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Abstracts

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ABSTRACTS

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

1. Light Chain and γ-Globulin Production. GEORGE ABRAHAM,* CHRISTINE WATERHOUSE,** JOHN VAUGHAN,** AND RAMON MEDALLE,* Rochester, N. Y.

Since synthesis of γG globulin is usually accompanied by excess light (L) chain production, impairment of the latter has been suggested as a limiting factor in certain patients with low immunoglobulin levels. This study describes the relationship between excess production of L chains and γG globulin synthesis in 12 subjects, 6 of whom were without disease. ^{125}I-labeled L chains and ^{131}I-labeled γG globulin were administered by single intravenous injection. Appropriate serum and urine samples were collected and aliquots counted. The samples were then precipitated with 5% trichloroacetic acid to determine protein-bound iodine label, and were chromatographed on a Sephadex G-100 column to obtain a purified pool of free L chains for quantitation by complement fixation. The plasma disappearance curves of labeled L chains showed virtual disappearance (<1%) in 2–3 days except in two uremic subjects. Labeled γ-globulin disappearance was followed from 30 to 40 days to ensure observation of the final decay rate. Production rates were measured by integration of specific activity curves. γG synthesis ranged from 32 to 57 mg/kg per day in normal subjects. These figures were always higher than clearance rates calculated from serum values and urinary iodide. The discrepancy was clearly related to sequestration of 25–40% of the label outside the vascular system. Suggestive evidence for adsorption of γG on leukocyte membranes was obtained by direct splenic counting and isolation and counting of the circulating white cells. The ratio of excess L chain to γ-globulin production ranged in normal subjects from 0.05 to 0.24 with mean of 0.16. The two uremic subjects showed high values suggesting that the low γG levels were related either to heavy (H) chain deficiency or failure of union of the component parts.

2. Identification of *Proteus mirabilis* for Epidemiological Purposes. JONATHAN L. ADLER,* JOHN P. BURKE,* AND MAXWELL FINLAND,** Boston, Mass.

In tracing the source of an outbreak of serious proteus infections in a nursery, it was necessary to evaluate methods for the precise identification of the organisms cultured. Biochemical characteristics, bacteriophage and bacteriocine typing, Dienes tests, and susceptibility to appropriate antibiotics were used for this purpose to classify 194 strains of *P. mirabilis* isolated from infections at Boston City Hospital. Only 29 strains gave atypical biochemical reactions and did not conform to any specific phage or proticine type. Phage typing was the least helpful in distinguishing strains, since 50% of them were of one pattern. However, 76% of strains could be classified in 10 major proticine types. Dienes tests performed with pairs of organisms of each proticine type showed all but those of types 6 and 9 to be heterogeneous, with only 4–20% of pairs merging. Several distinct Dienes types were noted among merging pairs of each group. Organisms of different proticine types gave positive Dienes tests, that is, they did not merge. Strains within type 6 or 9 gave negative tests in 92% and 73% of pairs, respectively, suggesting these two types were each composed predominantly of the same or closely related strains. Strains of types 6 and 9 were also similar in their resistance to the penicillins and aminoglycosides; they were nontypable by available phages, and were predominantly associated with nosocomial infections. Proticine typing, together with the Dienes test and susceptibility to antibiotics, thus proved useful in separating and classifying specific strains of *P. mirabilis*, and for identifying endemic strains. (Aided by Research and Training Grants from NIH.)

3. Phenobarbital (PB): an Effective Form of Therapy in Primary Biliary Cirrhosis. W. H. ADMIRAND* AND K. BAUER,* San Francisco, Calif. (introduced by V. Sborov**).

It has previously been demonstrated that PB reduces hyperbilirubinemia and pruritis in patients with intrahepatic cholestasis and benign recurrent cholestasis, but the mechanism is not known. We have shown that PB is also effective in the treatment of primary biliary cirrhosis, and have investigated the mechanism of this therapeutic effect. Seven patients with characteristic clinical, chemical, and histologic features of primary biliary cirrhosis were studied. PB was administered (30–60 mg q.i.d.) for 3 months. Serum and stool samples were obtained weekly before, during, and after therapy. Bile salts (BS) were measured by gas-liquid chromatography; serum bilirubin and alkaline phosphatase were also determined. The mean serum BS concentration, 70.7 ±22.3 μg/ml before treatment, decreased to 18.0 ±12.4 μg/ml during therapy. This decrease was maximal within 1-3 wk after starting PB and was associated with cessation of pruritis. 2 wk after stopping PB serum BS returned to approximately pretreatment levels (58.2 ±12.6 μg/ml) and pruritis recurred. The ratio cholate/chenodeoxycholate was 2.3 and did not change significantly with PB. Pretreatment serum bilirubins were elevated in three patients and decreased 17, 24, and 46% respectively during therapy. Serum alkaline phosphatase activity also decreased during treatment (21–53%). PB resulted in a marked increase in fecal BS excretion from 126 ±63 mg/day pretreatment to 387 ±82 mg/day during therapy. This study shows that PB relieves pruritis and lowers serum bilirubin and alkaline phosphatase in patients with primary biliary cirrhosis. The concomitant decrease in serum BS and increase in fecal BS excretion during therapy suggests that PB enhances biliary excretion of BS.

4. Intravascular Fibrin Deposition in Renal Disease. NORMA ALKJAERSIG,* ALAN ROBSON,* JULIE INGELFINGER,* AND ANTHONY FLETCHER,** St. Louis, Mo.

Evidence has accumulated that intrarenal glomerular fibrin deposition may plat a significant role in the genesis of rena

damage in certain forms of chronic renal disease. A new method for detecting intravascular fibrin deposition, plasma fibrinogen chromatography, has been employed in the study of 82 children with renal disease. Plasma fibrinogen chromatography detects the presence of specific fibrinogen-fibrin complexes and fibrinogen derivatives produced in vivo during intravascular fibrin deposition and/or its lysis. The method has been demonstrated to be of sufficient sensitivity to detect clinically silent thrombosis (Fletcher et al., *Trans. Amer. Ass. Physicians*, 1970). Biopsy confirmation of diagnosis was obtained in the majority of 30 children with acute glomerulonephritis, 24 with chronic glomerulonephritis, and 17 with nephrotic syndrome. Serial studies were performed for periods of up to 15 months. In 12 children with acute glomerulonephritis followed from the acute phase until recovery, initial chromatograms showed evidence of intravascular fibrin deposition followed later by lysis and a return to normal chromatographic findings with clinical recovery. While chromatographic anomalies were less prominent in those with idiopathic nephrotic syndrome, 20 of the 24 patients with chronic glomerulonephritis showed evidence of fibrin deposition or resolution, either on initial examination or subsequently, abnormal chromatographic findings appearing to correlate with activity of the disease. In four acutely ill children with severe "progressive" glomerulonephritis, plasma fibrinogen chromatographic assays were used to control anticoagulant therapy. Each child showed a good clinical response and improvement in chromatographic findings. Our findings suggest that intravascular fibrin deposition (presumably intrarenal) almost invariably occurs in acute glomerulonephritis and frequently in chronic glomerulonephritis; its control may be of therapeutic importance. (Supported by HE-03745 and NIH-69-2263.)

5. Evidence for Diagnostic Uniqueness of Protein and Water Clearance in Human Pleural Effusions. JAMES C. ALLEN,* WILLIAM D. LERNER,* AND MICHAEL A. APICELLA,* Buffalo, N. Y. (introduced by Morris Reichlin).

Experimental studies have suggested different pathways for clearance of protein and water from the pleural space. Since etiology influences composition of pleural effusions in man, the possibility that differential effects on protein and water clearance were being exerted was entertained. 14 patients with cytologically proven pleural malignancy and effusion, and 13 with effusions due to congestive heart failure (CHF) were studied. Clearance of human serum albumin-^{125}I (protein marker) and ethylenediaminetetraacetate-^{51}Cr (water marker) was monitored by serial sampling after transthoracic injection. Intrapleural half-lives of each isotope were determined. Effusion volumes were calculated by extrapolation and were equivalent in the two groups. Protein and water half-lives were indistinguishable between the two diagnostic categories, but significant differences ($P < 0.05$) appeared when water and protein clearances were calculated. Thus, average protein and water clearances in malignancy were 67.3 mg/min and 5.5 ml/min respectively; in CHF the corresponding values were 20.2 and 2.8. These represent a daily unilateral pleural fluid outflow in CHF (expressed as serum) of about 4000 ml based on water clearance and 400 ml based on protein clearance, stressing the different mechanisms controlling the two moieties. The data also suggest that protein and water clearance are subject to different effects in the two diagnoses. Thus, the protein clearance to water clearance ratio in malignant effusions was 12.3, but only 6.4 in effusions due to CHF ($0.1 > P > 0.05$). When studies on effusions less than 1000 ml were considered, this difference was significant ($P < 0.05$). These data support the concept that characteristic pathophysiologic defects may be operative in pleural effusions associated with different disease states.

6. Soy Proteins: Another Rare Cause of the Flat Intestinal Lesion. MARVIN E. AMENT* AND CRYUS E. RUBIN,** Seattle, Wash.

This is a prospective study of the pathogenesis of a violent gastrointestinal reaction to a new feeding in an infant. Within 24 hr of changing this 6-wk-old infant's formula to soy milk he developed sequentially: fever, leukocytosis, cyanosis, vomiting, massive blood-tinged mucoid diarrhea, dehydration, and metabolic acidosis. All symptoms disappeared after discontinuing soy milk. When the infant was 6 months old and asymptomatic, a single test feeding of soy milk was given; proximal jejunal biopsies were taken with a miniaturized biopsy tube before and 12 and 24 hr afterwards. Before soy milk, proximal jejunal biopsies were normal, but afterwards they became acutely inflamed and flat (polymorphonuclear leukocytic infiltration and loss of villi). All previous symptoms recurred within 4 hr of this feeding but immediate parenteral fluid and electrolyte replacement prevented dehydration. Total hemolytic complement did not change after soy milk exposure nor did circulating eosinophils increase, despite leukocytosis with shift to the left. Lactase deficiency developed transiently. A single test feeding of soy protein isolate (Mead Johnson and Co.) was given 4 months later, when the proximal jejunal biopsies were normal and the child was well. The same systemic and intestinal responses occurred and similar laboratory abnormalities developed. Clinical recovery was complete in 24 hr and jejunal villi regenerated within 4 days. Test feedings of soy-lecithin, gluten, and cow's milk neither altered jejunal structure nor produced symptoms. No consistent changes in the rectal mucosa were observed after oral or rectal administration of soy protein isolate or cow's milk. This is the first documentation of a reversible flat jejunal lesion developing in response to feeding soy proteins to a susceptible infant. (Supported by Research Grant CA04320 and General Research Support Grant 5 SO1 RR05655 from NIH.)

7. Plasma α_2-Macroglobulin: the Primary Inhibitor of Human Thrombin. DUDLEY B. ANDERSON* AND SANDOR S. SHAPIRO, Philadelphia, Pa.

The capacity of normal blood to neutralize thrombin has been ascribed to at least three well defined plasma proteins—α_2-macroglobulin (α_2M), α_1-antitrypsin (α_1-AT), and antithrombin III (AT-III)—as well as to the fibrin clot itself. We have studied the neutralization of thrombin in plasma using radioactive human thrombin purified by ion-exchange chromatography and gel filtration from tissue-activated human prothrombin-^{125}I. Varying quantities of thrombin were added to normal human plasma to achieve concentrations over the entire potential physiologic range (0–> 250 U/ml) and, after total neutralization of thrombin, clot radioactivity was measured and clot supernatants were gel filtered on Sephadex G-200 columns. Over the entire range of measurement a linear

relation was found between initial thrombin concentration and radioactivity in the clot, accounting for approximately 13% of the thrombin added. The remaining radioactivity was found largely in the macroglobulin peak on gel filtration, with some trailing into the 7S area. The height of the radioactive macroglobulin peak was linearly related to the initial thrombin concentration over the entire range studied. Throughtout the entire macroglobulin peak radioactivity was precipitable by antiserum to human α_2M. No radioactivity precipitated with antisera to α_1-AT or AT-III. The majority of the radioactivity in the 7S shoulder was not precipitable by α_2M antiserum, but also could not be identified with either α_1-AT or AT-III. Although it is quite possible that these latter two proteins play some role in physiologic thrombin neutralization, it is concluded that the major antithrombin of human plasma is α_2M. (Supported by grants from NIH.)

8. Regulation of Cholesterol Storage in White Adipose Tissue. A. ANGEL* AND J. FARKAS,* Toronto, Canada (introduced by C. H. Hollenberg).

