Activation. ARTHUR R. THOMPSON,* Seattle, Wash. (introduced by Clement A. Finch**).

Direct evidence bearing on the mechanism of middle phase intrinsic blood coagulation has been obtained with highly purified clotting factors. Factors VIII, IX, and X were purified from bovine plasma and are homogeneous by polyacrylamide-gel electrophoresis, sedimentation analysis, and column chromatography. Bovine thrombin (2500 NIH units/mg protein) was obtained by affinity chromatography with *p*-chlorobenzylamido-ε-aminocaproyl agarose. Considerable purification was also made with factor XI_a from human serum and hirudin from crude leech preparations. Factor IX is rapidly activated by factor XI_a in the presence of calcium ions. Factors IX and IX_a migrate identically as one band on polyacrylamide-gel electrophoresis, both contain carbohydrate and readily bind to heparin-agarose in the presence of calcium, and both have molecular weights around 90,000. In the presence of phospholipid and calcium, factor IX_a slowly activates factor X. Addition of factor VIII greatly accelerates this reaction. With thrombin-modified factor VIII ($VIII_t$), the rate of factor X activation can be increased another fortyfold. The addition of hirudin to factor VIII or $VIII_t$ in the presence of factor IX_a, calcium, and phospholipid has no effect on factor X–activating activity. Hirudin, however, does inhibit the modification of factor VIII by thrombin. These data suggest that the middle phases of intrinsic blood coagulation involve the following steps. Factor IX is activated by factor XI_a with only minor changes in the protein. Factor IX_a then interacts with factor VIII in the presence of phospholipid and calcium to form a product capable of activating factor X. When thrombin is generated, it further accelerates the middle phases of blood coagulation by increasing the activity of factor VIII. (Supported by Grant HE 11857 from NIH.)

308. Plasma Lipoprotein Abnormalities in Patients with Malabsorption. G. R. THOMPSON,* P. MILLER,* AND T. SHIMOYAMA,* London, England (introduced by K. J. Isselbacher).

Low serum lipids are a feature of the malabsorption syndrome but no data exist on individual lipoproteins in such patients. In this study, the concentration of total cholesterol, triglyceride, and phospholipid in very low, low, and high density lipoproteins (VLDL, LDL, and HDL) in fasting plasma from 14 control subjects and 18 patients with various intestinal disorders was measured after preparative ultracentrifugation. Male patients, in whom steatorrhea happened to be most marked, had 50% lower levels of LDL total lipid than controls, whereas females showed a 25% decrease in HDL. In contrast, VLDL levels tended to be normal or even raised. Calculation of the molar ratios of cholesterol: triglyceride:phospholipid revealed a major difference in LDL between controls (71: 6: 23) and patients (65: 13: 22), but VLDL composition was unchanged. The very significant decrease in the proportion of cholesterol in LDL ($P < 0.01$) was reflected in a lowered ratio of cholesterol: apoprotein, although the phospholipid: apoprotein ratio remained normal. Another striking finding was the decreased percentage of linoleate in lipoprotein fatty acids, especially in cholesterol esters. Changes in LDL concentration and composition were proportional to the degree of steatorrhea, irrespective of whether this was due to intraluminal defects or mucosal abnormalities. High levels of VLDL in some patients imply carbohydrate induction, presumably due to relatively less severe malabsorption of carbohydrate than of fat. The observed decrease in cholesterol and reciprocal increase in triglyceride content of LDL in patients with steatorrhea is the converse of what has been described in familial hyperbetalipoproteinemia. These results suggest that LDL lipids can be extensively modified by impaired absorption of cholesterol and long-chain fatty acids, and emphasize the important influence of intestinal lipid transport on serum lipoproteins in man.

309. Chemical Synthesis of a Bilirubin Diglucuronide. RICHARD P. H. THOMPSON* AND ALAN F. HOFMANN, Rochester, Minn.

Recent evidence from Leuven and Zurich that bilirubin is excreted in human bile conjugated to different carbohydrates emphasizes the need for synthetic bilirubin conjugates of defined chemical structure. Such compounds would permit unequivocal identification of the carbohydrates, and the configuration and position of their linkage in natural conjugates. Lability of the ester linkage of the conjugates and instability of bilirubin makes conventional synthesis impossible. We therefore sought a bilirubin derivative in which the carboxyl groups of bilirubin are activated, permitting coupling with the C1 hydroxyl group of glucuronic acid under mild conditions. Bilirubin was reacted with carbonyldiimidazole in dimethylformamide, and the "activated" bilirubin diimidazole (BIm_2) crystallized. Its structure was inferred from thin-layer chromatography (TLC), infrared spectroscopy, and elemental analysis, and confirmed by formation with methanol of bilirubin dimethyl ester. Bilirubin-^{3}H was crystallized from canine bile, converted to BIm_2-^{3}H, and reacted with the tetrabutylammonium salt of glucuronic acid-^{14}C. TLC showed the formation of monoglucuronide and then bilirubin diglucuronide (BG_2), their mobilities being identical with those of the conjugates in human bile. BG_2 was purified by solvent partition, and crystallized, or precipitated as a heavy metal salt. The ratio of glucuronic acid-^{14}C: bilirubin-^{3}H was about 2. When protonated, BG_2 was soluble in water or ethanol, and reacted directly with diazotized sulfanilic acid, forming an

azopigment identical on TLC with the predominant azopigment formed from bile. The ethyl anthranilate azopigment of BG_2, however, contained several components by TLC, suggesting the synthetic product was a mixture of BG_2 isomers, i.e., conjugates differing in the position of the ester linkage onto glucuronic acid. This is the first chemical synthesis of bilirubin glucuronide, and gives promise of a general method for the preparation of acyl glucuronides. (Support by NIH Grant AM6908.)

310. Intercellular Space Formation in Isolated Toad Bladder. C. CRAIG TISHER* AND BYRON P. CROKER, JR.,* Durham, N. C. (introduced by R. R. Robinson).

Recent reports describe the formation of enlarged intercellular spaces in toad bladder epithelium in the presence of vasopressin and the absence of net fluid transport. Although these results have been ascribed to the action of vasopressin as a smooth muscle relaxant, we have found that the method of fixation (addition of 1% glutaraldehyde to the serosal bathing solution) creates an osmotic gradient that can induce transepithelial water flux during early fixation. Sacs prepared from *Bufo marinus* urinary bladders were bathed in isosmotic Ringer's solution. In the presence of vasopressin and the initial absence of an osmotic gradient enlarged intercellular spaces were present when tissue was fixed in 1% glutaraldehyde applied to the serosal surface, but were absent when 1% glutaraldehyde was applied to both sides of the membrane simultaneously or when 50 mM NaCl was added to the mucosal bathing solution while 1% glutaraldehyde was added to the serosal surface. In tissue fixed in 1% osmium tetroxide, enlarged intercellular spaces were observed with vasopressin in the presence but not the absence of an osmotic gradient (dilute mucosa). Contrary to a previous report, neither atropine (1 mmole/liter) acting as a smooth muscle relaxant nor ATP (5 mmoles/liter) caused enlargement of intercellular spaces with or without an osmotic gradient. In our study ATP was found to cause smooth muscle contraction rather than relaxation as previously reported. We conclude that (*a*) vasopressin-induced intercellular spaces are formed by net transepithelial water flux occurring either before or during fixation; (*b*) smooth muscle relaxation alone does not cause enlarged intercellular spaces; and (*c*) ATP causes smooth muscle contraction rather than relaxation in the toad bladder. (Supported in part by USPHS Grants AM13845, 5T GM00726, and AM10698.)

311. Iron Chelation by Penicillamine. GLENN TISMAN,* JACK PEISACH,* AND VICTOR HERBERT, Bronx and New York, N. Y., and Murray Hill, N. J.

Numerous reports suggest iron deficiency may occur with long-term penicillamine therapy. To help throw light on possible mechanisms, interaction of penicillamine with iron was studied using optical and electron paramagnetic resonance (EPR) spectroscopy. Penicillamine chelation of ferrous (Fe^{+2}) and ferric (Fe^{+3}) iron was observed. Addition of Fe^{+2} to penicillamine in water produces a colorless solution which turns purple when adjusted to pH 8. Addition of Fe^{+3} to penicillamine produces a blue color which fades within seconds; subsequent adjustment to pH 8 produces a purple complex, dependent upon presence of molecular oxygen, which fades gradually. Spectrophotometric analysis of the purple Fe^{+2}-penicillamine mixture by the method of Job revealed two complexes, one absorbed maximally at 490 mμ when mixed in a molar ratio (iron: penicillamine) = (1:4), and the second absorbed maximally at 565 mμ when mixed in a molar ratio (iron: penicillamine) = (1:2). Chemical and low temperature (1.4°K) EPR studies revealed reduction of Fe^{+3} to Fe^{+2} by penicillamine, analogous to penicillamine reduction of Cu^{+2} to Cu^{+1}. Preliminary studies using short-term bone marrow cultures suggest penicillamine (0.1 and 1.0 mg/ml) may inhibit intracellular transport of transferrin-delivered ^{59}Fe. Using dextran-coated charcoal to separate free from bound iron, penicillamine appeared to reduce intracellular incorporation of iron into large complexes. These findings suggest reductive chelation of iron may occur during penicillamine therapy; this could help produce iron deficiency. The findings support possible value of oral penicillamine in therapy of iron overload and acute iron toxicity. Since ribonucleoside diphosphate reductase is iron dependent, these studies may also help explain our recent finding of inhibition by penicillamine of DNA synthesis by human bone marrow in vitro. (Supported in part by USPHS Grants AM 15163 and 15164, by VA, and by HRC of NYC.)