In adult primates and rats, white adipose tissue has been shown to contain more than 20% of total body exchangeable cholesterol. The present study was undertaken to characterize this storage pool and to delineate factors regulating its formation. Analysis of collagenase digests of epididymal fat taken from 500-g rats raised on Purina Chow showed that 90% of tissue cholesterol was in isolated adipocytes and, on analysis of subcellular fractions, more than 85% of the cholesterol was in the bulk liquid compartment, of which more than half was in esterified form. In contrast, 65–80% of the cholesterol extracted from adipocyte ghosts and isolated plasma membrane fraction was free. The cholesterol:DNA ratio of white fat increased linearly with age and weight, despite a constant fasting serum cholesterol level. After 4–6 days starvation, the cholesterol:glyceride ratio increased, indicating preferential mobilization of glyceride. The cholesterol content of total white adipose tissue dissected from obese hyperglycemic mice (Ob/Ob) exceeded that of their normal littermates 6- to 8-fold. In fat tissue of rats raised on formula diets containing 0–5% cholesterol, cholesterol accumulation occurred in the absence of a dietary source, and the amount of cholesterol stored in adipose tissue increased progressively with increasing dietary cholesterol levels. Isolated fat cells prepared from human omental and rat epididymal fat failed to convert labeled glucose or acetate into cholesterol. Nevertheless, uptake by human fat cells of cholesterol added to the medium was rapid and esterification was observed. These data imply that accumulation of adipocyte cholesterol is time and diet dependent and is derived from circulating lipoprotein particles, rather than *in situ* synthesis. (Supported by MRC and the Banting Research Foundation.)

9. Hormonal Control of Glutamine Levels. THOMAS T. AOKI,* WALTER A. MULLER,* ERROL B. MARLISS,* AND GEORGE F. CAHILL, JR., Boston, Mass.

In postabsorptive man, glutamine is produced by muscle and removed by splanchnic bed and kidney. Its renal extraction is increased with augmented ammoniagenesis, yet its plasma level remains relatively constant in spite of acidosis or alkalosis. Glucagon and insulin have been shown to alter other amino acid levels. The effect of these hormones on enzymatically determined glutamine levels was therefore studied. In five otherwise normal obese subjects fasted 3–4 wk, glucagon (0.1 mg/day given intravenously for 4 days) decreased plasma glutamine from 268 ±33 μmoles/liter to 180 ±34 μmoles/liter and to 174 ±42 μmoles/liter (both $P < 0.25$, paired t test) by 24 and 48 hr. By the 4th day, it returned to 233 ±52 μmoles/liter. Unaffected were levels of circulating β-hydroxybutyrate, acetoacetate, glucose, free fatty acids, and insulin. Urine β-hydroxybutyrate and acetoacetate were constant. In three subjects fasted 6 wk, insulin (20 U given intravenously over 24 hr) decreased glutamine 365 ±61 μmoles/liter to 176 ±72 μmoles/liter ($P < 0.005$, paired t test). Serum insulin rose 5 ±1 to 10 ±4 μU/ml ($P < 0.05$, paired t test) and glucose fell 70 ±6 to 28 ±5 mg/100 ml. FFA, blood β-hydroxybutyrate and acetoacetate, and urinary total nitrogen, ammonia nitrogen, and ketoacid levels were not significantly changed by this small dose of insulin. Urea nitrogen excretion did decrease ($P < 0.05$, paired t test). We conclude that insulin and glucagon in physiological concentrations are both capable of markedly altering circulating glutamine levels, suggesting a role of these hormones in controlling glutamine metabolism.

10. Effects of Cyclic Adenosine Monophosphate (AMP) and Dibutyryl Cyclic AMP in Antidiuretic Hormone–Deficient and Antidiuretic Hormone–Resistant Diabetes Insipidus. SUSAN AVERY,* CHARLES M. CLARK, JR.,* CARL TRYGSTAD,* AND NORMAN H. BELL,* Indianapolis, Ind. (introduced by Harvey Feigenbaum).

The renal effects of antidiuretic hormone (ADH) on urinary concentrating ability appear to be mediated through the adenyl cyclase system. ADH-resistant diabetes insipidus (RDI) could result from a defect in the formation of cyclic adenosine 3′, 5′-monophosphate (cyclic AMP), the response to cyclic AMP, or both. To investigate this, the effects of cyclic AMP and dibutyryl cylic AMP were determined in male patients with classic sex-linked recessively transmitted RDI and in patients with ADH-deficient diabetes insipidus (DI) by standard clearance techniques. All patients had normal renal function as judged by clearances of inulin. Cyclic AMP (0.1–0.6 mg/kg per min) and dibutyryl cyclic AMP (0.1 mg/kg per min) were given by constant intravenous infusion after control periods. Dibutyryl cyclic AMP increased C_{H_2O} and increased phosphate clearance without changing glomerular filtration rate (GFR) in each subject examined. Whereas cyclic AMP decreased C_{H_2O} and increased urine osmolality without changing GFR in the patients with DI, it did not alter C_{H_2O}, urine osmolality, or glomerular filtration rate in the patients with RDI. The results suggest that normally there are three distinct renal responses to cyclic nucleotides which are clearly dependent on the analogue used: one to increase C_{H_2O}, one to increase phosphate clearance, and one to concentrate the urine. The demonstration that patients with RDI, unlike those with DI, are unable to increase urinary concentration after cyclic AMP suggests a defect in the response to cyclic AMP. Whether this is coupled with a defect in the formation of cyclic AMP in response to ADH is yet to be determined.

11. The Metabolism of 25-Hydroxycholecalciferol-^{3}H (25 OHD$_3$-^{3}H) in Uremia. LOUIS V. AVIOLI, SOOK WON LEE,* AND HECTOR DELUCA,* St. Louis, Mo., and Madison, Wis.

Chronic uremia in man and animals results in a resistance to the biological effects of vitamin D_3 (D_3) and an abnormality in D_3 metabolism. Since it has been established that 25OHD$_3$ is one of the biologically active circulating metabolites of D_3, experiments were designed to study its metabolic fate in the uremic state. 25OHD$_3$-^{3}H was administered by jugular puncture to animals with experimentally induced chronic uremia and their pair-fed, age-matched controls. 24 hr later the animals were sacrificed and urine, plasma, small intestine, liver, and muscle harvested for measurements of DNA, protein, and radioactivity. 25OHD$_3$-^{3}H metabolites were separated and isolated by silicic acid gradient elution techniques and liquid-liquid partition chromatography. Whereas less than 4% of the injected radioactivity was normally excreted in 24 hr, over 20% was recovered from the urine of uremic animals. In each instance, the majority of the excreted radioactivity was characterized as unaltered 25 OHD$_3$-^{3}H. 3.9 and 1.9% of the injected radioactivity was recovered from the intestine of normal and uremic animals respectively. The decrease in intestinal radioactivity was due primarily to a decrease both in 25OHD$_3$-^{3}H and one of its polar metabolites. These alterations in 25OHD$_3$-^{3}H metabolism were also associated with a net decrease in intestinal protein synthesis as reflected by tissue protein:DNA ratios. Whereas the hepatic concentration of 25OHD$_3$-^{3}H and its esterified derivatives were normal in the uremic animals, the muscle and bone content of 25 OHD$_3$-^{3}H were decreased. These studies demonstrate that the chronic uremic state results in an impairment of intestinal protein synthesis and a derangement in the metabolism and distribution of 25OHD$_3$-^{3}H. As such, they offer a reasonable explanation for the malabsorption of calcium and its resistance to vitamin D_3 manifested by patients with chronic renal disease.

12. A New Method of Measuring Pulmonary Diffusing Capacity in Patients with Diffuse Lung Disease. BRIAN AYOTTE,* WOLFGANG O. FRIESEN,* GUNNAR ROSENHAMER,* AND MALCOLM B. MCILROY,** San Francisco, Calif.

Alveolar (end-tidal) and arterial oxygen tensions are measured during a short period of alveolar hypoxia ($P_{A_{O_2}}$ 40–60 mm Hg) lasting about 20 sec. Respired P_{O_2} is measured with a rapidly responding oxygen electrode and Pa_{O_2} measured in brachial arterial blood. An ear oximeter is used to ensure that arterial oxygen saturation stays constant during arterial sampling. After end-tidal and arterial P_{O_2} have been measured during several periods of hypoxia at rest, the procedure is repeated during exercise on a cycle ergometer. Short-term hypoxia serves to reduce or eliminate alveolar-to-arterial P_{O_2} difference due to shunt or mismatching of ventilation with perfusion. We assume that any A−a difference which appears during short-term hypoxia during exercise results from diffusion block. We studied five patients with diffuse lung disease in whom the A−a difference breathing air averaged 30 mm Hg at rest and 47 mm Hg during exercise at 200 kg/min ($\dot{V}_{O_2}$ 711 ml/min). In four patients the A−a difference during short-term hypoxia ranged from +3 to −3 mm Hg at rest (mean 0 mm Hg) and increased during exercise to 21–26 mm Hg (mean 24 mm Hg). We measured cardiac output during exercise by a nitrous oxide rebreathing method and calculated $D_{L_{O_2}}$ from the A−a difference during exercise using the Bohr integration procedure. The values for $D_{L_{O_2}}$ (14.0, 24.0, 11.3, and 15.9 ml/min per mm Hg) were not greatly different from the values for single breath D_{LCO} measured at rest (14.9, 22.7, 15.0, and 16.9 ml/min per mm Hg). (Supported in part by Grant HE-06285 from the National Heart and Lung Institute.)

13. Reduced Pyridine Nucleotide (RPN) Content in G6PD-Deficient Granulocytes (PMN): an Explanation for Their Defective Bactericidal Function. ROBERT L. BAEHNER,* RICHARD B. JOHNSTON, JR.,* AND DAVID G. NATHAN, Boston, Mass.

Totally G6PD-deficient PMN (0% G6PD PMN) have normal NADH oxidase activity but lack a respiratory burst; hexose monophosphate shunt (HMPS) is not stimulated; H_2O_2 is not produced and nonperoxide-forming bacteria are not killed. Bactericidal H_2O_2 is produced during phagocytosis from NADH by NADH oxidase, but the above data suggest that NADPH produced via the HMPS might also contribute to the RPN pool (NADH + NADPH) from which H_2O_2 is generated. To examine this, 5% G6PD PMN with metabolic and bactericidal defects similar to but milder than 0% G6PD PMN were studied. Low total RPN production in intact 5% G6PD PMN was detected by incubation with 2 mM methylene blue (MB) and formate-^{14}C. MB is reduced by PMN RPN diaphorase to MBH which is autoxidized to H_2O_2. The amount of H_2O_2 formed determined from catalatic formate-^{14}C oxidation is a function of total RPN production. Formate-^{14}C oxidation in control, 5% G6PD PMN, and 0% G6PD PMN was 1.16 ±0.26, 0.48, and 0.45 mμmoles mg^{-1} 30 min^{-1} respectively and was normal (0.84) in 20% G6PD PMN which function normally. The pools of NAD:NADH and NADP:NADPH in PMN extracts were determined fluorometrically. The NAD:NADH pool was larger than NADP:NADPH pool in all PMN's tested (9.7 ±4.3 vs. 4.3 ±1.1 mμmoles/10^8 PMN). NADPH content of 5% G6PD PMN (0.50) was markedly reduced compared to 20% G6PD PMN (2.00) or controls (2.40 ±0.66 mμmoles/10^8 PMN). NADH was also depressed in 5% G6PD PMN (1.0) compared to 20% G6PD PMN (2.0) or controls (2.5 ±0.79 mμmoles/10^8 PMN). This decreased RPN pool may limit the production of bactericidal H_2O_2 by oxidase which could explain the defective metabolic and bactericidal function observed in G6PD PMN. (Supported by grants from NIH, the American Heart Association, and the John A. Hartford Foundation.)

14. The Role of Thrombin in Platelet Aggregation. NANCY BAENZIGER,* GRAEME BRODIE,* LEWIS R. CHASE,* AND PHILIP W. MAJERUS, St. Louis, Mo.

Thrombin rapidly induces aggregation of human platelets. We have postulated that the substrate for thrombin is a protein of the platelet surface membrane. Using sodium dodecylsulfate–polyacrylamide-gel electrophoresis to separate membrane proteins, we have recently described a membrane protein which is rapidly hydrolyzed when *intact*, but not disrupted, platelets are incubated with thrombin (0.1–1 U/ml).