312. Long-Term Follow-Up of Symptom-Free Asthmatic Subjects and Bronchial Sensitivity to Methacholine. ROBERT G. TOWNLEY,* UNG YUN RYO,* AND BANN KANG,* Omaha, Nebr. (introduced by Robert P. Heaney).

Patients with bronchial asthma who are currently symptomatic all have an exquisite bronchial sensitivity to histamine and methacholine. In an attempt to determine if the bronchial sensitivity is permanent or reversible, we measured the bronchial sensitivity to methachcline in 41 patients who had had symptoms for a mean of 8.5 yr, but have had no asthma symptoms for 1–21 yr (mean 6.8 yr). 19 nonallergic controls and 33 allergic rhinitis subjects were similarly studied. All subjects were challenged with methacholine, 5 mg/ml using a vaponephrin nebulizer activated at 6 liters/min of air. FEV_1 and VC were recorded before and after 1, 3, 6, 10, 20, 40, and 80 inhalations or until FEV_1 falls more than 20% of control value. Only one nonatopic control subject had a positive response (greater than 15% decrease in FEV_1) and this required 160 inhalations of methacholine. Four of 33 hay fever subjects had a positive methacholine response; two of these subsequently have had slight asthma symptoms and both responded to 10 inhalations. Only 3 of 41 former asthmatics who had "outgrown" their asthma had a normal methacholine response. The other 38 all responded to 40 inhalations or less with a mean of 10 inhalations inducing—a mean decrease in FEV_1 of 42%. The results indicate that most symptom-free asthmatic subjects are highly sensitive to aerosolized methacholine even though they have been free of symptoms for many years. The methacholine challenge test appears to provide a useful index of the permanent or transient nature of bronchial sensitivity in the symptom-free asthmatic. It may also serve to predict which individuals with only allergic rhinitis will become asthmatic. (Research supported by NIH Grant A1 07433.)

313. Studies of Serum Gastrin Concentrations in Patients with Duodenal Peptic Ulcer Disease before and after Vagal Transection. WALTER L. TRUDEAU* AND JAMES E. MCGUIGAN, Gainesville, Fla.

Duodenal peptic ulcer disease is frequently characterized by increased rates of gastric hydrochloric acid secretion. Gastric acid secretion appears important in the initiation and perpetuation of duodenal ulcer disease. Gastrin is the most potent agent known in stimulating gastric acid secretion, and in man plays a prime role in the regulation of gastric acid secretion. Vagal stimulation evokes gastrin release and consequent stimulation of gastric acid secretion. The major surgical procedures for duodenal ulcer disease are directed to the reduction of the rate of gastric acid secretion, either by vagal transection and pyloroplasty or vagal resection and antrectomy. In the present study fasting serum gastrin concentrations were measured by radioimmunoassay in 65 patients receiving vagal transection and pyloroplasty and 41 patients receiving vagotomy and removal of the antrum. Serum gastrin concentrations were measured and compared before and 6 months after surgery. The effects of vagal transection with pyloroplasty and vagal transection with removal of the antrum on serum gastrin concentrations were compared. The mean fasting serum gastrin concentrations for the patients receiving vagal resection and pyloroplasty were 106 $\pm$15.3 pg/ml before surgery and 106 $\pm$10.6 pg/ml after surgery. The mean fasting serum gastrin concentration for the patients receiving removal of the antrum and vagal nerve transection were 104 $\pm$24.2 pg/ml before surgery and 24 $\pm$5.1 pg/ml after surgery. These results indicated no reduction in fasting serum gastrin concentration after vagal transection and pyloroplasty for duodenal ulcer disease. Conversely, substantial and significant ($P < 0.02$) reductions in fasting serum gastrin levels were found after vagal transection and antral removal. (Supported by NIH Grant AM13711 and ACS Grant T394C.)

314. Measurement of Prolactin Activity in Human Plasma by New Biological and Radioreceptor Assays. ROGER W. TURKINGTON, Durham, N. C.

Prolactin activity in human plasma samples has been characterized quantitatively in two new assay systems. Biological activity has been determined by the induction of the specific milk protein casein in organ cultures of mouse mammary gland. Binding activity has been studied utilizing the competitive displacement of ^{125}I-labeled prolactin from specific receptor sites on membrane structures derived from mammary epithelial cells. Both assays were highly specific for the measurement of authentic ovine prolactin, although chorionic somatomammotrophin showed equal biological activity and nearly equal competitive displacement in the binding assay. Human growth hormone showed 10–20% of the potency of ovine prolactin in both assays. The activities of both these interfering hormones could be removed by treatment of the plasma with specific antisera. The sensitivity of both the radioreceptor and biological assays was 2 ng/ml of standard prolactin, and the precision was $\pm$10% (SD) in the physiological range. Elevated prolactin concentrations in plasma were found in patients with various types of pituitary tumors, with galactorrhea, and during treatment with various tranquilizer drugs. The measurement of chorionic somatomammotrophin levels in plasma provided a sensitive correlate of the course of choriocarcinoma in both male and female patients. These studies provide evidence that prolactin has a specific cellular site for exerting its biological effects, they provide clinical data showing good agreement between measurements based upon molecular binding and upon biological activity, and they provide new techniques for the study of prolactin in physiological and pathological conditions.

315. Serum Thyroglobulin Levels in Healthy Subjects and Thyrotoxic Patients. R. P. ULLER,* A. J. VAN HERLE,* N. L. MATTHEWS,* AND J. BROWN,** Los Angeles, Calif.

Thyroglobulin is present in the sera of normal people and in significantly larger quantities in patients with thyrotoxicosis. A radioimmunoassay has been developed to measure serum thyroglobulin levels in human blood. Using this technique, thyroglobulin was detectable in the sera in 15 of 32 euthyroid subjects (47%). The range was 9.5 ng/ml to 61 ng/ml with a mean of 21.4 $\pm$3.7 ng/ml (SEM). Serum thyroglobulin levels were measured sequentially in the sera of eight patients with thyrotoxic Graves' disease. Four underwent thyroidectomy. Preoperative levels were 117 ng/ml, 250 ng/ml, 250 ng/ml, and 36,500 ng/ml. There was an immediate postoperative elevation in thyroglobulin levels to 300, 2900, 34,000, and 38,000 ng/ml respectively, followed by a dramatic fall reaching a normal range in about 3 wk. One patient was treated with ^{131}I. The pretreatment thyroglobulin level was 640 ng/ml rising to a high of 1350 ng/ml 72 hr after ^{131}I administration, followed by a two-step fall reaching a level of 44 ng/ml 8 wk after treatment. Three patients were treated with propylthiouracil. They have been followed for 3–14 months. Pretreatment levels were 460 ng/ml, 162 ng/ml, and 155 ng/ml. All became euthyroid but continued to have elevated thyroglobulin levels. We conclude that thyroglobulin is detectable in sera of euthyroid individuals but is significantly elevated in all thyrotoxic patients studied. No overlap of serum thyroglobulin levels between thyrotoxic and normal patients was noted. Surgery and ^{131}I treatment were followed by a fall of thyroglobulin to normal levels and correlated well with the PBI. Patients treated with antithyroid drugs maintained elevated levels of thyroglobulin despite being clinically euthyroid and having normal conventional thyroid function tests. (Supported by USPHS Grant 5035.)

316. Lack of Thyroid Peroxidase Activity: a Cause of Congenital Goitrous Hypothyroidism. LUBOMIR VALENTA,* HANS H. BODE,* AUSTIN L. VICKERY,* AND FARAHE MALOOF,** Boston, Mass.

The concept that a defect in the thyroid peroxidase could be the etiologic basis for a total block in the biosynthesis of thyroxine has been entertained, but never established. A 50-yr-old female presented with goitrous cretinism. Serum total T_4 was 0.5 μg/100 ml; free T_4, 0.1 ng%; total T_3 = nondetectable; butanol insoluble iodine = 0.4 μg/100 ml, and serum thyroid-stimulating hormone (TSH) 50 ng/ml. Thyroid ^{131}I uptake peaked at 5 hr (18%) and fell to 6% in 24 hr. Only inorganic ^{131}I was detected in the serum and urine. All the thyroidal ^{131}I was dischargeable by thiocyanate. Salivary/

serum ^{131}I ratio was 20/1. Histologically the tissue revealed microfollicular structure interspersed with areas of macro and normal follicles with periodic acid–Schiff (PAS)-positive colloid. The ^{127}I content of the thyroid was very low (0.6–1.6 μg/g), 99% of which was trichloroacetic acid (TCA)-soluble and dialyzable. Thyroglobulin (19S), identified by sucrose gradient density centrifugation, comprised 10–50% of the soluble proteins (60–100 mg/100 g thyroid). Peroxidase activity was absent in all subfractions of thyroid tissue, before and after dialysis, as measured by using guaiacol, iodide (10^{-4} mole/liter), and thiocyanate (10^{-4} mole/liter) as electron donors in the presence of H_2O_2 (10^{-4} mole/liter). The activity of catalase, a peroxidase inhibitor, was not elevated (ΔA_{240}/min per mg = 0.49) compared to normal human tissue (0.64). Guaiacol oxidation was not detectable even with added azide (2×10^{-5} mole/liter) which inhibits catalase activity. Incubation of the patient's thyroglobulin with horseradish peroxidase (8.6 μg) and H_2O_2 (10^{-4} mole/liter) led to iodination. Deiodination of DIT-^{125}I and protease activity were normal. The patient's saliva contained normal peroxidase activity as evidenced by guaiacol oxidation (ΔA_{470}/min per mg = 14.0), iodination of BSA (60 mμmoles ^{127}I incorporated per mg protein), and thiocyanate oxidation (300 mμmoles/mg protein). Lack of thyroid peroxidase activity is the basis for the iodine organification defect. (Research supported by USPHS Grant AM 13916.)

317. Blood Flow Distribution at Rest and during Exercise in Severe Chronic Anemia in Conscious Dogs. STEPHEN F. VATNER,* CHARLES B. HIGGINS,* AND DEAN FRANKLIN,* La Jolla, Calif. (introduced by John B. West).

Although it is known that cardiac output increases in anemia to satisfy the metabolic demands of the peripheral tissues, the pattern of altered blood flow distribution at rest and during exercise is unclear. After recovery from instrumentation with Doppler flow probes on the left circumflex coronary, mesenteric, renal, and iliac arteries, and pressure gauges in the aorta, six dogs were chronically bled from an initial hematocrit of 40% to 14% over 4–6 wk while volume was replaced with the harvested plasma and dextran. Mean arterial pressure remained unchanged (99 mm Hg), heart rate increased from 71 to 128 beats/min, renal flow increased 9%, mesenteric flow 31%, iliac flow 106%, and coronary flow 193%. In severe anemia catecholamines could not increase coronary flow further, indicating near maximal dilatation of that bed. Measurements of regional flows and arterial pressure were radiotelemetered from 11 normal dogs running spontaneously 15–25 mph behind a mobile recording unit in the field for distances averaging 1.5 miles. During this severe exercise renal and mesenteric flows did not decrease, but in anemia renal and mesenteric flows decreased by 64% and 61% respectively even during mild exercise (3–5 mph). Thus, the increased cardiac output in chronic anemia in conscious dogs at rest is distributed to the heart and limbs in preference to the viscera. Furthermore, contrary to traditional concepts, blood flow is not diverted from the inactive viscera during severe exercise in normal dogs, but when the hypoxic stress induced by severe anemia is added, significant reductions of visceral flows occur even during mild exercise.

318. Uptake and Metabolism of Circulating Lipoprotein Triglyceride by Arterial Wall. A. VOST,* Montreal, Canada (introduced by D. G. Cameron**).

The association of atherosclerosis with elevated plasma triglyceride concentrations is well recognized but the role of plasma triglyceride in arterial metabolism is unknown. To compare aortic metabolism of plasma triglyceride fatty acid (TGFA) and FFA, palmitic acid-^{3}H was injected intravenously in rabbits either as FFA or chylomicron TGFA and aortas were removed 10 min later; TG formed 80% of aortic lipid ester radioactivity (ALER) in TGFA-^{3}H experiments and only 42% in FFA-^{3}H experiments. To explore this difference, rabbit abdominal aortas were perfused *in situ* for up to 2 hr with defibrinated blood-buffer containing ^{3}H- or ^{14}C-labeled glycerol, FFA, or chylomicron triglyceride–glycerol or -FA. Triglyceride formed >83% of ALER after perfusion with triglyceride (labeled ester glycerol or FA) whereas, after labeled free glycerol or FFA, most ALER was recovered in phospholipids. Aortic TG synthesis rates from glycerol and FFA were <5% of lipoprotein TG uptake rates. Thus aorta takes up lipoprotein TG but without hydrolysis. Aortic TG uptake increased linearly with time and in aortic outer segment (capillary-perfused) it increased with increasing perfusate [TG]; in inner segment (avascular) TG uptake approached maximal at physiological concentrations ([TGFA] $\geq$2 mmoles/liter). Perfusion of FFA (^{3}H- and ^{14}C-labeled palmitic acid) and chylomicron TG–FA-^{3}H was followed by prolonged unlabeled perfusion; measurement of $^{3}H/^{14}C$ ratios demonstrated that aortic FFA was derived from plasma FFA and from hydrolysis of triglyceride taken up from perfusate. In summary lipoprotein TG is taken up by aorta as intact molecules but some TG is hydrolyzed in aorta; the rates of TG uptake greatly exceed rates of aortic TG synthesis. TG uptake by inner aortic segment approaches saturation at physiological perfusate TG concentration.

319. The Effect of Peritubular Capillary Flow Rate and Pressure on Glomerulotubular Balance. TAKAO WADA,* HAGOP S. AYNEDJIAN,* AND NORMAN BANK, New York.

In order to study the role of peritubular capillary pressure on glomerulotubular balance independent of changes in renal hemodynamics and circulating hormones, we measured fractional reabsorption of water and single nephron GFR (SNGFR) in end-proximal tubular convolutions of rat kidneys during microperfusion of the peritubular capillaries. Pooled rat plasma was used to perfuse the vascular "stars" at rates from 400 to 800 nl/min. Hydrostatic pressure in the perfused capillary branches and in the adjacent tubular lumen ranged from 14 mm Hg at the lower perfusion rates to 23 mm Hg at the higher rates. In 24 paired recollections made during two different capillary perfusion rates, SNGFR was 29 nl/min during perfusion at the lower rate and fell to 23 nl/min at the higher perfusion rate ($P < 0.01$ by paired t test). In spite of this fall in SNGFR, fractional reabsorption fell from 56.4% $\pm$2 SEM to 38.1% $\pm$3.9 SEM ($P < 0.001$). Absolute reabsorption rate fell from 16.8 nl/min $\pm$1.5 to 9.28 nl/min $\pm$1.2 ($P < 0.001$). The effect was independent of the sequence of change in capillary perfusion rates, and if perfusion rate was kept constant, fractional reabsorption did not change sig-

nificantly upon recollection. These observations demonstrate a dissociation between GFR and fractional reabsorption of glomerular filtrate by the proximal tubule unrelated to changes in over-all renal hemodynamics, circulating hormones, or protein concentration of the capillary fluid. The initiating stimulus for the change in fractional reabsorption was the change in capillary flow rate and hydrostatic pressure. We conclude that this pressure, extrinsic to the tubule, is an important controlling mechanism for glomerulotubular balance. (This work was supported by grants from NIH and NYHA.)

320. Role of Hepatic Phagocytosis in Increasing Heavy Metal Toxicity. HENRY N. WAGNER, JR., AND VICTOR EVDOKIMOFF,* Baltimore, Md.

Last year we reported the finding of a fortyfold increase in the toxicity of the heavy metal, indium, when administered to mice in the form of colloidal indium hydroxide particles rather than in ionic form. This suggested that, contrary to accepted belief, the phagocytic function of the reticuloendothelial system (RES) might at times have deleterious effects, rather than serve exclusively as a protective mechanism. Recent experiments indicated that blockade of the reticuloendothelial system in mice resulted in a decrease in the toxicity of indium hydroxide particles. To produce RES blockade, preliminary injections of nontoxic doses of gallium hydroxide, which is accumulated by the reticuloendothelial cells of the liver, spleen, and bone marrow, were administered before administering various doses of indium hydroxide. Gallium blockade of the RES decreased hepatic uptake of subsequently administered radioactive indium hydroxide particles. With increasing blockading doses of gallium hydroxide, there was a decrease in the toxicity of subsequently injected indium hydroxide. The LD_{50} of indium hydroxide increased from 13 $\pm$1 (SD) μg to 39 $\pm$5 (SD) μg/mouse. Indium hydroxide had no effect on the metabolism of phagocytic cells in vitro. We postulate that the role that hepatic phagocytosis plays in enhancing indium toxicity is that it results in chemical quantities of indium in the vicinity of hepatic parenchymal cells that produce toxicity. Our results support the hypothesis that hepatic phagocytosis of colloidal indium hydroxide plays a role in the toxicity of this heavy metal, since blockade of its uptake by the hepatic RES decreased its toxicity. Studies are in progress to determine whether hepatic phagocytosis affects the toxicity of other metals, such as lead and mercury. (Supported by USPHS Grants GM 10548 and 5T01 EC 00051-11.)

321. Biofunctional Conformation of Neurohypophyseal Hormones. RODERICH WALTER* AND IRVING L. SCHWARTZ,** New York.

The biologically active conformation of oxytocin and its congeners is proposed on the basis of studies involving high resolution (220 MHz) NMR, circular dichroism, partition chromatography, and countercurrent distribution. This conformation consists of a maximally cooperative, intramolecularly stabilized topological unit which is essentially featureless on one side of the molecule while the contours of the other side are highly differentiated by the C-terminal tripeptide, the side chains of asparagine and glutamine, and by the aromatic moiety of tyrosine. The side chains of each of the constituent amino acid residues play a critical role in the formation and intramolecular stabilization of the preferred backbone conformation with the exception of the side chains in positions 3, 4, 7, and 8 which are the distinguishing topological features and the primary determinants of differential hormonal specificity. Thus only the amino acid side chains in these latter positions can be expected to reveal the natural selection of neurohypophyseal peptides in the course of evolution. On the basis of the conformation it can be predicted that, biofunctionally, structural modifications can be grouped in three general categories: (*a*) modifications affecting the stabilization of the peptide backbone, which extensively perturb the spatial relationships among all the constituent amino acids and, hence, affect both affinity and intrinsic activity uniformly; (*b*) modifications which, while retaining the stability of the backbone conformation, alter the steric environment and charge distribution of limited surface areas, and thereby can affect affinity and intrinsic activity differentially; and (*c*) modifications changing the steric and electronic requirements of moieties comprising the active surface of neurohypophyseal peptides without perturbing the peptide backbone and, hence, affecting intrinsic activity without altering affinity. To our knowledge, this is the first identification of the conformation of a peptide hormone in solution and, in turn, the first analysis of the specific three-dimensional molecular structural requirements for hormonal activity.