The time course for the hydrolysis of the thrombin-sensitive protein (TSP) from intact platelet membranes ($t_{\frac{1}{2}}$ 15 sec to 1 min) suggests that hydrolysis of TSP may trigger platelet aggregation. TSP has a molecular weight of 190,000 and is not fibrinogen. Several investigators have reported that agents which increase cellular cyclic AMP (cAMP) (theophylline and prostaglandin PGE_1), prevent thrombin-induced aggregation, suggesting that a fall in cAMP may trigger platelet aggregation. We have examined the effects of thrombin on basal and PGE_1- or fluoride-stimulated adenyl cyclase activity in membranes from sonicated platelets. Incubation of *intact* platelets with thrombin (0.2–1 U/ml) at 37°C caused rapid inactivation of adenyl cyclase ($t_{\frac{1}{2}}$ approximately 30 sec); however, no inactivation occurred when disrupted platelets (sonicated) were incubated with thrombin. The activities of other membrane enzymes including Mg^{++}-Ca^{++} ATPase, Na^{+}-K^{+} ATPase, phosphodiesterase, and 5′-nucleotidase were not affected by thrombin treatment of intact platelets. When platelets were preincubated with PGE_1 (10^{-6} mole/liter) and subsequently incubated with thrombin, TSP was not hydrolyzed and adenyl cyclase was not inhibited. The rapid hydrolysis of TSP and inactivation of adenyl cyclase which occur in parallel under a variety of conditions at physiological concentrations of thrombin suggest that thrombin may initiate platelet aggregation by inactivating adenyl cyclase. Whether TSP is adenyl cyclase or another membrane protein involved in this reaction remains to be determined. (Supported by grants from NIH and ACS.)

15. Periodic Hormonogenesis: a Progress Report. RICHARD E. BAILEY,* Portland, Ore. (introduced by Monte A. Greer**).

"Periodic hormonogenesis" is a newly discovered phenomenon of periodicity in hormone production by a neoplasm. The original description of this entity is in press (*J. Clin. Endocrinol. Metab.*).The propositus had a very slow growing, carcinoid-type, adrenocorticotrophic hormone (ACTH)-producing, malignant bronchial adenoma. The salient features in her case were: Cushing's syndrome, as well as the superior vena caval syndrome, periodicity in the adrenocortical production of cortisol and 17-ketosteroids with an approximate 18-day cycle, intermittently normal and elevated cortisol production, autonomy of production of ACTH-like material by the tumor for stimuli commonly used to test pituitary-adrenal feedback mechanisms, intermittent hypokalemia, intermittent hypertension, cyclical variations in renal sodium excretion, and suppression of aldosterone production in spite of marked and prolonged dietary sodium restriction. Recent observations establish the tumor had biologically active ACTH as well as the previously reported radioimmunoassayable ACTH, and contained 167 μU/g, as measured with an isolated adrenal cell preparation (Sayers) after $3\frac{1}{2}$ yr storage. Also, the excretion rates of 3α-17α-21-trihydroxy-5α-pregnan-20-one (THS) and 3α, 21-dihydroxy-5α-pregnan-20-one (THDOC), compounds regulated by ACTH secretion, were very high at the time of maximal cortisol production. This phenomenon deserves emphasis because of the expectation that additional cases of hormone production rhythms will be discovered in a variety of neoplasms, the implications for understanding the biology of hormone-producing tumors, and the diagnostic problems which are presented. (Research supported by grants from NIH, Oregon Heart Association, American Heart Association, and Medical Research Foundation of Oregon.)

16. Hormonal Control of Hepatic Heme Catabolism. ARNE F. BAKKEN,* M. MICHAEL THALER,* AND RUDI SCHMID,** San Francisco, Calif.

In newborn and adult rats, activity of hepatic heme oxygenase (HO), the substrate-inducible enzyme responsible for the conversion of heme to bilirubin, is increased 2- to 3-fold by starvation or by hypoglycemia produced by insulin or mannose. This increase in activity is prevented or reversed with glucose, whereas in fed animals glucose, with or without insulin, has no effect. These observations suggested that HO may be responsive to hormones released during hypoglycemia. Glucagon and epinephrine given intraperitoneally increased HO 2- to 6-fold; effects were additive, reproducible with cyclic AMP, and not prevented by glucose. Arginine, which causes endogenous glucagon release, stimulated HO 4-fold. Nicotinic acid, which counteracts the lipolytic activity of epinephrine and glucagon, did not block their effect on HO, thus excluding plasma free fatty acid as a possible regulatory mediator. Thyroxin, another cyclic AMP–mediated hormone, and hydrocortisone had no effect on HO activity. Puromycin, but not actinomycin, abolished enzyme stimulation by glucagon and epinephrine. In contrast, substrate (heme)-mediated induction is blocked by both inhibitors, indicating that control of HO activity occurs at more than one level of enzyme synthesis. HO activity may play an important role in regulating hepatic bilirubin production. In rats whose heme had been labeled with glycine-2-^{14}C, glucagon or epinephrine enhanced production of carbon monoxide-^{14}C up to 4-fold. Since CO is produced during catabolism of heme to bilirubin by HO, the increase in CO resulting from hormone treatment reflects stimulated bilirubin production. These findings suggest that the rate of bilirubin formation in the liver is under hormonal control, which may explain both the rise in serum bilirubin in Gilbert's syndrome during fasting, and the development of unconjugated hyperbilirubinemia in hypoglycemic infants and in adults infused with mannose.

17. Observations Indicating Cholera-Induced Secretion Originates from the Crypts of Lieberkühn. J. G. BANWELL,* G. M. ROGGIN,* J. H. YARDLEY,* AND T. R. HENDRIX,** Baltimore, Md.

To extend observations that fluid secretion in cholera originates from crypt cells, paired in vivo rabbit jejunal loops were pretreated with either hypertonic sodium sulfate (2100 mOsm/liter) or isotonic electrolyte solution (290 mOsm/liter) and subsequently exposed to cholera toxin or uninoculated medium (syncase). Exposure of rabbit intestinal loops to hypertonic Na_2SO_4 damage produced consistent morphologic alteration in villus crest cells and inhibition of glucose absorption without morphologic damage to the crypts. Net fluid secretion was calculated from changes in concentration of a poorly absorbable marker (PSP) in perfused loops. Mean fluid production in response to cholera toxin was not different in loops pretreated with Na_2SO_4 or isotonic electrolyte solution (0.54 ± 0.05 vs. 0.40 ± 0.05 ml/cm per 2 hr) ($P > 0.2$). On the other hand, pretreatment with hypertonic Na_2SO_4 inhibited glucose absorption ($P < 0.001$). PSP recovery (95-

100%) was comparable in both groups of animals. Therefore, morphologic damage to the villus crest by hypertonic Na_2SO_4 which inhibited glucose absorption did not affect cholera toxin–induced intestinal secretion. However, pretreatment of rabbits with cycloheximide (20 mg/kg), which produced morphologic damage to crypt cells, prevented the secretory response to cholera toxin in loops preexposed to hypertonic Na_2SO_4 or isotonic electrolyte solution (0.15 ± 0.03 vs. 0.13 ± 0.07 ml/cm per 2 hr). These data are consistent with the hypothesis that intestinal fluid after exposure to cholera toxin originates in the intestinal crypts.

18. Cortisol-Induced Alteration in Capacity for α-Amino-isobutyric Acid (AIB) Transport in Human Leukemic Lymphocytes and Granulocytes. DANIEL T. BARAN,* MARSHALL A. LICHTMAN,* AND WILLIAM A. PECK,* Rochester, N. Y. (introduced by Robert I. Weed).

Since the effect of glucocorticoids on leukemic lymphocytes (LL) may be due to changes in their plasma membrane function, we have examined the in vitro effect of cortisol on their active transport of AIB-^{14}C. We have also studied AIB transport in human leukemic myeloblasts (LM) in order to explain their well known resistance to glucocorticoids. Initial rates (V_0) of AIB accumulation in LL were 1–10 μmoles/kg cell H_2O per min and inhibition by 1–10 μM cortisol was 30–55% in each of 12 patients. V_0 and the degree of cortisol inhibition were similar in the same patient at different times and did not correlate with clinical course or WBC. Cortisol inhibition was (*a*) noncompetitive, (*b*) evident after 60 and maximal at 180 min, (*c*) present at equilibrium (120 min of label) as well as during V_0, (*d*) unaccompanied by changes in sodium gradients, (*e*) unaffected by absence of extracellular glucose, and (*f*) present in treated cells reincubated for 60 min in cortisol-free medium. Simultaneous blockade of protein synthesis with puromycin or cycloheximide prevented appearance of cortisol inhibition. LL and LM each responded to low extracellular sodium concentration (25 mmoles/liter) or ouabain treatment (10 mmoles/liter) with a 45% decrease in active AIB transport. In striking contrast with LL, however, LM from each of two patients responded repeatedly to cortisol treatment (1–10 μmoles/liter) with increased AIB accumulation (+20, +70%); moreover, this stimulation was prevented by simultaneous inhibition of protein synthesis. Hence, LL demonstrates a characteristic transport potential and cortisol sensitivity for each patient. Cortisol decreases their capacity for AIB transport indirectly by a process which requires new protein synthesis. Differential sensitivity of amino acid transsystems in LL and LM may account for differences in their sensitivity to glucocorticoids in vivo.

19. Validation of a New Uniplane Cine-Angiographic Technique for Estimating Ventricular Volume. ROBERT BARNDT,* EARL C. HARRISON,* DONALD W. CRAWFORD,* L. JULIAN HAYWOOD,* FRANCIS Y. K. LAU,* AND DAVID H. BLANKENHORN,** Los Angeles, Calif.

Careful correction for nonparallel ray magnification is critical in estimating left ventricular end diastolic volume (LVEDV) by angiographic methods. A practical uniplane technique has been developed by adaptation of a standard C-arm mounted image amplifier system. This technique involves triangulating on the center of the heart in the AP (0°), RAO (30°), and LAO (45°) positions utilizing a center marker on the input phosphor shield, a protractor with one degree graduations on the C-arm pivot, and centimeter scales on the vertical and horizontal carriages. After the angiographic procedure, identical settings are used to position and photograph a 1 inch steel sphere in the former position of the center of the left ventricle. LVEDV is calculated from the tracing of the 30° RAO projection using the formula for volume of a prolate spheroid. The diameter of the sphere on cine compensates for left ventricle to input phosphor distance, projector focus, and screen distance. Latex reproductions of four swine ventricles were distended with 40 different known volumes of contrast media over the range of 80–360 cc. Correlation between calculated (y) and known volume (x) was $y = x + 2.3$ cc ($r = 0.986$, SEE = 12.1 cc). Sequential studies of 30 patients with coronary atherosclerosis comparing thermodilution and angiographic LVEDV (range 147–430 cc) yielded a correlation $r = 0.974$, SEE = 18.1 cc. This method, which compares favorably with biplane methods, can be applied to any standard cine-angiographic equipment to allow accurate estimation of LVEDV. (Research supported by Grant RR 43 from NIH.)

20. The Effect of pH on Endochondral Calcification In Vitro. URIEL S. BARZEL,* Bronx, N. Y. (introduced by Louis Leiter**).

This study was undertaken as part of an investigation into the relationship of bone and acid-base metabolism. Femoral primordia of 16- and 17-day rat embryos were grown for 6 days in organ culture in a CO_2 incubator. The pH of the bicarbonate-buffered culture medium was controlled by varying the CO_2 flow into the incubator. Growth in length and weight was observed over a pH range extending from 6.5 to 7.6; it was more marked at the more alkaline pH levels. 16-day primordia, which do not have a calcified epiphysis, failed to calcify at pH 7.2 and below. 17-day primordia demonstrated some calcification below pH 7.2. All primordia demonstrated progressive, histologically normal, endochondral calcification at increasing pH levels above 7.3. Relative to the growth in length and weight, the increment in calcification was much larger at the higher pH levels. Thus, 16-day primordia had 0.920 μg calcium per mg bone at pH 7.6, 0.453 at pH 7.3, and none at pH 6.8. In 17-day primordia the comparable values were 0.931, 0.643, and 0.163 respectively. It may be concluded that bone as an organ is metabolically responsive to acid-base changes. Raisz (1970 *N. Engl. J. Med.* **282**: 909) demonstrated that bone resorption is optimal at pH below 7.4 and this study demonstrates that bone formation is optimal at pH above 7.4. (Supported by a grant from the John A. Hartford Foundation, Inc.)

21. Cytotoxic Properties of Streptococcal M Proteins. EDWIN H. BEACHEY,* PATRICIA W. BELEW,* AND GENE H. STOLLERMAN,** Memphis, Tenn.

Purified streptococcal M proteins produce cytotoxic effects upon platelets and polymorphonuclear leukocytes (PMN) in fresh human blood which can be neutralized by homologous M antibody only. M protein fractions prepared from Types 5, 6, 12, 24, 30, and 56 streptococci were mixed with samples of

fresh human blood. Immediately after mixing, phase-contrast microscopy revealed clumping of platelets. PMN surrounded the platelet clumps within 5 min. M24 caused minimal clumping at 0.1 μg/ml and lysed clumped platelets at higher concentrations. At 10 μg/ml, each M protein tested reduced PMN motility and inhibited phagocytosis. M protein produced similar effects upon washed platelets and PMN resuspended in fresh or heated serum, but not when resuspended in Tyrode's gelatin buffer. The effect of M protein on the motility of PMN in fresh blood samples could be estimated rapidly and simply by incubating centrifuged M protein–blood mixtures in glass capillary tubes and measuring PMN migration. With this technique, sigmoid dose-response curves were obtained for each serotype. The minimal inhibitory dose varied from 0.2 μg/ml (M24) to 2.5 μg/ml (M56). The magnitude of the cytotoxic effect was proportional to the purity of the M lots tested. Cytotoxicity was abolished by absorption of M protein with homologous, but not heterologous, M-type antiserum. Trypsin, but not lysozyme, digestion also abolished cytotoxicity. These results demonstrate toxic properties of highly purified M protein which are distinct from those of cell wall mucopeptide. Toxicity appears inseparable from the type-specific M determinant. Inherent toxic properties of M protein molecules rather than impurities, therefore, may account for the inflammatory effects of M protein vaccines. (Supported by grants from NIH and VA.)

22. Ventricular Tachycardia after Release of Coronary Artery Occlusion in Conscious Dogs and the Antiarrhythmic Effect of Atropine. G. DAVID BEISER,* DOUGLAS R. ROSING,* RICHARD B. KARSH,* AND STEPHEN E. EPSTEIN, Bethesda, Md. (introduced by Albert Sjoerdsma**).

Arrhythmias occurring after acute myocardial infarction have been generally attributed to persistent myocardial ischemia secondary to coronary artery occlusion. To evaluate the mechanisms and treatment of such arrhythmias, we have studied 15 closed-chest conscious dogs in which acute myocardial ischemia was produced by inflating a balloon catheter previously implanted around the left anterior descending coronary artery just distal to its first diagonal branch. Of the 15 dogs, eight did not develop arrhythmias during the 1st hr of occlusion. In seven of these eight, however, sudden *release* of occlusion was followed within 15 sec to 3 min by ventricular arrhythmias which progressed rapidly to ventricular tachycardia (VT). In all these dogs, reocclusion of the coronary artery abolished VT within 30 sec. Reocclusion for 10 min followed by successive periods of release and reocclusion consistently reproduced this phenomenon. In four of five dogs atrial pacing at rates of 105–130 was successful in overriding release-induced VT or preventing its appearance. Likewise, administration of atropine just before release of occlusion in doses that increased heart rate from an average of 76 ±6 (SE) to 112 ±3 prevented VT in all seven dogs. It may be inferred from these results that (*a*) sudden reperfusion of a previously ischemic region of myocardium, as may occur in man by lysis or dislodgment of a clot, may be responsible for some of the serious arrhythmias seen in acute myocardial infarction, and (*b*) increasing the heart rate by administration of atropine may be successful in the prevention of these arrhythmias.

23. Decreased Human Systolic Blood Pressure in Essential Hypertension by Operant Conditioning. HERBERT BENSON,* DAVID SHAPIRO,* BERNARD TURSKY,* AND GARY E. SCHWARTZ,* Boston, Mass. (introduced by Walter H. Abelmann**).

Persistent arterial hypertension had been induced in monkeys by behavioral means. These same monkeys were then trained to lower their mean arterial blood pressure by operant conditioning techniques. In the present study, operant conditioning techniques were employed to decrease systolic blood pressure in six patients with essential hypertension. Median systolic blood pressure was recorded by use of an automated constant cuff pressure system. Control blood pressures were measured on each of 5–15 separate days before the operant conditioning training began, representing in each patient the median systolic blood pressure of between 7500 and 22,500 heart beats. During the subsequent training sessions, the absence of a Korotkoff sound (which indicated that arterial blood pressure was less than cuff pressure) was reinforced with the appearance of a light and a tone. After each 20 reinforcing events, a photographic slide, equivalent to the award of a small sum of money, was shown to the patient. Median control systolic blood pressure of the six patients during the last five sessions before training was 167.4 mm Hg. During the last five conditioning sessions, median systolic blood pressure decreased to 151.1 mm Hg ($P < 0.05$). Systolic blood pressure decreased 33, 30, 17, 16, 3, and 0 mm Hg in the individual patients. Therefore, in patients with essential hypertension, systolic blood pressure can be significantly decreased by operant conditioning techniques. (Research supported by grants from NIH, NIMH, and Hoffman-La Roche, Inc.)

24. Inhibition of Red Cell Enzymes by 2,3-Diphosphoglycerate (2,3-DPG). E. BEUTLER, Duarte, Calif.

Recent investigations have emphasized the important role of 2,3-DPG in regulating the position of the oxygen dissociation curve of hemoglobin. It has also been found that 2,3-DPG inhibits diphosphoglyceromutase and there are conflicting reports regarding its inhibition of hexokinase. We have investigated the effect of physiological concentrations of 2,3-DPG on the activity of all of the glycolytic enzymes of the red cell. 2,3-DPG was found to inhibit hexokinase (Hx) competitively with adenosine triphosphate (ATP), phosphofructokinase (PFK) competitively with fructose-6-P, aldolase competitively with fructose-1-6-di-P, phosphoglucomutase competitively with glucose-1-6-di-P, and glyceraldehyde phosphate dehydrogenase (GAPD), noncompetitively. The K_i values for inhibition of these enzymes at pH 7.80 were found to be 1.5, 1.5, 1.5, 0.15, and 14 mmoles/liter respectively. Supplementation with additional magnesium only partially relieved inhibition of PFK, and no relief of inhibition was observed in the case of the other enzymes. No inhibition of any other glycolytic enzyme or of NADP glutathione reductase, NADPH or NADH diaphorase, glucose-6-P dehydrogenase, or 6-phosphogluconate dehydrogenase was found. NADH glutathione reductase activity was, however, inhibited, providing a possible explanation for the failure of the NADH-linked pathway of glutathione reduction to function in vivo. At physiological substrate concentrations

and at a free 2,3-DPG concentration of 4.5 mmoles/liter there would be about 75% inhibition of PGM, PFK, and aldolase, 50% inhibition of Hx, and 25% inhibition of GAPD. A regulatory effect of 2,3-DPG on several successive critical reactions of glycolysis before 2,3-DPG formation could form the basis of an effective method of regulation.

25. Increased Actomyosin Adenosine Triphosphatase (ATPase) in Hearts of Conditioned Rats. ASHOK BHAN* AND JAMES SCHEUER,* Pittsburgh, Pa. (introduced by Monto Ho).

Previous studies from this laboratory demonstrated improved function and contractility in isolated hearts from conditioned rats. In view of the evidence that actomyosin ATPase is related to myocardial contractility, hearts of 11 rats which were conditioned by swimming 150 min/day, 5 days/wk for 8 wk (CH) were compared with hearts of matched sedentary controls (SH). Heart to body weight ratios were 2.51 ±0.10 mg/g in SH and 3.13 ±0.11 mg/g in CH ($P < 0.01$). Actomyosin was isolated by extraction with 0.6 M KCl, 0.05 M Tris at pH 7.0 for 20 hr and by repeated precipitation with water and centrifugation at 13,000 *g*. Calcium-activated ATPase activity, studied in the presence of 10^{-2} M Ca^{++} and 180 mM K^+ at pH 7.6, was 18.7 ±1.3 μmoles P_i/g heart per min in SH and 25.0 ±0.9 μmoles/g per min in CH ($P < 0.01$). Magnesium-activated ATPase activity, studied in the presence of 10^{-3} M Mg^{++}, 60 mM K, and 10^{-4} M Ca^{++} at pH 6.8, was 1.85 ±0.07 μmoles P_i/g per min in SH and 2.65 ±0.27 μmoles/g per min in CH ($P < 0.05$). Inclusion of 10^{-3} M azide did not inhibit ATPase activity. Specific activity (μmoles P_i/mg actomyosin protein) of ATPase under both ionic conditions in seven pairs of CH and SH was also significantly elevated in CH ($P < 0.01$). The results suggest that, in rats, physical training and improved myocardial performance are accompanied by a rise in actomyosin ATPase. The increased specific activity of ATPase suggests that this may be due to a qualitative change in the contractile proteins. In view of the fact that actomyosin ATPase is depressed in myocardial hypertrophy due to chronic systolic overload, the present findings also suggest that myocardial hypertrophy found in physical conditioning, may be basically different from that found in pathological cardiac states. (Research supported by a grant from AHA.)

26. Human Plasma Very Low Density Lipoprotein (VLDL) Metabolism. DAVID BILHEIMER,* SHLOMO EISENBERG,* AND ROBERT I. LEVY, Bethesda, Md.

The metabolic fates of the several apoproteins recently identified in VLDL are unknown. To investigate VLDL apoprotein metabolism, we have labeled VLDL in the protein moiety with ^{125}I. 45–55% of the ^{125}I was isolated with an apoprotein identical with the principle protein of LDL (apoLDL), and 25–35% was attached to apoLP-ala and apoLP-glu, small proteins found also in high density lipoproteins (HDL). Upon incubation of VLDL-^{125}I with plasma, apoLP-ala and -glu rapidly exchanged between HDL and VLDL. apoLDL-^{125}I remained within VLDL. When isologous VLDL-^{125}I was injected intravenously in three normal and six hyperlipoproteinemic subjects, the same rapid redistribution of apoLP-ala and -glu between VLDL and HDL occurred. In all studies, ^{125}I in VLDL rapidly declined. Radioactivity sequentially appeared in the intermediate lipoprotein fraction of salt (d 1.006–1.019) (peak at 6–12 hr with rapid decline) and in LDL (d 1.019–1.063) (peak at 24 hr with slow decline). This sequence was accelerated by increasing VLDL lipolysis through heparin administration. In one patient with type I hyperlipoproteinemia, in whom lipolysis is deficient, these interconversions were markedly delayed. Analysis of the distribution of apoproteins-^{125}I revealed that the proportion of ^{125}I in VLDL associated with apoLDL declined to < 5% by 24 hr, and that found with apo-ala and -glu increased from 20–30% at time 0 to > 85% at 24 hr. This rapid disappearance of apoLDL-^{125}I from VLDL entirely accounted for the enrichment of the intermediate and LDL fractions. These studies indicate that some VLDL apoproteins (apoLP-ala, -glu) exchange readily between lipoproteins and persist longer in plasma than apoLDL. By contrast, apoLDL does not exchange but moves unidirectionally from VLDL to LDL in association with lipolysis.

27. Effect of Lipids on Growth Hormone Secretion in Humans. WILLIAM G. BLACKARD, EDGAR H. HULL, JR.,* AND ALFREDO LOPEZ-S,* New Orleans, La.

To determine the effect of elevations of plasma lipids on growth hormone secretion in humans, paired insulin hypoglycemia tests and paired arginine infusion tests were performed on eight and six normal female volunteers respectively. On 1 of the 2 test days for each growth hormone stimulus, subjects were given 60 g corn oil (Lipomul) 3 hr before testing followed by intravenous heparin (5000 U) at the time of insulin or arginine administration. Lipomul plus heparin administration inhibited both insulin- and arginine-induced plasma human growth hormone (HGH) elevations with almost complete suppression of the response to arginine. The plasma HGH inhibition was associated with elevation in plasma triglycerides and inhibition of plasma FFA depression after insulin or arginine. Neither the hypoglycemic response to insulin nor the blood glucose and plasma immunoreactive insulin (IRI) responses to arginine were altered by Lipomul plus heparin administration. In four additional subjects in whom Lipomul was given without heparin, the elevated plasma triglyceride values were not associated with suppression of arginine-induced plasma HGH elevations. This latter observation suggests that the elevation in plasma FFA is responsible for suppression of growth hormone secretion by Lipomul plus heparin. These studies indicate an important role of plasma FFA even at physiological concentrations in regulation of growth hormone secretion. (Research is supported by a grant from NIH.)

28. Beta Cell Activity in Insulin-Treated Diabetics. M. B. BLOCK,* A. H. RUBENSTEIN,* M. MAKO,* AND D. F. STEINER,* Chicago, Ill. (introduced by H. T. Ricketts**).