322. Studies of Platelet Function in Congenital Afibrinogenemia. HARVEY J. WEISS, New York.

Studies of hemostasis were performed in two patients, ages 19 and 23, with congenital afibrinogenemia (plasma fibrinogen 1.2 and 1.6 mg/100 ml). The prolonged bleeding time in both patients was shortened by transfusions of fibrinogen. Adhesion to connective tissue was normal. Retention of platelets in glass bead filters was consistently decreased (0–10% compared with normal values of 33–81%) and was markedly increased to 68% by pretreating the beads with purified fibrinogen. ADP-induced platelet aggregation in citrated platelet-rich plasma (PRP) was moderately decreased and corrected to normal values by addition of 2.5 mg/100 ml fibrinogen. Maximum aggregation was achieved with a final fibrinogen concentration of 10–20 mg/100 ml. Epinephrine-induced aggregation was markedly abnormal and neither a primary nor secondary wave occurred with epinephrine concentrations of 5–50 μmoles/liter. The platelet release reaction was abnormal when induced by kaolin and epinephrine but normal with connective tissue. Abnormalities in the release reaction were only inconstantly corrected by addition of fibrinogen in vitro. In contrast to the abnormalities in platelet aggregation obtained with citrated PRP, aggregation was consistently normal in heparinized PRP. This suggests that the cation requirements for platelet aggregation may be increased with suboptimal concentrations of fibrinogen. The abnormalities in citrated PRP were not corrected, however, by addition of either Ca^{++} or Mg^{++}. The studies leave unanswered the question of the fibrinogen requirement for platelet aggregation, but indicate that fibrinogen is required as a cofactor in the interaction of platelets with some surfaces. (Supported by Career Scientist Award I-639 from the Health Research Council of the City of New York.)

323. Controlling Factors during Intestinal Fat Absorption in Man. JORDAN B. WEISS* AND PETER R. HOLT, New York.

Human studies of triglyceride absorption rates have been limited since multiple complex physicochemical processes are involved. Meaningful analyses require steady-state conditions to determine rates, and correlations of these rates with quantifiable compartments. Using intraduodenal perfusion, homogenized meals containing 6% or 12% corn oil, protein, and carbohydrate were administered through a triple-lumen tube at 2.2 ml/min. Polyethylene glycol and glyceryl triether-^{3}H were used as dilution markers. For each meal five samples were collected at 25-min intervals from portals 15 cm and 55 cm from the infusion sites. Samples were analyzed for pH, total and micellar fat, bile salts, pancreatic enzymes, and markers. Steady-state triglyceride absorption rates in normals were 1.75 ±0.10 (SD) mg/min per cm intestine during perfusion of 7.92 g/hr and increased proportionately with higher fat perfusions indicating that maximal absorptive capacity was not reached. Regardless of amounts of triglyceride perfused, absorption rates correlated positively with mean micellar fatty acid (FA) concentration ($r = 0.688$, $P < 0.05$). The oil-micellar partition of FA was dependent on micellar bile salt concentrations. Per cent absorption also correlated with bile salt concentration ($r = 0.868$, $P < 0.01$). Negative correlations between absorption rate and total (oil + micellar) FA concentration were found. These data suggest that absorption rate is dependent upon availability of micellar fat. The estimated minimum bile salt concentration needed for micellar solubilization of FA was 0.73–1.01 mmole/liter. During intraluminal bile salt depletion, lipolysis was slowed but transfer of FA from oil to micellar compartment was rate limiting; intestinal mucosal absorption rates were normal for the concentrations of micellar fat achieved. We suggest that this method permits detailed analysis of rate-controlling factors during intestinal fat digestion and absorption and allows the mechanisms altered in a particular patient with steatorrhea to be precisely defined.

324. Molecular Basis of Gouty Inflammation. GERALD WEISSMANN, GIUSEPPE RITA,* AND ROBERT B. ZURIER,* New York.

Gouty inflammation results from the interaction of crystalline monosodium urate (MSU) with polymorphonuclear leukocytes (PMN'S) which release inflammatory and chemotaxis-generating factors. Although MSU has been shown to be hemolytic (Wallingford and McCarty. 1971. *J. Exp. Med.* **133**: 100) no direct interaction of the crystals has previously been found with lysosomes, into which they are taken and which contain inflammatory substances. Since isolated lysosomes are usually prepared in sucrose, it appeared possible that vicinal hydroxyl groups of the sugar might interfere with H-bonding between MSU and biomembranes. Consequently, crystalline MSU (1–30 μ) was prepared, examined by X-ray diffraction (major d spacings in A = 9.31, 7.56, 4.69, 3.16, 2.66), and exposed to erythrocytes, isolated lysosomes from rabbit liver and PMN's, and liposomes (model lipid spherules of phosphatidyl choline: cholesterol: dicetylphosphate). MSU (10–100 μmoles/ml) induced concentration-dependent lysis of isolated liver and leukocyte lysosomes suspended in phosphate-buffered saline (pH 7.4) or acetate buffer (pH 4.5) with release of β-glucuronidase and aryl sulfatase (e.g. 260% of controls at 60 min). As predicted, sucrose (0.25–0.025 M) prevented membrane-lytic effects of MSU in each system, as did Tris buffer, also rich in OH groups. Unlike nystatin, vitamin A, and etiocholanolone (other lysosome-labilizing agents) only MSU and silica (an H-bonding, membrane-lytic crystal) ruptured lysosomes in saline but not sucrose or Tris; plasma inhibited membrane lysis. Lysis was not due to dissolved uric acid, since lysosomes separated from crystals by dialysis membranes remained intact. MSU and silica (6 μmole/μmole lipid) disrupted artificial liposomes *but only if these had cholesterol preincorporated.* These data suggest that ingested, plasma-free, MSU kills leukocytes by means of direct interaction (H-bonding) between crystals and the cholesterol-rich membranes of secondary lysosomes which perforate from within to release hydrolytic enzymes and chemotactic factors.

325. Human Lymphocyte Transformation Induced by Autologous Lymphoblasts. MARC E. WEKSLER* AND GARY BIRNBAUM,* New York (introduced by Henry O. Heineman**).

Lymphoblasts derived from the peripheral blood of four healthy volunteers have been established in continuous culture. Irradiated lymphoblasts markedly stimulate autologous lymphocyte transformation as measured by thymidine incorporation in a one-way mixed leukocyte reaction. Cell-free supernatant does not produce this stimulation. Allogeneic lymphoblasts are more potent stimulators of transformation than are allogeneic lymphocytes; however, no direct relationship exists between the increase in thymidine incorporation induced by allogeneic lymphocytes and that induced by allogeneic lymphoblasts derived from the same donor. Autologous lymphoblasts appear as potent as allogeneic lymphoblasts in stimulating lymphocyte transformation. Bromodeoxyuridine (BUdR) is known to alter cell surfaces. Addition of BUdR to the culture medium (10 μg/ml) does not alter the growth characteristics of lymphoblasts but reduces their ability to stimulate autologous lymphocyte transformation. BUdR at this concentration has no effect on lymphocyte transformation. Receptor sites on the surface of leukemic cells allow their agglutination by concanavalin A (Con A). These sites are not expressed on normal cells but are exposed by treatment with trypsin. We find that Con A agglutinates lymphoblasts under conditions which do not result in the agglutination of blood lymphocytes. Further, incubation of lymphoblasts with Con A appears to impair their capacity to stimulate autologous lymphocyte transformation. Incubation of blood lymphocytes with trypsin does not expose sites which stimulate autologous lymphocyte transformation. In conclusion, an antigenic determinant is present on lymphoblasts which is recognized as "foreign" by autologous lymphocytes. The expression of this determinant is depressed by incubation of lymphoblasts with BUdR or Con A. The immune reaction of autologous lymphocytes toward this antigenic determinant on lymphoblasts may limit their proliferation in vivo and may reflect the activity described by the term "immune surveillance." (Research supported by grant from ACS.)

326. Relationship of Tissue Oxygen Tension to Erythropoiesis. LOUIS F. WERTALIK,* KURT VON MAUR,* ROBERT D. RINGLE,* AND STANLEY P. BALCERZAK,* Columbus, Ohio (introduced by Charles A. Doan**).

To determine whether erythropoiesis is solely regulated by the level of tissue oxygen (O_2) two different means for inhibiting erythropoiesis, polycythemia and hyperoxia, were compared for their effect on tissue O_2 and erythropoiesis. Tissue O_2 was measured by the skin bubble technique; erythropoiesis was quantitated by plasma iron turnover (PIT) and red cell incorporation of radioiron (% Inc.). 6 days after rats were either (*a*) made polycythemic by transfusion (Hct $\leq$ 60%), (*b*) exposed continuously to 60% O_2, or (*c*) subjected to both hyperoxia and polycythemia, values were as follows:

	PIT (mg/day)	% Inc.	P_{O_2} (mm Hg)
Polycythemia	0.395 ±0.067	17.7 ±3.9	33 ±6.4
Hyperoxia	0.415 ±0.089	32.6 ±12.6	54 ±9.5
Polycythemia + Hyperoxia	0.272 ±0.054	18.9 ±4.3	55 ±6.3
Control	0.555 ±0.102	46.4 ±4.9	25 ±5.2

Polycythemia or hyperoxia caused significant suppression of PIT compared to controls ($P < 0.001$). Degree of suppression for these two groups was similar despite much greater increase in skin bubble P_{O_2} for hyperoxia compared to polycythemia ($P < 0.001$). Maximal inhibition of PIT was not obtained by either hyperoxia or polycythemia alone as demonstrated by significantly greater reduction in PIT when animals were subjected to both simultaneously ($P < 0.001$). Despite greater inhibition of PIT, skin bubble P_{O_2} in this latter group was similar to that found with hyperoxia alone. Values for % Inc. suggested that effective erythropoiesis was even less closely related to tissue O_2 tension than total erythropoiesis as measured by PIT. Polycythemia inhibited red cell production more than hyperoxia and the combination had little additional effect on synthesis of red cells. These results suggest that polycythemia inhibits erythropoiesis by factors in addition to tissue O_2 tension and raise the possibility of a regulatory role for these factors.