The discovery of proinsulin and recognition of its intracellular conversion to insulin and C-peptide, which are stored and subsequently secreted together, has provided, for the first time, alternative methods for monitoring β-cell function in insulin-requiring diabetics, in whom insulin antibodies hinder measurement of immunoreactive insulin (IRI). By use of an antiserum which reacts with antigenic determinants in human

C-peptide and this region in human proinsulin and its intermediates, we have measured C-peptide reactivity (CPR) in unextracted plasma and characterized its components. During oral glucose tolerance tests (OGTT) in five healthy subjects plasma CPR was 1.0 ±0.23 ng/ml fasting and peaked at 3.1 ±1.0 ng/ml (60 min). These values were positively correlated with IRI concentrations. CPR was not detected during OGTT's in five newly diagnosed, untreated diabetics with fasting blood sugars (FBS) > 200 mg/100 ml and unmeasurable IRI. Temporary recovery of β-cell activity in one of these patients, measured by rising CPR levels, correlated with clinical remission and withdrawal of insulin therapy. Plasma CPR was undetectable in five insulin-requiring (10–20 yr), ketosis-prone juvenile diabetics with insulin antibodies. In striking contrast, fasting CPR was 4.4 ±2.7 ng/ml in 9 of 12 insulin-treated adult onset diabetics (FBS > 150 mg/100 ml), and rose to 7.4 ±3.0 ng/ml 2 hr after glucose. The relative contribution of proinsulin and C-peptide to these levels was determined by gel filtration of acid-ethanol–extracted samples. The patients with high CPR were characterized by ease of control, and absence of ketosis and insulin reactions, whereas the clinical course of the remaining three patients, with undetectable CPR, resembled the ketosis-prone juvenile diabetics. These observations indicate that significant residual β-cell secretory activity is present in many insulin-requiring diabetics and is of importance in determining their lack of lability and clinical mangagement. (Supported by NIH Grant AM-13-941.)

29. Neutrophil Kinetics in a Tumor-Induced Leukemoid Reaction. D. R. BOGGS, E. MALLOY,* S. S. BOGGS,* AND R. E. LEE,* Pittsburgh, Pa.

5 days after subcutaneous transplantation of a breast carcinoma into CE mice, mature blood neutrophils began to rise (3000 to 118,000/mm³ by 18 days) and then remained stabile until death (blood change first described by Delmonte). Ratio of neutrophil concentration in 1st–20th drop of blood from the orbital sinus was 0.75 ±0.05 with tumor vs. 0.62 ±0.04 in controls. This, plus pathological observations indicating that vessels in lung, liver, and spleen contained a higher concentration of neutrophils than other organs, indicates marginated neutrophils were disproportionately increased as compared to circulating neutrophils. Total mature neutrophils and immature precursors washed from the humerus increased from 4.0 to 5.2 × 10^6 3 days after tumor transplant, by 5–7 days reached 7.4 × 10^6, remained stabile through 14 days (7.2 × 10^6), and then declined (5.4–4.7 × 10^6 on days 18–35). Tritiated thymidine, 10 mCi, was injected 14 days after transplant. Peak per cent labeled blood neutrophils occurred at 4 days in tumor-bearing and control mice, but the subsequent slope of decline was slower in tumor-bearing mice. This, plus the continuing rise in blood neutrophils after marrow expansion ceased, suggests prolonged neutrophil survival. Intraperitoneal endotoxin injection decreased neutrophils per humerus from 4.4 to 3.1 × 10^6 in controls (30% loss) and from 7.8 to 4.3 × 10^6 in 14-day tumor-bearing mice (45% loss). After such injection peritoneal neutrophil mobilization in controls was 7.0 ±0.05 × 10^6 vs. 9.3 ±2.6 × 10^6 with tumor. Thus, neutrophil migration into exudates is not appreciably increased simply because blood neutrophil concentration is increased. These results support suggestions that leukemoid reactions in man, characterized by extreme elevation of mature neutrophils, represent stimulation of orderly neutrophil production with resultant increased neutrophil survival and disproportionately increased marginated neutrophils. (Research supported by NIH and ACS.)

30. The Effect of Calcium Absorption upon Release of Gut Glucagon-Like Immunoreactivity (GLI): Evidence for Intestinal Influence upon Calcium Homeostasis. INGOLF BÖTTGER,* GERALD R. FALOONA,* AND ROGER H. UNGER,** Dallas, Tex.

Both Potts and Munson have suggested that gastrointestinal glucagon-like immunoreactivity (GLI) might play a role in homeostasis of ingested calcium by stimulating thyrocalcitonin release. To test this possibility, the effect of intestinal calcium absorption upon GLI release was studied in conscious dogs. Within 20 min after intraduodenal administration of 4.5 mmoles/kg of $CaCl_2$ (30% more than daily calcium requirements) mean GLI, measured by radioimmunoassay in inferior vena caval plasma, rose significantly ($P < 0.001$) from a base line of 2.2 ±0.2 ng/ml to 4.3 ±0.3 at 45 min as calcium rose. Pancreatic glucagon, measured by specific immunoassay, did not change, proving the nonpancreatic origin of the GLI. Insulin and glucose remained constant in all experiments. 2.25 mmoles of $CaCl_2$ per kg (30% less than daily calcium requirements) caused a smaller but significant rise from 2.0 ng/ml to 3.0 ng/ml ($P < 0.02$). In control experiments designed to exclude the possibilities that absorption of water, solute, or Cl might account for the effect, it was found that neither tap water nor amino acid solution raised GLI significantly; 4.5 mmoles/kg of $MgCl_2$ caused significantly less GLI release than $CaCl_2$ ($P < 0.02$). It is concluded that GLI is released after intraduodenal administration of $CaCl_2$ in dogs. It is possible that GLI plays a role in the homeostasis of ingested calcium, perhaps as an afferent limb of an "entero-parafollicular cell axis," which limits hypercalcemic change after large calcium loads by signaling the regulators of calcium *during*, rather than *after*, absorption of the magnitude of the incoming load. (Supported by NIH grant.)

31. Pharmacological Modification of Histamine-Mediated Airway Responses. A. BOUHUYS, J. S. DOUGLAS,* AND A. R. GUYATT,* New Haven, Conn.

In spontaneously breathing, unanesthetized guinea pigs, histamine airway constriction is inhibited by atropine and potentiated by propranolol (1968. *Clin. Res.* 16: 560). We now report that similar interactions occur in man. Histamine airway constriction was induced by aerosol inhalation (2 min) in three healthy subjects and five hemp workers. Measurements of flow rates on expiratory flow-volume curves (1969. *J. Clin. Invest.* 48: 1159) showed graded responses to increasing histamine doses. 90–120 min after 40 mg propranolol orally, similar histamine doses caused increased effects in all subjects (dose ratios 1.7–6.5). Similar experiments in one healthy subject and three hemp workers demonstrated protection against histamine bronchoconstriction by atropine (1 mg subcutaneously) (dose ratios 0.2–0.6). Propranolol also

potentiated (4/4 hemp workers and 2/3 healthy subjects), and atropine protected against (3/3 hemp workers) the airway constrictor effect of hemp dust exposure in hemp fiber processing factories (1970. *J. Clin Invest.* **49**: 106). This dust probably exerts its effect (chest tightness and decreased flow rates) through histamine release in the lungs. Thus, airway constriction induced by exogenous or endogenously released histamine in man appears to be subject to pharmacological modulation. The net effect of histamine appears to depend on the balance between vagal and sympathetic stimuli which impinge on airway smooth muscle. Since histamine and the autonomic transmitter substances are considered to act on different cell surface receptors, their interaction probably takes place within the cell. We speculate that this interaction may result from synergistic or antagonistic effects of the mediating substances on intracellular concentrations of cyclic 3',5'-AMP. (Supported by USPHS Grant EC-00159.)

32. Lymphocyte Cyclic Adenosine Monophosphate (AMP) Synthesis and Inhibition of Phytohemagglutinin-Induced Transformation. HENRY R. BOURNE,* LOIS B. EPSTEIN,* AND KENNETH L. MELMON, San Francisco, Calif.

Recent data has shown that cyclic AMP inhibits function of several formed elements of the blood: platelet aggregation, basophil histamine release, and neutrophil candidacidal activity. This study was designed to investigate the effect of cyclic AMP on transformation of lymphocytes. We examined the effect of several compounds on (*a*) cyclic AMP synthesis in pure suspensions of human lymphocytes (as measured by the conversion of adenine-^{3}H to cyclic AMP-^{3}H), and (*b*) the transformation of lymphocytes grown in short-term cultures either in the presence or absence of PHA (as measured by the incorporation of thymidine-^{3}H into DNA). The results demonstrated that although two prostaglandins (PGE_1 and PGE_2) and epinephrine stimulated cyclic AMP synthesis in resting lymphocytes they did not trigger transformation and DNA synthesis in cultures of lymphocytes prepared without PHA. Conversely, PHA had no detectable effect on lymphocyte cyclic AMP synthesis, although it did demonstrate its well known mitogenic effect on lymphocytes in culture. Furthermore, the presense of PGE_1 and PGE_2 (10^{-6} mole/liter), epinephrine (10^{-4} mole/liter), theophylline (10^{-4}–10^{-3} mole/liter), and dibutyryl cyclic AMP (10^{-4}–10^{-3} mole/liter) in cultures of lymphocytes which contained PHA resulted in a marked (50–100%) diminution in lymphocyte transformation. These results indicate, therefore, that cyclic AMP is not an intracellular mediator of the mitogenic effect of phytohemagglutinin. They suggest, however, that the inhibiting effect of prostaglandins, catecholamines, methylxanthines, and dibutyryl cyclic AMP on lymphocyte transformation might be mediated through cyclic AMP. (Research supported by grants from NIH.)

33. Leukocyte Cyclic Adenosine Monophosphate (AMP) Inhibits Antigenic Histamine Release. HENRY R. BOURNE,* LAWRENCE M. LICHTENSTEIN, AND KENNETH L. MELMON, San Francisco, Calif., and Baltimore, Md.

Recent inferential evidence, based on effects of catecholamines, methylxanthines, and dibutyryl cyclic AMP, suggests that cyclic AMP may regulate antigenic release of inflammatory mediators from leukocytes in vitro. As a more rigorous test of this hypothesis we have measured the ability of a series of prostaglandins, biogenic amines, and amine antagonists to stimulate synthesis of cyclic AMP by sonicated or intact preparations of human leukocytes, by use of radioactive adenosine triphosphate (ATP) or adenine as precursors. In addition we have examined the effects of these compounds on release of histamine produced by exposure of cells from pollen-sensitive donors to purified pollen antigens. For every compound or combination tested, the ability to stimulate synthesis of leukocyte cyclic AMP correlates, at comparable concentrations, with the ability to inhibit antigenic histamine release: PGE_1 and PGE_2 are almost equipotent (maximal effect at 10^{-6} mole/liter), while $PGF_{1\alpha}$ is inactive (up to 10^{-5} mole/liter). Exogenous histamine stimulates cyclic AMP synthesis and inhibits release of intracellular histamine (50% of maximum effect at 10^{-6} mole/liter). The order of potency of adrenergic agonists (isoproterenol $\geq$ epinephrine > norepinephrine > phenylephrine) is characteristic of effects mediated by a beta-adrenergic receptor (50% of maximal isoproterenol effect at 10^{-7} mole/liter). Propranolol completely abolishes the effects of the catecholamines, but not of the prostaglandins, while phentolamine antagonizes neither. Antigenic stimulation of histamine release can be separated into two phases by the presence or absence of divalent cations (Mg^{++} and Ca^{++}). Like the methylxanthines and dibutyryl cyclic AMP, agents which stimulate cyclic AMP synthesis act to inhibit histamine release in the first, or Ca^{++}-Mg^{++}–independent stage. These results indicate that intracellular cyclic AMP acts as a "second messenger" in leukocytes to inhibit antigenic release of histamine. (Research supported by grants from NIH.)

34. Control of Total Peripheral Resistance (TPR) in Malignant Nephrosclerosis and the Anephric State. JOHN D. BOWER,* THOMAS G. COLEMAN,* AND ALLEN W. COWLEY, JR.,* Jackson, Miss. (introduced by Herbert G. Langford**).

Nonuremic patients maintained on chronic hemodialysis for end-stage nephrosclerosis in the malignant phase of essential hypertension were studied before and after bilateral nephrectomy. Direct intraarterial blood pressure and cardiac outputs were performed through the arteriovenous (A-V) shunt. Before bilateral nephrectomy the mean arterial pressure averaged 145 mm Hg, the cardiac index was 1.86 liters/min per m^2 and the total peripheral resistance was 0.052 mm Hg/ml per min. Control of blood pressure could not be achieved with standard anti-hypertensive therapy. Minimal addition volume depletion by dialysis and ultrafiltration produced a hypotensive shock-like state. Isuprel infusion (0.05 μg/kg per min) produced a marked increase in cardiac output and a fall in total peripheral resistance. After bilateral nephrectomy the blood pressure fell, the cardiac output increased, and the total peripheral resistance decreased within a week. During a follow-up period the mean arterial pressure averaged 102 mm Hg, the total peripheral resistance averaged 0.023 mm Hg/ml per min, and the cardiac index averaged 3.07 liters/min per m^2. These changes are all statistically significant ($P < 0.02$). Plasma renin fell from high to low

levels and was significant. Previous studies from this laboratory (*Circulation.* **42**: 509) have shown that increasing weight in the anephric state by an average of 7% above optimal weight initially results in an increase in cardiac output and a rise in blood pressure. Subsequently the total peripheral resistance rises and the cardiac output falls toward normal. Clinically this is similar to the malignant phase of essential hypertension but it differs in that the cardiac output is not severely depressed and that it is readily reversible with volume control. These data suggest that tissue autoregulation of blood flow is operative in the anephric state but that it is overridden in the malignant phase of essential hypertension possibly by renin. The only way to control blood pressure in the patient with end-stage kidney disease in the malignant phase of essential hypertension is bilateral nephrectomy.

35. Potassium: Is It the Adrenal Glomerulotrophin? J. E. BOYD,* W. P. PALMORE,* AND P. J. MULROW, New Haven, Conn.

Our previous studies indicate that renin-angiotensin is not the major regulator of aldosterone secretion in the rat: (*a*) chronic angiotensin II infusions do not simulate the effects of sodium depletion upon aldosterone secretion; (*b*) nephrectomy does not lower the high aldosterone secretion of the sodium-depleted rat; (*c*) sodium depletion by dialysis increases aldosterone secretion in nephrectomized rats. Subtle changes in K must be able to stimulate the zona glomerulosa, since the chronically K-loaded rat requires the adrenal gland for adaptation, yet fasting plasma K is normal. Our recent data suggest that subtle changes in K balance also mediate the adrenal response to sodium depletion. (*a*) Small but significant increases in plasma [K] occur during sodium depletion. (*b*) K depletion prevents the aldosterone response to sodium depletion. (*c*) K loading mimics sodium depletion by increasing aldosterone secretion, by stimulating the last step in aldosterone biosynthesis, and by increasing zona glomerulosa width, and, in fact, plasma [K] is elevated in the potassium-loaded rat if measured while the rat is active at night. These effects of K loading can occur without any change in sodium or water balance, or in plasma renin activity. (*d*) The rat adrenal gland in vitro is capable of responding to such small increments in [K] (0.35 mEq/liter) with an increased aldosterone production. We hypothesize that K acts through activation of Na-K adenosine triphophatase leading to increases in intracellular K. We find this enzyme in the adrenal gland and its activity is greatest in the outer portions of the gland. Ouabain blocks the stimulating effect of K upon aldosterone production in vitro. These data suggest that intracellular potassium and not a decrease in membrane potential is critical in the regulation of cell growth and steroidogenesis in the zona glomerulosa. (Supported by NIH grants.)

36. Structure-Binding Relationships in Thyroxine-Binding Prealbumin. WILLIAM T. BRANCH,* HAROLD EDELHOCH,* AND JACOB ROBBINS,** Bethesda, Md.

Relationships between thyroxine binding and conformation were studied in a purified commercial preparation of human thyroxine-binding prealbumin (TBPA). Molecular weight of 53,500 by equilibrium sedimentation in water compared to 14,000 in 6 M guanidine suggested four subunits of identical size. Studies with UV circular dichroism and by fluorescence quenching of tryptophan revealed one thyroxine-binding site with $K_a \sim 10^8$ LM^{-1}. Affinity and number of sites were apparently unchanged between pH 10 and 7.4, whereas binding decreased significantly beyond these limits. 1-Anilino-8-naphthalenesulfonate (ANS) bound to TBPA with increased fluorescence quantum yield and with a 44 nm shift of fluorescence emission maximum toward the ultraviolet, characteristics which indicate nonpolarity of its binding site. From ANS titration curves, two binding sites with $K_a \sim 10^5$ LM^{-1} were calculated. Occupancy of the single thyroxine site displaced ANS competitively from both sites, thereby furnishing independent measurements of thyroxine affinity. Protein structure was studied by UV difference spectroscopy, intrinsic protein fluorescence, circular dichroism, and polarization of 1-dimethylaminonaphthalene-5-sulfonyl chloride (DNS) fluorescence. No change in molecular conformation could be detected over the pH range 3.5–12. Subunit structure remained intact in 8 M urea or 4.5 M guanidine at neutral pH and at pH 2.2 in aqueous solution. Our studies are compatible with a tetrameric structure of remarkable stability and with a single hydrophobic crevice for thyroxine binding. Changes in binding affinity do not appear to depend on conformational changes in the protein.

37. Decreased Pulmonary Oxygen Toxicity in Rats. RICHARD E. BRASHEAR* AND ROBERT E. DEATLEY,* Indianapolis, Ind. (introduced by Paul J. Fouts**).

The pulmonary pathology and death in respiratory failure that occurs after exposure to excessive amounts of oxygen is relatively unalterable. This is a study of altered pulmonary oxygen toxicity by pretreatment of Sprague-Dawley rats (333 ±33 g) with 10% oxygen and 90% nitrogen. 15 of 16 rats breathing > 99% oxygen at ambient pressure expired after 66 ±7 (mean ±SD) hr with gasping respirations. At autopsy there was extensive pleural fluid and the lungs were dark red-black in color. The single survivor was sacrificed at 192 hr. Six rats that initially breathed 10% oxygen for 120 hr all subsequently survived breathing > 99% oxygen and were sacrificed after 240 hr. 16 rats were placed in chambers with compressed air and 16 in chambers with 10% oxygen for 120 hr at which time eight compressed air–treated rats and eight 10% oxygen–treated rats were placed in > 99% oxygen. Seven of eight compressed air–treated rats in > 99% oxygen expired at 62 ±10 hr; one survivor was sacrificed at 336 hr. The eight rats pretreated with 10% oxygen and then placed in > 99% oxygen all survived and were sacrificed at 336 hr. Eight compressed air–treated rats and eight 10% oxygen–treated rats were maintained in compressed air for 336 hr and none expired. The 10% oxygen–treated rats placed in > 99% oxygen lost approximately 1/3 of their body weight during 336 hr of breathing > 99% oxygen. Pretreatment with 10% oxygen may induce enzymatic and/or subcellular changes that prevent the development of fatal pulmonary oxygen toxicity in rats.

38. Interrelationships of Aldosterone Excretion, Plasma Renin Activity, Serum Electrolyte Concentrations, Plasma Volume, and Arterial Pressure in Hypertension. EMMANUEL BRAVO,* HARRIET DUSTAN,** AND ROBERT TARAZI,* Cleveland, Ohio.

Elevated arterial pressure, abnormalities of plasma volume, and exaggerated natriuresis characterize the hypertensive state. Less well defined are abnormalities of the renin-aldosterone system. Since sodium deprivation and loading influence all these functions it seemed likely that any effects of manipulating sodium intake might reflect closely integrated responses of these pressure, volume, renal excretory, renal endocrine, and adrenal steroid systems. To investigate such interrelationships, nine hypertensive patients were studied during 7 days of a 9 mEq sodium diet supplemented during the last 3 days by a 0.9% NaCl infusion (25 ml/kg). Measurements made before and after 4 days of sodium deprivation and 3 days of loading included arterial pressure (MAP), plasma volume (PV), serum Na and K, plasma renin activity (PRA), and aldosterone excretion (AER). Sodium excretion during loading was expressed as per cent of amount given (%NaEx). Rise in AER with deprivation was directly related to PV reduction ($r + 0.76$, $P < 0.01$); the ability to excrete a salt load (%NaEx) after deprivation was inversely related to MAP reduction ($r - 0.635$, $P < 0.05$), PRA increase ($r - 0.692$, $P < 0.05$), and actual level of AER ($r - 0.591$, $P < 0.05$). Although %NaEx during loading did not correlate with AER, suppression of AER was inversely related to changes in serum K ($r - 0.603$, $P < 0.05$). Correlation coefficients among the other parameters strongly suggested close functional interrelationships although the group was too small for coefficients to be statistically significant. These data strongly suggest that AER and PRA responses to manipulation of sodium intake in hypertensive patients are dependent on arterial pressure and plasma volume levels achieved, as well as serum electrolyte concentrations, and that the handling of sodium during these manipulations is a function of all these factors. (Research supported by grants from NIH and AHA.)

39. Total and Free Triiodothyronine (T3) in Health and Disease. MILTON A. BRENNER* AND KENNETH STERLING,** New York.

The free thyroxine concentration of serum has been found to be closely correlated with clinical status and hormone turnover. The importance of T3 in thyroidal economy has been demonstrated with the availability of methodology for its determination in serum. The T3 fractions of sera have been measured by equilibrium dialysis and magnesium precipitation, as described for the determination of free T4, except that T3-^{125}I was added to sera. In normal sera the free T3 percentage averaged 0.27%, approximately 7 times as great as the mean normal fraction of free T4. The absolute free T3 concentration was given by the product of the free fraction and the total T3 concentration; the mean normal value was 612 pg/100 ml ±152 (SD). In sera from subjects in the last trimester of pregnancy, an essentially normal absolute free T3 value (627 pg/100 ml ±76) was computed since it represented the product of an increased total T3 concentration and a diminished free fraction. In thyrotoxicosis the mean absolute free T3 value was 2175 pg/100 ml ±1289 (SD) and in frank hypothyroidism the mean was 129 pg/100 ml ±61 (SD). In male subjects who received estrogen there was a rise in total T3 concentration concomitant with elevation of TBG capacity. Conversely, androgen administration with diminished TBG capacity was associated with reduction of the total T3 concentration. Despite the weaker binding of T3 by TBG in comparison with its binding of T4, it was concluded that TBG plays a significant role in the determination of diffusible T3, which is believed to be the active form of the hormone.

40. Effect of Local Anesthetics on Insulin Secretion by Pancreatic Pieces. RUBIN BRESSLER AND KLAUS BRENDEL,* Tucson, Ariz.

Because the pancreatic islets are innervated by parasympathetic and sympathetic fibers, and in vitro insulin secretion is calcium dependent, a study was undertaken to ascertain the effect of local anesthetics (LA) on glucose- and β-methylcholine (mecholyl)–stimulated insulin secretion by pancreas pieces. The results showed that different classes of LA affected insulin secretion differently. Nonantiacetylcholine type LA with uncharged amino groups (Holocaine, propranolol) depressed glucose-induced insulin secretion progressively as their concentrations were raised (10^{-3} to 1 mmole/liter). The inhibition was more marked at lower calcium concentrations (3.0 vs. 0.5 mmoles/liter). The antiacetylcholine type LA (mepivicaine, lidocaine) with charged tertiary amino groups had complex effects on insulin secretion. At low concentrations (10^{-4} mmole/liter) they acted as anticholinergic agents in their inhibitions of mecholyl-stimulated insulin secretion. Complete blockade of mecholyl-stimulated secretion was found at calcium concentrations of 0.5 and 3.0 mmoles/liter. This concentration of LA did not, however, depress glucose-induced insulin secretion. At LA concentrations of 10^{-3} mmole/liter inhibition of glucose-stimulated insulin secretion occurred, and was more marked at lower calcium concentrations (3.0 vs. 0.5 mmoles/liter). At still higher LA concentrations (0.05–1.0 mmole/liter) the inhibitory effect on glucose-induced insulin secretion diminished, disappeared, and became a stimulatory effect, which was abolished by the addition of atropine. These data suggest that LA inhibit insulin secretion in response to glucose and mecholyl in different ways. The inhibition of mecholyl-stimulated insulin secretion was not reversed by raising the calcium concentration, whereas the higher concentrations of calcium lessened the inhibition of glucose-stimulated insulin secretion due to both Holocaine (no charged nitrogen) and mepivicaine and lidocaine (charged nitrogens). LA with charged nitrogens behaved both as anticholinergic agents and cholinergic agonists, whereas Holocaine showed neither action. (NIH HE 13636, AM 14977.)

41. Effects of Dietary Cholesterol on Sterol Synthesis and Release by the Perfused Rat Liver. LEE A. BRICKER,* Miami, Fla. (introduced by W. J. Harrington**).

The interaction between dietary cholesterol and hepatic sterol synthesis may have major implications in the genesis of atherosclerosis. A new approach to hepatic sterol metabol-

ism, utilizing an *in situ* perfusion system, was employed in this study. It was designed to permit comparison of the effects of low (LCD) and high cholesterol diets (HCD) on independently assessed hepatic synthesis of sterols and their release to blood and bile. Newly synthesized (NS) digitonin-precipitable sterol was released to the perfusion medium ("blood"), excreted into a bile cannula, or retained in liver; the relative proportions of these sterol pools were assessed, as were appearance times and synthesis rates of NS sterol in blood and bile. NS bile acid was similarly monitored in bile. Rats were kept on diet for 3–4 wk before study. After the 3-hr perfusion liver concentrations of free and total sterols and their specific activities were determined. HCD rat livers released 2–4 mμmoles/hr of NS sterol into "blood" during perfusion; by contrast, LCD rat livers released 150–600 mμmoles/hr. No NS sterol appeared in "blood" until 35 min after acetate-^{14}C infusion had begun. All livers contained 7–12 × the amount of NS sterol (65–95% esterified) as did "blood" at 3 hr. NS biliary sterol accounted for < 0.5% of total NS sterol by LCD rat livers, but up to 6% of that by HCD rat livers, there being no reflection of dietary influences, in contrast to the marked suppression by HCD of NS sterols in liver and "blood." Further, NS bile acid in bile similarly failed to evidence suppressibility by HCD, but consistently was 75–200 × greater than NS sterol in bile, and appeared in bile before NS sterol was detectable either in bile or "blood." The data suggest that the liver stores most of its NS sterol regardless of diet, and that the striking suppressive effect of HCD on hepatic sterol synthesis is closely reflected in circulating blood. Further, biliary sterols and bile acids appear to be synthesized from acetate via an independent pathway not inhibited by dietary cholesterol.