327. Iron and Liver Damage. MUNSEY S. WHEBY,* Charlottesville, Va. (introduced by Julian I. Kitay).

The relationship between excessive iron deposition and liver cell damage in hemochromatosis remains controversial. In hemochromatosis the periphery of hepatic lobules is the site of greatest iron concentration and cellular damage. Previously we showed in animals and man that when plasma transferrin is saturated with iron most iron absorbed from intestine is deposited in liver on the first portal circulation. The present study was designed to determine the site of deposition in liver of the absorbed iron. In normal rats plasma transferrin was saturated with a constant intravenous infusion of iron. Ferrous iron (5 μg) labeled with either ^{59}Fe or ^{55}Fe was then injected into a duodenal loop. After a 30 min absorption period the intact loop was removed. Total absorption and deposition of ^{59}Fe in liver was determined by whole-body counting. Radioautographs of liver sections were made from rats given ^{55}Fe. Results: (*a*) 97% of iron absorbed while transferrin was saturated was deposited in liver. Viviperfusion failed to remove this iron indicating fixation to liver cells. Iron reaching liver cells under these conditions is not bound to transferrin and may be toxic. (*b*) ^{55}Fe radioautographs show definite localization of absorbed iron mainly to parenchymal cells in the periphery of hepatic lobules. This is identical with the site of heaviest iron concentration and greatest cellular damage seen in hemochromatosis. Thus, these studies suggest the hypothesis that daily absorption of iron while transferrin is saturated (as in hemochromatosis) repeatedly exposes liver cells to ionic iron which produces damage particularly in the portal areas. (Research supported by Grant AM11258-04 from NIH.)

328. Adrenergic Stimulation of Ventilation in Man. ROBERT C. WHEELER,* DONALD D. HEISTAD,* ALLYN L. MARK,* FRANÇOIS M. ABBOUD, AND PHILLIP G. SCHMID,* Natick, Mass., and Iowa City, Iowa.

The mechanism by which catecholamines stimulate ventilation in man is not known. We have measured ventilatory responses of 12 normal men to intravenous infusions of norepinephrine and isoproterenol before and after propranolol. Minute volume ($\dot{V}_E$) increased significantly ($P < 0.01$) during infusions of 5 and 10 μg/min norepinephrine from a control value of 5.4 ±0.2 (SE) liters/min to 6.5 ±0.2 and 6.6 ±0.2 respectively. During infusions of 1 and 2 μg/min of isoproterenol $\dot{V}_E$ increased ($P < 0.01$) from 5.6 ±0.3 to 7.5 ±0.2 and 8.0 ±0.2. After the administration of propranolol (12 mg intravenously) the ventilatory effect of the catecholamines was blocked ($P < 0.01$). $\dot{V}_E$ was 5.2 ±0.2 after propranolol and increased to only 5.6 ±0.2 and 5.5 ±0.2 during infusion of the two doses of norepinephrine. Corresponding values before and during infusions of isoproterenol were 5.2 ±0.3 before and 5.6 ±0.2 and 5.9 ±0.2 during isoproterenol. Decreases in end-tidal P_{CO_2} in response to norepinephrine and isoproterenol were also blocked by propranolol. The hyperventilatory response to hypoxia (breathing 10.5% oxygen) was not altered by propranolol. The possibility that the ventilatory response to norepinephrine and isoproterenol is mediated through activation of chemoreceptors was tested in three men. 100% oxygen was administered to suppress chemoreceptors. Increases in $\dot{V}_E$ and decreases in end-tidal P_{CO_2} in response to norepinephrine and isoproterenol were markedly attenuated. We conclude that a beta adrenergic mechanism mediates the ventilatory response to norepinephrine and isoproterenol but not to hypoxia. Suppression of the chemoreceptors by breathing 100% oxygen attenuates adrenergic hyperventilation.

329. Rigid Hemoglobinopathies: Structural Differences in Sickled Human and Deer Erythrocytes. JAMES WHITE AND ULYSSES SEAL,* Minneapolis, Minn.

Deer erythrocyte sickling does not produce disease, and is caused by increased oxygen tension and high pH, conditions opposite to those which induce human sickling. However, sickling in the animal is considered a model for the human disorder due to similarities in red cell distortion and a report (1963. *Exp. Mol. Pathol.* 2: 173) which indicated that the fine structure of sickled deer erythrocyte was identical with that of sickled human cells. Recent investigations have shown that

polymers of human HbSS have a unique rod-like structure. It seemed unlikely that the structural basis for a rigid human Hb syndrome could be the same as that for benign sickling in deer. RBC's and cell-free solutions of Hb were prepared from deer in whom sickling and Hb types had been established, and sickling was induced by high oxygen pH and other methods, including a new technique used to gel normal human Hb. The structure and organization of Hb polymers in thin sections of sickled deer erythrocyte did not resemble the 170–190 A rods grouped parallel and equidistant to each other in human sickle cells. Hb polymers in crescentric deer cells were packed closely together in wall to wall contact. Cross-sections revealed circular profiles resembling microtubules, but with amorphous material in their central cores. Match-stick type sickled deer erythrocyte contained elongated bars with a crystalline substructure. Cell-free solutions of deer Hb formed gels consisting entirely of microtubules which crystallized into structures identical with the bars in match-stick deer cells. The sol-gel-crystal transformation characterizing sickling in deer erythrocyte and Hb solutions is strikingly similar to the process of Hb polymerization in gels of normal human Hb, but does not resemble the sickling phenomenon observed in erythrocytes of Hb gels of patients with sickle cell anemia.

330. Cardiac Performance after Fatty Acid and Glucose Infusions: the Influence of Hypoxia. KERN WILDENTHAL* AND GEORGE F. VASTAGH,* Dallas, Tex. (introduced by Jere H. Mitchell).

It has been suggested that elevations in free fatty acids (FFA) may depress the heart and that high glucose may be beneficial, especially during hypoxia or ischemia; experiments in vitro have not consistently supported that hypothesis, however. This study was made to test the influence of glucose and FFA in vivo, under normal and low oxygen tensions. Isotonic glucose or sodium octanoate was infused into open-chest anesthetized dogs breathing 100% O_2. Aortic pressure and cardiac output were constant. In six dogs receiving glucose (8 mmole/kg; blood glucose = 250–350 mg/100 ml) there were no significant changes in heart rate, left ventricular max dp/dt, and end-diastolic pressure (EDP). Similarly, no significant changes occurred in six dogs after 800 μmole/kg octanoate (plasma FFA = 1000–2000 μmoles/liter); with doses over 1600 μmole/kg heart rate and max dp/dt usually fell and EDP rose slightly. Responses were not influenced by vagotomy and propranolol pretreatment, nor by adding insulin and potassium to the glucose solution. In other experiments, 10 closed-chest anesthetized dogs were given either octanoate (800 μmole/kg) or glucose (8 mmole/kg). The dogs were then ventilated with 4% O_2 + 96% N_2 (arterial P_{O_2} = 15–25 mm Hg). All dogs initially developed tachycardia and hypertension, followed by progressive bradycardia and hypotension. Cardiovascular depression was more rapid in five dogs receiving octanoate, and they died after 26 ±3.4 (SEM) min; five dogs pretreated with glucose survived for 43 ±6.1 min ($P < 0.05$). The results suggest that although moderate variations in plasma FFA and glucose have little or no effect on the performance of well oxygenated hearts, dogs with elevated FFA are less able to maintain normal cardiovascular function during hypoxia than are dogs with high glucose. (Supported by NIH Grant HE 06296.)

331. Influence of Hyperosmolality on Myocardial Ischemia. JAMES T. WILLERSON,* W. JOHN POWELL,* TIMOTHY E. GUINEY,* JAMES J. STARK,* CHARLES A. SANDERS,* AND ALEXANDER LEAF,** Boston, Mass. (introduced by Marian W. Ropes**).

Hypoxia interferes with the active extrusion of intracellular sodium and is accompanied by intracellular edema. Hyperosmolar agents reduce cellular edema and in the brain prevent capillary obstruction and improve blood flow after ischemia. The influence of hyperosmolar agents on myocardial ischemia was evaluated in anesthetized dogs. Mannitol was infused into the aortic root of 15 isovolumic hearts (IVH) and of 15 dogs on right heart bypass (RHBP) for 13–30 minutes before ischemia. Mean serum osmolality increased 42 mOsm and 18 mOsm in the two preparations, respectively. Ischemia was produced by reversibly ligating the proximal left anterior descending coronary artery (LAD). Ventricular function curves (RHBP data) were unchanged after mannitol in four normotensive dogs without LAD occlusion. At normal systemic pressures mannitol significantly improved the depressed ventricular function curves which attended LAD occlusion. Left ventricular end-diastolic pressures (LVEDP's) were lower at three comparable levels of stroke work (SW) ($P < 0.005$, 0.025, and 0.05) and SW's higher at comparable LVEDP's ($P < 0.025$, 0.01, and 0.025). To investigate further the effect of mannitol on the ischemic myocardium ST segment elevation was assessed by epicardial EKG mapping in the IVH. ST elevation after 14 min of ischemia was substantially reduced by mannitol ($P < 0.025$). These EKG changes were associated with significant increases in total coronary blood flow ($P < 0.005$). Collateral coronary blood flow to the ischemic area was consistently increased in seven IVH's studied with ^{85}Kr washout ($P < 0.001$). Thus, increases in serum osmolality of 18–42 mOsm/liter by mannitol result in the following beneficial changes during myocardial ischemia: (*a*) improved myocardial function, (*b*) reduced ST segment elevation, (*c*) increased total coronary blood flow, and (*d*) increased collateral coronary blood flow. (Research supported by NIH Grant HE-06664.)

332. The Prevalence of Plasma Lipid Abnormalities in Men and Women of the Central Valley, California. PETER WOOD,* MICHAEL STERN,* ABRAHAM SILVERS,* JOBST VON DER GROEBEN,* AND GERALD REAVEN, Palo Alto, Calif.

The prevalence of plasma glyceride (PG) elevations, and of hyperlipoproteinemic patterns has not been examined in a large, free-living population. A study was carried out to provide this information for a sample of the population of Modesto, Calif., aged 25–79, including only individuals free from overt diabetes, electrocardiographic abnormalities, and elevated diastolic blood pressure. Plasma was taken after a fat-free breakfast from 494 males (mean age 46 ±12) and 503 females (mean age 48 ±13). PG concentration exceeded 200 mg/100 ml in 15% of males, 6.4% of females; for individuals aged 50–59, 22% of males and 7.6% of females exceeded this level. PG was significantly higher in men than in women in all age decades before the seventh. Plasma cholesterol (PC) exceeded 275 mg/100 ml in 4.5% of males, 6.2% of females; significant sex differences were absent or minor except in the

eighth decade. The prevalence of hyperlipoproteinemic patterns (Fredrickson) in the entire sample was: type I, absent; II, 3.7%; III, 0.2%; IV, 8.9%; V, 0.2%; not classified, 1.5%. Type II was more common in women (4.6%) than in men (2.8%); however, type IV was more common in men (13%) than in women (4.8%). Frequency of type IV patterns for those aged 50–59 was 20% for men, 3.4% for women. We conclude that (*a*) type IV pattern, with elevated PG level, was the most common abnormality, and was 3 times as common in men as in women; (*b*) PC levels were not different for men and women, confirming some earlier studies; and (*c*) sustained elevation of PG, rather than PC, in men is a possible factor in the well known male preponderance in coronary heart disease. (Research supported by grants from NIH.)

333. Plasma Prokininogenase (Prekallikrein): Isolation and Activation. KIRK D. WUEPPER* AND CHARLES G. COCHRANE, La Jolla, Calif.

The bradykinin-releasing enzyme of rabbit plasma was isolated in precursor form by ammonium sulfate precipitation, chromatography on DEAE- and CM-Sephadex, and block electrophoresis in Pevikon. The final product, prokininogenase (PK), was recovered in 8% yield, approximately 6000-fold purified from starting plasma, and gave a single band in polyacrylamide-gel electrophoresis (PAGE). The proenzyme had a mol wt of 99,900, calculated from sedimentation and diffusion coefficients and migrated as a gamma$_1$-globulin. Activation of PK by the prokininogenase activator (PKA), isolated from rabbit or human plasma, or trypsin resulted in limited proteolysis of PK at neutral pH. Cleavage of PK by PKA or trypsin was demonstrated immunochemically or by PAGE in alkaline or acid media. The larger fragment was kininogenase, which also hydrolyzed lysine and arginine esters and had a calculated mol wt 86,300. Kininogenase was inhibited noncompetitively by diisopropyl fluorophosphate (DFP), Trasylol, and SBTI but not by PMSF, TLCK, LBTI, or OMTI. A logarithmic (Hill) plot distinguished kininogenase inhibition by SBTI ($\lambda = 0.9$) from Trasylol ($\lambda = 2.9$); both substances inhibited trypsin ($\lambda = 0.8$) similarly. Purified PKA demonstrated enzymatic activity, hydrolyzing certain lysine esters, but not arginine esters. The proteolytic and esterolytic capacity of PKA was inhibited by DFP, the trypsin inhibitor from lima beans or phenylmethyl sulfonyl fluoride. The activation of prokininogenase occurs, therefore, due to the enzymatic action of PKA. (Supported by grants from NIH and NMSS.)

334. Disassociation of the Suppressive Effects of Triiodothyronine on Thyroid Iodine Accumulation and Hormonal Release in Patients with Increased Thyroxine-Binding Globulin. TOSHIHIDE YAMAMOTO,* APOSTOLOS VAGENAKIS,* LEWIS E. BRAVERMAN, AND SIDNEY H. INGBAR, Boston, Mass.

In normals given thyroid-suppressive doses of triiodothyronine (T_3), serum PBI decreases into the hypothyroid range within 2–3 wk. In pregnancy, prolonged treatment with large doses of T_3 produces lesser decreases in PBI, suggesting abnormal resistance to pituitary-thyroid suppression (Raiti et al. 1967. *N. Engl. J. Med.* **277**: 456). We have studied T_3 effects in two patients with idiopathic and four with estrogen-induced increase in serum thyroxine (T_4)–binding globulin (TBG), comparable to that found in pregnancy. During pronounced suppression of 24 hr thyroidal ^{131}I uptake (mean ±SD, 5 ±2%), stable serum T_4 decreased with a half-time ranging between 25 and 65 days (mean 39 ±16). In contrast, half-times of ^{131}I-labeled T_4, measured concomitantly, ranged between 5.0 and 11.4 days (mean 8.1 ±2.6). Stable/labeled half-time ratios averaged 4.8 ±1.2. Such discrepancies were not seen in two athyreotic patients with estrogen-induced increase in TBG. Here, stable and labeled T_4 half-lives after substitution of T_3 for L-thyroxine therapy approximated 9 days, with stable/labeled half-time ratios of 1.1 and 1.2. We conclude the following. The retarded decrease of serum T_4 in pregnant patients given T_3 does not indicate pituitary resistance to suppression, since comparable retardation was seen in other states associated with increased serum TBG, in which pronounced suppression of ^{131}I uptake could be demonstrated. The retarded decrease in serum T_4 is due to continued entry of T_4 into the blood, rather than a marked slowing of peripheral T_4 turnover, as indicated by studies with ^{131}I-labeled hormone. The unusually slow decrease in serum T_4 during T_3 suppression in patients with increased TBG suggests that the latter is in some way responsible for the continued entry of T_4 into the blood. The thyroid is doubtless the source of this stable T_4, since athyreotic patients with increased TBG did not display retarded stable T_4 disappearance when T_4 therapy was withdrawn.

335. Studies on Glutathione Reductase Activity in Red Cells of Patients with Severe Metabolic Disorders. YOSHIHITO YAWATA* AND KOUICHI R. TANAKA, Torrance, Calif.

Glutathione reductase (GR) plays an important role in the protection of protein in red cells against oxidation. Recently, it has been reported that GR is present in at least two forms: the active form with flavine adenine dinucleotide (FAD) and the inactive form without FAD. Thus, the effect of metabolic stress on GR activity in red cells in relation to these two forms and the mechanism of the increased GR activity were studied. GR activity was assayed after incubation of hemolysates at 37°C with or without 1 μM FAD for 30 min. Mean GR activity in red cells from 30 normal adults was 3.04 ±0.36 IU (the active form) and total GR was 4.38 ±0.37 IU. Thus, 69.4% of total GR was in the active form. In contrast, GR activity in the active form and per cent saturation were: 4.76 ±0.49 and 89.3% in eight severe uremics; 4.82 ±0.58 and 91.3% in seven severe cirrhotics; and 5.18 ±0.51 and 95.2% in seven cases of G6PD deficiency, respectively. Thus, GR is almost completely saturated with FAD in red cells in these patients. To clarify the mechanism of increased GR saturation with FAD, intact red cells of eight normal adults were incubated at 37°C up to 15 hr with or without 6×10^{-6} M methylene blue (MB), 12×10^{-3} M sodium ascorbate (SA) (pH 7.4), or 6×10^{-3} M acetylphenylhydrazine (APH) in the presence of 1.0×10^{-6} M riboflavin and 11.2×10^{-3} M glucose. After washing the red cells, GR was assayed in the hemolysates with or without FAD. The degree of saturation of GR with FAD was higher in the presence of riboflavin plus MB (97.4%), SA (96.5%), or APH (94.2%), than that with riboflavin alone

(84.3%). Thus, saturation per cent of GR was increased by addition of these well known activators of pentose phosphate pathway activity. In conclusion, there is increased GR activity with a high degree of saturation with FAD in red cells of severe uremia, cirrhosis of liver, and G6PD deficiency. This enhanced association with FAD appears to be an important controlling mechanism for GR activity and is independent of their various causes. (Research supported by grant from NIH.)

336. Demonstration of a Characteristic Protein in Plasma of Febrile Patients with Hodgkin's Disease. CHARLES YOUNG* AND SADIE HODAS,* New York (introduced by Joseph Burchenal**).

Pursuant to the report by Sokal and Shimaoka that urine from febrile patients with Hodgkin's disease contains a pyrogen (1970. *Nature (London).* **215**: 1183), we have consistently demonstrated the presence of a cationic protein (CP) (pI $\sim$ 10) of low molecular weight ($\sim$15,000) in the urine of 15 febrile Hodgkin's patients. This protein was not found in urine from afebrile patients (10) or normal individuals (7). On cationic electrophoresis at pH 4.3 in 6 M urea-containing acrylamide gels CP migrates well beyond the bulk of urinary proteins but short of muramidase. CP has been extensively purified from urine by chromatography on QAE-Sephadex and used as an internal marker in subsequent electrophoretic studies. A protein band with an electrophoretic migration identical with that of CP was characteristically present in CSF (8), plasma from febrile patients with Hodgkin's disease (26), and Hodgkin's disease tumor homogenates (6), but could not be identified in plasma from normal individuals (13), afebrile patients with advanced cancer (9), or afebrile patients with Hodgkin's disease in clinical remission (10). In plasma from febrile patients and in tumor homogenates, but not in urine or CSF, a second cationic band was present migrating just proximal to CP, thus giving a doublet appearance. The two proteins appear to be linked metabolically in that the entire doublet has disappeared from plasma after therapeutic response to vinblastine or procarbazine. A marked reduction has also occurred within 6 hr in one patient when defervescence was produced by infusion of cycloheximide, an inhibitor of protein synthesis. The data strongly suggest that these cationic proteins are pertinent to Hodgkin's disease activity. (Supported by NCI CA-08748, ACS-T40, and the Bodman Foundation.)