42. Effect of Hydrochlorothiazide on Calcium Metabolism. ARNOLD S. BRICKMAN,* JACK W. COBURN,* MARCEL KOPPEL,* MUNRO PEACOCK,* AND SHAUL G. MASSRY,* Los Angeles, Calif. (introduced by Seymour Dayton**).

In man, thiazide diuretics increase serum calcium and cause hypocalciuria. Although hemoconcentration and decreased urinary losses may contribute to the former, thiazide-induced hypercalcemia occurs in patients with negligible renal function. The hypocalciuria has been ascribed to enhanced tubular calcium reabsorption caused by diuretic-induced volume depletion. Alternatively, augmented secretion or action of parathormone could produce both effects. Studies were undertaken to evaluate the effect of hydrochlorothiazide (200 mg/day × 4) on serum and urinary calcium in relation to the presence or absence of (*a*) parathormone and (*b*) volume depletion. Subjects included normals, hyperparathyroid, and vitamin D–treated hypoparathyroid patients receiving normal sodium diets. In other normals, daily sodium losses during thiazides were quantitatively replaced; in other studies, the effect of comparable sodium losses produced by furosemide was evaluated. Mean serum calcium and phosphorus levels rose similarly and significantly in all thiazide treatment groups. Although marked sodium losses occurred in those receiving normal sodium, significant hypocalciuria and phosphaturia developed in normal and hyperparathyroid but not in hypoparathyroid patients. Furosemide administration augmented urinary calcium despite sodium losses and volume depletion; serum calcium was unchanged. With replacement of sodium losses, urine sodium was 3–4 times control throughout thiazides and urinary calcium did not fall. Immunoreactive parathormone levels did not change consistently when such measurements were made. These observations indicate that increases in serum calcium and phosphorus caused by thiazides do not depend upon parathormone release, occur in the hormone's absence, and can develop independent of hypocalciuria. Thus, direct skeletal action(s) of thiazide is plausible. Volume depletion may be implicated in contributing to hypocalciuria, but present studies suggest that this thiazide effect is also dependent upon the presence of parathormone.

43. Thyrotoxicosis: a Disease of Defective Thymic-Dependent Lymphocytes. JEROME I. BRODY AND SIGMUND GREENBERG,* Philadelphia, Pa.

The purpose of this study was to define the in vitro and in vivo immune responses of lymphocytes from patients with thyrotoxicosis with the idea that their potential aberrant behavior might provide an immunogenic basis for this endocrine disorder. Lymphocytes from treated and actively hyperthyroid patients were placed in cell culture in duplicate sets of two vials each in order to concurrently assay the biochemically interdependent reactions of lymphocyte carbohydrate metabolism and DNA synthesis. The first culture of each group was left as an unstimulated control while the second was provoked with phytohemagglutinin. After a 48 hr culture period, glucose-1-^{14}C was added to the first group of cultures, and generated $^{14}CO_2$ and separated radioactive glycolytic intermediates were quantitated as indicators of sugar utilization via the hexose monophosphate shunt and Embden-Meyerhof pathways, respectively. The second group of cultures received thymidine-^{14}C as a DNA precursor, and, after suitable incubation, polynucleotides were precipitated with trichloroacetic acid. All counting was done by scintillation spectrometry. As an in vivo experimental counterpart, all patients were given sensitizing and test doses of DNCB as a model antigen assaying delayed hypersensitivity. Lymphocytes from all patients responded normally to provocation with phytohemagglutinin by concordantly augmenting glucose consumption and DNA synthesis. Nevertheless, no patient, regardless of his clinical state, developed a positive reaction to DNCB. This discrepant in vitro and in vivo reactivity, also observed in primary lymphoid disorders such as the Wiskott-Aldrich syndrome, infers that uncontrolled growth and activity of thyroid epithelium, evolving ultimately to thyrotoxicosis, may occur because of defective immune surveillance and inherently distorted function of the thymic-dependent lymphocyte system. (Supported by grants from NIH.)

44. Hormone-Induced Lung Growth. JEROME S. BRODY,* WILFRIDO J. BUHAIN,* AND MARGARET J. OPPENHEIMER,* Philadelphia, Pa. (introduced by Ronald F. Coburn).

To study the effect of growth hormone excess on lung growth in the adult, an MtTF4 tumor which secretes growth hormone, prolactin, and adrenocorticotrophic hormone (ACTH) was transplanted into 11-wk-old female rats. These

rats, control rats, and tumor-bearing adrenalectomized rats were sacrificed 6 wk later and studies of organ weight, total lung capacity (TLC), lung compliance (C_L), and analysis of lung deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein were performed. Calculations of lung cell numbers were made from total lung DNA and alterations in lung cell size were evaluated with RNA:DNA and protein:DNA ratios. Alveolar numbers and alveolar size were calculated by morphometric techniques. Comparing tumor-bearing rats to control rats, there were significant increases in body weight, 283 ±26 g (2 SEM) vs. 161 ±9 g, and body length, 39 ±0.6 cm vs. 36 ±0.8 cm, with visceromegaly of all organs. In tumor-bearing rats, lung weight was significantly increased, 1.10 ±0.06 g vs. 0.86 ±0.04 g, as was TLC, 15.8 ±0.2 ml vs. 11.2 ±0.2 ml, and C_L, 0.89 ±0.09 ml/cm H_2O vs. 0.62 ±0.06 ml/cm H_2O. There were no changes in lung elastic recoil. The number of lung cells was significantly increased in tumor-bearing rats, 139 × 10^6 cells vs. 105 × 10^6 cells, but there was no change in the size of lung cells. The increase in lung size was associated with an increase in average alveolar size, 0.178 ±0.010 μμl vs. 0.132 ±0.018 μμl, but no increase in alveolar numbers. Adrenalectomy did not alter the tumor affect on lung growth. These studies show that the rat lung is capable of accelerated growth during adult life. This lung growth, apparently induced by growth hormone excess, is characterized by enlargement of existing alveoli produced by cellular hyperplasia. (Supported in part by NIH Grant HE-11767.)

45. Effects of Arvin on Platelets. C. H. BROWN,* W. R. BELL,* D. P. SHREINER,* AND D. P. JACKSON,** Baltimore, Md.

Thrombin induces biochemical and morphologic changes in platelets, presumably initiated by hydrolysis of a protein of the cell surface. Thrombin clots fibrinogen and releases fibrinopeptides A, AP, AY, and B. Arvin, an enzyme from Malayan pit viper venom, clots fibrinogen and releases fibrinopeptides A, AP, AY, but not B. The effects of arvin and thrombin on platelets have been compared. Clots formed by addition of arvin (5 U/ml) to citrated, human platelet-rich plasma did not retract. Arvin, in the presence of Ca^{++}, induced delayed aggregation of washed platelets, but other morphologic changes of viscous metamorphosis observed in thrombin-induced aggregation did not occur. Unlike thrombin, arvin did not cause release of 5-hydroxytryptamine, adenosine triphosphate, adenosine diphosphate, or K^+ from platelets. Lysates of washed platelets clotted in 3 min after addition of 5 U/ml of arvin or thrombin. Lysates of platelets preincubated with arvin in the absence of Ca^{++} were clotted by thrombin in > 6 min. Platelets preincubated with arvin aggregated with subsequent addition of thrombin and Ca^{++}, and caused retraction of thrombin-induced clots. Thrombocytopenia did not occur in six rabbits during 7 days of arvin-induced hypofibrinogenemia (fibrinogen levels 0–5 mg/100 ml). The rate of appearance and disappearance of labeled, circulating platelets was similar to controls when methionine-^{75}Se was injected 24 hr after induction of hypofibrinogenemia. These data suggest that if fibrinogen is a principal substrate for thrombin in its action on platelets, fibrinopeptide B must be cleaved for the entire complex of changes to occur; alternatively, hydrolysis of other proteins, e.g. factor XIII, which are unaffected by arvin may be involved. (Supported by NIH Grants HE-01601 and T1-AM-5260).

46. Marrow Response to Neutropenia during Hemodialysis. L. H. BRUBAKER* AND K. D. NOLPH,* Columbia, Mo. (introduced by C. E. Mengel).

Profound transient neutropenia is observed early in hemodialysis. The present studies were performed during seven coil dialyses (six cellophane, one cuprophane) in five patients with chronic renal failure to determine if rapid recovery reflects remobilization of sequestered N and/or if marrow reserves are utilized. Mean N count before dialysis was 3650 ±414 SEM (cells/mm^3) and by 30 min from the start of dialysis decreased ($P < 0.01$) in all studies to a mean low of 82 ±19. By 1½–3½ hr, N count invariably exceeded predialysis values with a mean high of 5744 ±856 ($P < 0.01$). In addition, one infected patient (B. E.) was studied twice (mean N counts: pre = 11,700, low = 1980, rebound 19,800). In five studies (not B. E.) 500 ml of blood was removed, the WBC labeled with DFP^{32} and reinjected 4–6 hr before dialysis. WBC specific activity (SA) was monitored thereafter until and throughout dialysis. The most remarkable finding was a steep decline in SA accompanying the decrease in N count early in dialysis. SA increased promptly as N count rebounded but peaked at levels less than predicted by normal kinetics. Essentially all labeled cells returned to circulating blood in ⅗; in ⅘, all but 20–25% returned. Dilution of SA with unlabeled cells occurred at the rebound peak. During the latter half of dialysis, labeled N disappearance was normal or slightly accelerated. These results show: (*a*) remobilization of most or all of sequestered N during recovery; (*b*) additional mobilization of a nonlabeled N pool (probably marrow), perhaps stimulated by transient leukopenia. They suggest that the N count is under immediate regulatory control by changes in marrow release.

47. Angiotensin II Vascular Receptors: Their Activity in Relationship to Sodium Balance, the Autonomic Nervous System, and Hypertension. HANS R. BRUNNER,* PAUL CHANG,* RONALD WALLACH,* JEAN E. SEALEY,* AND JOHN H. LARAGH,** New York.

During intravenous administration of varying doses of angiotensin II antibody to anesthetized rats we believe that we have discovered specific vascular receptors which compete with administered antibody to bind circulating angiotensin. This competitive phenomenon has been used to evaluate the affinity of these receptors for angiotensin. The apparent receptor affinity may be defined by the amount of antibody required to block the blood pressure response to exogenous angiotensin. Thus, when a large amount of antibody is required, the affinity of the vascular receptors is considered to be large. It was found that the receptor affinity varies directly with sodium intake, since the amount of antibody required was eightfold greater in normal rats on a high as compared with a low sodium intake. This sodium dependency was also demonstrated in renal hypertension, in nephrectom-

ized and in 11-deoxycorticosterone (DOC)-treated rats. Ganglionic blockade with pentolinium also increased antibody requirement eightfold. All of these changes were consistent, with no overlap observed in responses of individual animals from different groups. These observations may explain the known variation in pressor activity of angiotensin associated with changes in salt balance and ganglionic blockade. Receptor affinity was also greatly increased by experimental hypertensions. The amount of antibody required was enhanced in renal (twofold), DOC (fourfold), and genetic (fourfold) hypertension. The changes in apparent receptor affinity were not always related to measured endogenous angiotensin II levels. Accordingly, changes in the affinity of these receptors may be critically involved in normal blood pressure control and in the maintenance of various forms of experimental and clinical hypertension, even when circulating angiotensin II levels are normal. (Supported by NIH Grant HE-01275.)

48. Evidence for a Common Saturable Removal System for Dietary and Endogenous Triglyceride in Man. JOHN D. BRUNZELL,* DANIEL PORTE, JR., AND EDWIN L. BIERMAN, Seattle, Wash.