337. The Role of Micelle-Forming Properties of Bile Salts in Lipid Secretion into Bile. DAVID L. YOUNG,* Durham, N. C. (introduced by M. P. Tyor**).

It is now established that active transport of administered micelle-forming bile salts is associated with both increased bile formation and increased concentration of phospholipid (PL) in bile. The kinetics of lipid secretion is consistent with formation of a micelle-forming bile salt–PL complex at the canalicular membrane. Utilizing the ex vivo perfused rat liver, we have shown that administration of taurocholate (TC) caused a marked increase in bile flow and increased PL and TC concentration. Administration of the glycine conjugate of dehydrocholate (GDHC), a proven nonmicelle-forming bile salt caused a marked increase in bile flow comparable to that after TC administration, however, PL and TC concentrations were decreased. To prove that the increased flow and lowered PL concentration were due to secretion of GDHC into bile as an intact bile salt rather than an osmotically active, nonbile salt metabolite of GDHC, studies were done utilizing radiochemically pure glycine-GDHC-^{14}C(GDHC-^{14}C). It was shown that increased flow after GDHC-^{14}C administration occurs concomitantly with the rapid secretion of 95% of the administered GDHC-^{14}C as a single peak into bile. Both administered GDHC-^{14}C and GDHC-^{14}C recovered in bile chromatographed as single radioactive bands with R_f's identical with nonlabeled GDHC. The data confirm that the increased flow without increased lipid concentration is due to transport of an intact, nonmetabolized bile salt with poor micelle-forming properties and further supports the micelle-forming bile salt-lipid complex mechanism of PL transport into bile. (Research supported by Grant HE 11443 from NIH.)

338. Polymorphonuclear Leukocytes and Serum Factors in Human Immunity to *Pseudomonas aeruginosa*. LOWELL S. YOUNG* AND DONALD ARMSTRONG,* New York (introduced by Donald B. Louria).

Although *Pseudomonas aeruginosa* infections usually affect patients with underlying diseases, the association of specific immunologic deficits with increased susceptibility is less clear. We studied the interaction between *Pseudomonas* organisms, human polymorphonuclear leukocytes, and serum factors using serum bactericidal tests and quantitative tests of white cell phagocytosis and killing employing the differential centrifugation methods of Maaløe and Hirsch. 40 of 42 recent blood culture isolates of *Pseudomonas* were totally resistant to the action of fresh serum. Isolates from 11 patients surviving *Pseudomonas* bacteremia were not killed by fresh, autologous convalescent serum containing high titers of hemagglutinating and gel-precipitating antibody. However, serum from these 11 patients as well as others convalescing from nonbacteremic *Pseudomonas* infections contained high titers ($\leq$1:1280) of heat-stable opsonins which were predominately IgM in type, although IgG opsonizing activity was detectable. The use of functionally pure components of complement indicated that the first four components are critical for opsonization; less than 10% opsonizing activity remained in heated serum. Fresh normal serum promoted phagocytosis and intracellular killing of *Pseudomonas* but immunization of normal volunteers and cancer patients with the purified lipopolysaccharide antigens of Fisher, Devlin, and Gnabasik produced augmented opsonizing activity and was accompanied by rises in antibody measured by other techniques. Type-specificity of these opsonins was demonstrable and lipopolysaccharides could block phagocytosis. These findings suggest that the functioning antibody response to both natural *Pseudomonas* infection and immunization with purified antigens is production of heat-stable opsonins which require heat-labile serum factors for optimal effect. Essential to host defences are normally functioning polymorphonuclear leukocytes, as most invasive strains of *Pseudomonas* are serum resistant. (Supported by ACS and NIH grants.)

339. Synthesis and Degradation of Myosin in Cardiac Hypertrophy. R. ZAK,* V. ASCHENBRENNER,* AND M. RABINOWITZ, Chicago, Ill.

Accumulation of myofibrillar proteins in the hypertrophying heart may be secondary to increased synthesis, decreased degradation, or both. Changes in rates of cardiac myosin synthesis and breakdown were estimated at various times after surgical constriction of the ascending aorta of rats. Myosin was extracted with a modified Huxley's relaxing buffer, and isolated in a high degree of purity. Techniques for quantitation of cardiac myosin were developed using ATPase measurements, or isotope dilution with (carboxyl-^{14}C) *p*-chlormercuribenzoate coupled to myosin as marker. Synthesis was estimated from incorporation of 15-min pulses of leucine-^{3}H into myosin; and degradation from the decay of myosin specific radioactivity after administration of guanidoarginine-^{14}C or leucine-^{3}H 4 days before aortic constriction. Leucine-^{3}H incorporation into myosin is increased by 65–120% 48 hr after banding, peaks between 4 and 7 days (85–200% increase) and remained elevated at 10 days. The increase in incorporation was proportional to the degree of hypertrophy. In normal rats, cardiac myosin specific radioactivity reaches a peak 2 days after administration of leucine-^{3}H or guanidoarginine-^{14}C and then declines exponentially with a half-life of 11–12 days. With either isotope myosin specific radioactivities in banded animals were significantly higher than in sham-operated controls 2, 5, 7, and 10 days after aortic constriction. When correction is made for new myosin synthesis, degradation rate is almost completely shut off during the first 5 days after banding. No difference in myosin degradation rate was detected in rats banded 6 wk previously. It is concluded that early in the development of cardiac hypertropy after aortic constriction in rats there is a decrease in degradation as well as an increase in synthesis of myosin.

340. A Noninvasive Assessment of Left Ventricular Asynergy in Man. BARRY L. ZARET,* PETER J. HURLEY,* AND BERTRAM PITT,* Baltimore, Md. (introduced by Richard S. Ross**).

Quantification of left ventricular asynergy (LVA) is of value in estimating extent and location of injury and prognosis after myocardial infarction. LVA was assessed using intravenous Technetium 99M albumin (20 mCi) and an Anger scintillation camera. An ECG-triggered gating circuit selected 40- to 60-msec intervals from each cardiac cycle for imaging. Summed integrated end-diastolic (ED) and end-systolic (ES) cardiac images were obtained in the right anterior oblique position (RAO). From superimposed outlines of ED and ES images, cyclic changes in regional ventricular dimensions were measured. In 23 patients, per cent shortening from ED to ES of LV long axis (L), anterior (R_1), and posterior (R_2) hemiaxes was determined. In eight normal patients mean shortening was: L, 13%; R_1, 48%; R_2, 30%. In 15 patients with ischemic heart disease and LVA mean axis shortening was: L, 4%; R_1, 23%; R_2, 20%. These determinations were correlated with measurements made from single plane RAO LV contrast cineangiograms: L: $r = 0.92$, $P < 0.0005$; R_1: $r = 0.89$, $P < 0.0005$; R_2: $r = 0.87$, $P < 0.0005$. The location of the akinesis or dyskinesis in the 15 patients with LVA was: anterior wall 4, apex 5, inferior wall 2, generalized hypokinesis 4. The extent of the asynergic region determined by scintiphotography ranged from 15 to 59% of the LV circumference. The LV end-diastolic pressure (LVEDP) in patients with asynergy of less than 35% of the LV circumference averaged 11 mm Hg. In those with asynergy greater than 35%, the average LVEDP was 26 mm Hg ($P < 0.025$). This scintiphotographic technique offers a safe noninvasive means of evaluating left ventricular asynergy and estimating the size, location, and possible hemodynamic consequence of myocardial infarction in acutely ill patients. (Supported by Research Contract P. H. 43 67-1444 from the NHLI.)

341. The Concept of Local Determinants of Venous Volume: the Role of Nonadrenergic Factors in the Elevated Venous Tone of Congestive Heart Failure. ROBERT ZELIS,* ROBERT CAPONE,* EZRA A. AMSTERDAM,* AND DEAN T. MASON, Davis, Calif.

Although sympathetically mediated elevated venous tone had been considered an important compensatory mechanism in congestive heart failure (CHF), this concept has been recently challenged. To investigate venous compliance in CHF, venous volume of the elevated calf, with local venous pressure <1 mm Hg, was determined in 13 normal subjects and 10 CHF patients with a mercury-in-rubber strain gauge plethysmograph after volume equilibration at a congesting pressure of 30 mm Hg (VV[30]). VV[30] was considerably reduced in CHF (1.70 ml/100 ml) when compared with normals (4.63 ml/100 ml, $P < 0.01$) indicating increased venous wall tension in CHF. Moreover, this abnormality persisted despite calf alpha adrenergic blockade with intra-arterial phentolamine (normal 4.82, CHF 2.44, $P < 0.02$). Intra-arterial sodium nitrite ($NaNO_2$) (30 mg) produced a significant venodilation in both normal and CHF, before and after intra-arterial phentolamine. Note: although CHF veins dilated significantly more than normal, the CHF VV[30] was always significantly lower than normal. To maximally dilate the venous bed of CHF, 30 mg intra-arterial $NaNO_2$ was injected separately four times at 3-min intervals; after each injection VV[30] was 2.70, 3.14, 3.43, 3.62 in CHF and 5.10, 5.68, 6.10, 6.41 in normal. Since the normal and CHF changes in VV[30] induced with the last injection of sodium nitrite were similar, maximum venodilation had clearly been achieved. Moreover, the plateau of CHF maximal venodilation was considerably below normal ($P < 0.02$). These data indicate that, not only is venous tone elevated in CHF, but there is also an inherent venous stiffness, resistant to potent venodilator stimuli and independent of digitalis and sympathetic tone, thus demonstrating for the first time that local factors can significantly alter venous tone.

342. Stimulation of Glycogenolysis by Purified Cholera Enterotoxin. PHILIP D. ZIEVE,* NATHANIEL F. PIERCE,* AND WILLIAM B. GREENOUGH III,* Baltimore, Md. (introduced by Charles C. J. Carpenter).

Recent observations indicate that cholera enterotoxin stimulates adenyl cyclase and increases the level of cyclic AMP within the jejunal mucosa. In the current studies we

investigated the effect of enterotoxin on glycogenolysis, a process known to be mediated by cyclic AMP. When 100 μg of enterotoxin were administered intravenously to fasting dogs, serum glucose was 104 ±11, 110 ±12, and 151 ±14 mg/100 ml (mean ±SE) after 0, 1, and 2 hr and was 232 ±16, 140 ±6, and 136 ±11 mg/100 ml after 24, 72, and 120 hr ($P < 0.005$ comparing 0 value with those obtained from 2 to 20 hr). Control dogs given normal saline or inactivated enterotoxin showed no change ($P > 0.05$). When enterotoxin (2 μg) was administered intravenously to 12 mice, liver glycogen decreased 75% within 7 hr compared to controls ($P < 0.005$). Sonicated human platelets or homogenized rat liver were also depleted of glycogen after incubation at 37°C with enterotoxin (0.5 μg/ml) for 15 min. Rates of glycogenolysis were increased 36 ±5% ($P < 0.005$) and 61 ±27% ($P < 0.005$) respectively compared to controls incubated with buffer. Total phosphorylase activity was unchanged in lysates of platelets after incubation with cholera enterotoxin but phosphorylase *a* activity increased 14 ±5% ($P < 0.05$). These studies indicate that cholera enterotoxin stimulates glycogenolysis and that it may be a useful tool, therefore, for studying cyclic AMP–mediated processes. The very prolonged effect of the toxin suggests a mechanism of action that differs from that of most agents known to be mediated by cyclic AMP. (Supported by grants from NIH.)

343. Pancreatic Juice Ionized and Total Calcium in Dogs in Response to Secretin, Pancreozymin, and Calcium Infusions. MAURICE J. ZIMMERMAN,* EDWARD W. MOORE, DAVID A. DREILING,* AND HENRY D. JANOWITZ,** New York and Boston, Mass.

38 experiments were performed in five Thomas cannula dogs. Ionized calcium [Ca^{++}] was measured in pancreatic juice and serum by ion-exchange electrode and total calcium [Ca] by atomic absorption. Total protein was determined spectrophotometrically, volume by direct measurement and bicarbonate concentration [HCO_3^-] by AutoAnalyzer. In 11 experiments with single intravenous doses of secretin 5 μ/kg, and in 7 with secretin 5 μ/kg combined with pancreozymin (PZN) 2 μ/kg, basal [Ca^{++}] levels ranged between 0.39 and 1.22 mmole/liter, while basal [Ca] ranged between 1.00 and 3.38 mmoles/liter. After secretin, [Ca] fell to 0.2–0.3 mmole/liter, while [Ca^{++}] fell to virtually immeasurable levels of 0.075 mmole/liter. Pancreatic juice [Ca] varied inversely with pancreatic juice flow rate and [HCO_3^-]. At peak flow rates, [Ca] fell to extremely low levels, but returned to basal levels by 30–40 min. Plots of Q calcium against Q protein varied linearly over a wide range of values, as did plots of juice flow rate against Q calcium. The same general relationships held for secretin combined with PZN. Addition of PZN raised protein concentration and output as well as total calcium output while [Ca^{++}] remained in the range of 0.075 mmole/liter. In 20 experiments, the same dogs were made acutely hypercalcemic by constant intravenous infusion of 10% calcium gluconate solutions at varying rates, sufficient to raise serum [Ca] from 2.5–3.5 to 3.75 mmoles/liter. Secretin alone, or combined with PZN in the same dosages as above were administered as single intravenous injections and data obtained on volume, [Ca^{++}], [Ca], total protein, and [HCO_3^-]. Volume, [HCO_3^-], [Ca^{++}] and [Ca], and protein concentrations behaved as in experiments without infusion of calcium. Pancreatic juice [Ca] fell with the onset of secretion and juice [Ca^{++}] even with calcium being infused fell to 0.075 mmole/liter. These findings suggest that calcium is primarily secreted by the pancreas with protein (enzyme), and as suggested previously for gastic pepsinogen, may mean that stored pancreatic zymogen represents a calcium-enzyme polymerization. (Research supported by Grant AM-03889 from the NIAMD of the NIH.)

344. Participation of the Sixth Component of Complement in Normal Blood Coagulation and in the Acceleration of Coagulation Produced by Aggregated Gamma Globulin. THEODORE S. ZIMMERMAN,* CARLOS M. ARROYAVE,* AND HANS J. MÜLLER-EBERHARD, La Jolla, Calif.

Rabbits genetically deficient in the sixth component of complement (C6) showed retarded blood coagulation. Whole blood clotting time was prolonged and prothrombin consumption decreased. Prothrombin and partial thromboplastin time, specific clotting factor assays, and specific tests for platelet factor III activity were all normal. The prolonged clotting time and retarded prothrombin consumption could be corrected by the addition of isolated C6 in physiologic amounts to whole blood. C6 and C7 hemolytic activity, as well as immunologically determined C6 protein, were partially consumed during the clotting of whole human blood. No diminution of C2, C3, C4, C5, C8, or C9 hemolytic activity was observed. It is concluded that C6 and probably other complement components participate in normal blood coagulation. Aggregated gamma globulin, as well as antigen-antibody complexes, are known to activate the complement system. Heat-aggregated gamma globulin was found to accelerate prothrombin consumption in normal rabbit blood, but not in blood of C6-deficient animals. This accelerating effect on coagulation could also be demonstrated in platelet-poor plasma which had been collected without an anticoagulant in plastic tubes and separated from whole blood at 4°C. Thus, C6, and probably other complement proteins, are directly involved in the acceleration of blood coagulation by aggregated gamma globulin and presumably antigen-antibody complexes. (Supported by USPHS Grant Al-07007. Dr. Zimmerman is supported by a USPHS Training Grant from Case Western Reserve University.)

345. Deficient Chemotaxis of Leukocytes in Malignant Disease: Etiologic and Prognostic Features. MILUTIN ZIVKOVIC* AND JOHN BAUM,* Rochester, New York (introduced by John P. Leddy).

Chemotaxis of polymorphonuclear leukocytes (PMN) in malignant diseases has been studied by a modification of the Boyden technique allowing the use of small amounts of peripheral blood with good reproducibility. Of 39 patients (hospitalized and ambulatory) only eight had a chemotactic index (CIX) greater than 500 (normal mean 505; normal range 292–668). 26 patients with clinically active disease had an average CIX of 227 while 13 with clinically inactive disease averaged 576. CIX showed no relation to anti-tumor therapy. Seven patients with active disease who died during the study period had an average CIX of 184 (90–317). Prior studies had

shown that PMN's with low CIX from diabetic patients returned toward normal after treatment with insulin and glucose, while similar treatment of PMN's with low CIX from patients with infectious diseases and rheumatoid arthritis were unaffected by this therapy. To date, the PMN's of six patients with malignant disease have been tested for response to insulin and glucose. Three (with the lowest CIX) showed a return toward normal levels. We have thus demonstrated a deficiency of PMN function in malignant disease which, when marked, appeared to indicate a poor prognosis for survival and did not necessarily correlate with the clinical state of the patient at the time of testing. Decreased chemotaxis may be a specific metabolic defect in some patients since it was found that their cells could show increased activity after stimulation by insulin and glucose.

346. Morphologic Evidence for Two Types of Filaments in Human Platelets. DOROTHEA ZUCKER-FRANKLIN, New York.

Platelets possess a contractile protein which has the physical-chemical properties of actomyosin. Ultrastructurally, the isolated protein revealed 80-A filaments identical with those seen in the cytoplasm of intact platelets under experimental conditions. Studies were undertaken to better define individual components of this contractile system. In search for actin, use was made of the observation that purified heavy meromyosin (HMM) reacts with actin filaments to form "arrow head" complexes which can be resolved in tissue sections. Accordingly, glycerinated platelets were treated with rabbit muscle HMM (2 mg/ml) by a modification of the method by Ishikawa. Control and experimental platelets were fixed and embedded for electron microscopy. Glycerinated platelets showed mostly smooth appearing 80-A filaments which occurred in bundles or crisscrossed through the hyaloplasm. HMM-treated platelets showed filaments which were thicker (160–180 A) and "rougher" in longitudinal as well as cross-section. Arrays and single filaments resembling "arrow head" complexes were identified. Two methods were attempted to detect myosin subcellularly. A histochemical technique using lead phosphate to localize myosin ATPase associated reaction product predominantly with aggregates of thick filaments and plasma membranes. This method alone could not establish the presence of myosin since myosin ATPase cannot be specifically inhibited without affecting similar enzymes. Addition of 2 mM ATP in the presence of 0.05 M $MgCl_2$ consistently produced thick filaments which measured 250–300 A in width, often tapered at both ends, and had an axial periodicity of $\pm$110 A. They resembled those of artificially produced muscle myosin. The ultrastructural similarities between the filaments described here and those seen in purified contractile proteins from mammalian muscle as well as several primitive organisms suggest that the thin and thick filaments of platelets may also represent actin and myosin. (Research supported by grant from NIH.)