If lipoprotein lipase (LPL) is a saturable rate-limiting step for plasma triglyceride removal, then triglyceride concentration should increase disproportionately in relationship to increases in triglyceride turnover rate. When serial heparin triglyceride turnover measurements were performed in hypertriglyceridemic subjects ($n = 10$) on fat-free diets in isocaloric, hypercaloric, and hypocaloric states, a curvilinear relationship consistent with saturation kinetics was found between triglyceride turnover rate and concentration in each individual. Further, if dietary and endogenous triglyceride are removed in common by LPL, the presence of fasting chylomicronemia could be determined largely by changes in endogenous triglyceride levels. Selective alteration of endogenous triglyceride levels was accomplished by altering amount of dietary carbohydrate, keeping dietary fat constant. Subjects with endogenous lipemia (type IV pattern) overfed with carbohydrate developed higher triglyceride levels associated with fasting chylomicronemia (type V pattern). Conversely, lipemic subjects with fasting chylomicronemia cleared chylomicronemia with restriction of carbohydrate calories, despite constant dietary fat. This implicates a common saturable removal mechanism for dietary and endogenous triglyceride; types IV and V lipoprotein patterns relate to degree of saturation of this system. When lipemic subjects (n = 38) with normal postheparin lipolytic activity (PHLA) were arbitrarily divided by triglyceride turnover into two groups based on LPL removal V_{max} (independent of lipoprotein patterns), the group with higher V_{max} (endogenous lipemia) sustained circulating PHLA normally during 4–6 hr constant high dose heparin infusion, while group with lower V_{max} ("mixed lipemia") failed to sustain PHLA level ($-$ 31%, $P < 0.005$, endogenous vs. mixed), suggesting depletion of available tissue enzyme. Thus, triglyceride removal mechanisms appear to be saturable and involved in clearing of both endogenous and dietary triglyceride. Depletion of this system may characterize mixed lipemia. (Research supported by VA and NIH grants.)

49. Abattoir-Associated Brucellosis: Clinical and Immunologic Aspects. THOMAS M. BUCHANAN,* LUKE C. FABER,* AND STANLEY L. HENDRICKS,* Atlanta, Ga. (introduced by Richard M. Krause).

Brucellosis in the United States has become a predominantly abattoir-associated disease. These studies on the clinical features of brucellosis and the influence of naturally acquired immunity and resistance to the disease were done with employees from two abattoirs. Abattoir A had 203 cases in the period of 1960–1970; 53 of these were in 1970. Abattoir B had 323 cases in the same 11 yr. Patients at abattoir A were evaluated for signs, symptoms, severity of disease, antibody response, complications, reinfection, recrudescence, and effectiveness of treatment. Though initial symptoms were similar to those in cases reported by other investigators, the illness was less severe as measured by incidence of lymphadenopathy, splenomegaly, complications, and chronic disease. Only 19% of the 1970 patients had lymphadenopathy and 11% palpable splenomegaly. In none of the 203 patients were there complications, recrudescence, or chronic disease, and no deaths occurred. This may be due partly to early diagnosis and vigorous treatment. The median duration of illness before initiation of treatment was 13 days. Abattoir B employees who had serological evidence of prior subclinical brucellosis were observed for resistance to clinical infection. 85 persons who had not been clinically ill, but had been seropositive ($\geq$ 106) 5–15 yr previously, were matched with seronegative controls of the same sex, work location, and duration of employment. Both groups were followed from 5 to 15 yr. Clinical disease developed in only 2 of the 85 individuals who previously showed no signs of illness and were seropositive. However, 14 of 85 controls who were previously seronegative developed brucellosis ($x^2 = 8.35$, $P < 0.005$).

50. Importance of Nuclear Androgen Retention for Hormone Action. LESLIE P. BULLOCK,* RICHARD J. SHERINS,* AND C. WAYNE BARDIN,* Hershey, Pa., and Bethesda, Md. (introduced by Graham H. Jeffries).

The Stanley-Gumbreck male pseudohermaphroditic (Ps) rat is a rodent counterpart of testicular feminization. This rat has an inherited disorder of sexual development characterized by inguinal testes, a male genotype, but a female phenotype. Normal androgen-dependent differentiation does not occur due to end organ insensitivity to testosterone. Elucidation of the molecular basis of this genetic defect would help define the mechanism of androgen action. Androgen physiology of the Ps rat was therefore investigated. Testosterone enanthate (0.3 mg/day) stimulated DNA, RNA, and protein synthesis in preputial glands of normal rats but a 200-fold greater dose was required to produce a comparable effect in Ps animals. A similar dose-related response was observed in kidney and liver. Since androgen-dependent control of DNA, RNA, and protein synthesis were all affected, a defective pretranscriptional regulatory mechanism in the Ps rat was postulated. To investigate this possibility in vivo and in vitro testosterone metabolism was studied. Androgen insensitivity was not correlated with abnormal plasma steroid clearance or cellular uptake. Similarly, conversion of testosterone to dihydrotestosterone by preputial gland minces,

cytoplasm, and isolated nuclei was normal. By contrast, nuclear uptake and retention of dihydrotestosterone in liver and preputial gland of Ps rats were markedly reduced. In the normal rat progesterone stimulated preputial gland growth independent of its metabolism to androgens. The Ps animals, however, were unresponsive indicating that their steroid insensitivity was not limited to androgens. Glucocorticoid metabolism and estrogen response were normal. In conclusion: (*a*) androgen insensitivity in the Ps rat is correlated with inability to concentrate dihydrotestosterone at its active site in the nucleus; (*b*) androgen action is initiated in many tissues by a common mechanism regardless of whether the response is generalized growth or selective stimulation; and (*c*) androgens and progestins may share a common effector mechanism in some tissues.

51. Transfer Factor Therapy in Lepromatous Leprosy: an Evaluation. WARD E. BULLOCK,* JAMES FIELDS,* AND MICHAEL BRANDRISS,* Rochester, N. Y. (introduced by J. W. Hollingsworth).

Transfer factor (TF) or whole leukocytes from donors with delayed hypersensitivity to antigens of *Mycobacterium leprae* were employed in an effort to reconstitute delayed allergy in nine patients with lepromatous leprosy who were anergic to these antigens. A mean of 4.1×10^8 lymphocytes was given to five patients and four received TF from equivalent cell numbers. A 0.1 ml "local" injection was given intradermally on the forearm and the remaining volume (1.4–2.3 ml) delivered subcutaneously to deltoid sites. 1–6 days posttransfer, six of nine patients experienced erythematous and indurative changes within leprous skin infiltrates. By day 12, these exacerbated lesions regressed. Simultaneously, erythema nodosum was precipitated in four patients, and fever and arthralgias in three. Within 7 days posttransfer, five patients converted from anergy to weak allergic reactivity as measured by 48-hr skin test reactions to *M. leprae* antigens. Test reactions at "local" transfer sites and at "remote" sites were of equal magnitude. A sixth patient became skin test–positive at the "local" site only, reverting to negative 7 days later. Biopsies of skin tests performed pre- and posttransfer in three patients showed increased perivascular lymphocytic infiltration in two posttransfer specimens. Repeated lymphocyte cultures from the nine patients after transfer demonstrated no increase of thymidine-^{3}H uptake when incubated with antigens of *M. leprae*. All patients have been observed 1–3 yr after transfer experiments. As yet, neither dramatic deterioration nor improvement has been noted. The results indicate that TF may stimulate transient immunologic reactivity of low magnitude in lepromatous leprosy. The therapeutic promise of TF in single dosage appears limited. (Research supported by NIH Grant AI-07964.)

52. Role of Ribosomal Proteins in Genetic Control of Mammalian Protein Synthesis. EDWARD R. BURKA AND STEPHEN I. BULOVA,* Philadelphia, Pa.

In several mammalian cell lines free and membrane-bound ribosomes selectively synthesize different proteins. The mechanism by which a particular mRNA recognizes and interacts with a specific type of ribosome is unknown. In the mammalian erythroid cell, the two major classes of protein, globin and nonglobin protein (NGP), are synthesized predominently on free ribosomes and ribosomes bound to the cell membrane, respectively. The present studies have utilized this system to determine whether integral ribosomal components play a role in determining specificity in protein synthesis. Free and membrane-bound reticulocyte ribosomes were prepared in the presence of 0.2% deoxycholate. Free ribosomes (protein:RNA 1.9) incorporated 70 ±7% of valine-^{14}C into soluble globin peptides in the cell-free system, while the soluble protein synthesized by membrane-bound ribosomes (protein:RNA 4.7) was more than 90% NGP. Ribosomal proteins, solubilized in detergent and 0.1 M salt, were electrophoresed on 7.5% polyacrylamide gels and the patterns compared with each other and those of globin and solubilized erythrocyte membrane proteins. Neither type of ribosome was contaminated with globin. Membrane-bound ribosomes yielded 19 protein fractions, 7 of which appeared to be of membrane origin. 10 distinct protein fractions of membrane-bound ribosomes were identified which were not present in free ribosomes. Free ribosomes yielded more than 30 protein fractions, only 10 of which were electrophoretically identical with proteins of membrane-bound ribosomes. Differences in the RNA of the two types of ribosomes could not be discerned by polyacrylamide electrophoresis. The finding that the two types of ribosomes in the mammalian reticulocyte, each of which predominently synthesizes a different class of protein, have different protein constituents and similar RNA suggests that ribosomal protein in some manner determines the specific mRNA which attaches to the polyribosome complex. (Supported by NIH.)

53. Prediction of the Response of Acute Myeloid Leukemia to Cytosine Arabinoside. C. P. BURNS,* S. A. ARMENTROUT,* AND R. L. STJERNHOLM,* Cleveland, Ohio (introduced by O. D. Ratnoff**).

Cytosine arabinoside is an anti-metabolite used in the treatment of acute myeloid leukemia, which prevents mitosis by inhibition of DNA synthesis. 11 adults with this disease received 15 courses of cytosine arabinoside therapy (4 mg/kg per day by 8 hr infusion) until bone marrow remission or intolerable toxicity occurred. DNA synthesis was measured in the patients' peripheral blood leukocytes, and incubated in serum obtained before and 2 hr after the first cytosine arabinoside infusion was begun. The clinical response of treated patients could be related to the degree to which the serum obtained during therapy inhibited DNA synthesis. When DNA synthesis was depressed by 75% or more, each of four patients had peripheral blood and bone marrow remissions. In contrast, none of three patients in whom DNA synthesis was depressed less than 50% achieved bone marrow remission. The absolute number of immature forms in the blood fell twice as rapidly in the group in which DNA synthesis was depressed more than 75%, as compared to the group in which DNA synthesis was depressed less than 50%. Eight patients comprised an intermediate group in which DNA synthesis was depressed between 50% and 75%. Four of these patients achieved remission. Thus, the measurement of DNA synthesis by leukocytes from patients with acute myeloid leukemia

before and during the first infusion of cytosine arabinoside may provide a clue to the effect of this anti-leukemic therapy. (Supported by ACS Grants CI-1 and T-485A.)

54. Specific Removal of Antibody and Antigen In Vivo. JEAN-CLAUDE BYSTRYN,* ISAAC SCHENKEIN,* AND JONATHAN W. UHR, New York.

A solid-phase immunoadsorbent was developed by suspending in an agar-gel bromoacetyl cellulose (BAC) coupled to BSA or rabbit IgG with anti-BSA activity. In vitro experiments showed that binding of antibody or antigen to the respective immunoadsorbent in gel was highly specific, and that labeling of the immunologic reagents with radioiodine did not affect their binding to the immunoadsorbents. Purified rabbit anti-BSA-^{125}I was removed in vivo from four passively immunized rabbits by extracorporeal circulation of their blood through columns lined with BAC-BSA-^{131}I in agar gel. After 30–60 minutes of circulation, 79–83% of the circulating anti-BSA antibodies had been removed by this procedure, whereas the level of a control antibody to bacteriophage was decreased by only 8–14%. Sacrifice of the animals after the procedure revealed that no measurable ^{131}I radioactivity had been released from the immunoadsorbent (10^8 cpm) into their blood or organs. In a similar experiment, BSA-^{125}I previously injected into two rabbits was specifically removed by extracorporeal circulation through columns lined with BAC–anti-BSA in agar gel. Thus, this technique allows the rapid and specific removal of either antibody or antigen. Therefore, it may be possible to remove from the circulation unwanted antibodies such as auto-antibodies responsible for immune disease or "enhancing" antibodies in certain tumors, as well as other molecules, provided antibody can be formed to them. (This work was supported by grants from USPHS AI-0834, TI-AM-5326, NSF GB-7473-X, and USAMRDC DADA 17-69-C 9177.)

55. Phosphatidic Acid Metabolism in Human Platelets. FRANK L. CALL II* AND WILLIAM J. WILLIAMS, Syracuse, N. Y.

Phosphatidic acid is an early intermediate in platelet phospholipid metabolism. Therefore, two enzymes (phosphatidic acid phosphatase and diglyceride kinase) which could be active in the turnover of this substance were examined in human platelets. Platelet suspensions were prepared from freshly drawn human blood (anticoagulated with acid-citrate-dextrose) by differential centrifugation at room temperature. The enzymes were demonstrated in intact platelets but were considerably more active in platelets disrupted by freezing and thawing or by sonication. Both enzymes were particulate bound, and the experiments summarized here were performed on washed, resuspended platelet particles collected at 100,000 *g* for 90 min. Phosphatidic acid phosphatase activity was assayed by determination of inorganic phosphate released from phosphatidic acid. For the assay of diglyceride kinase activity, diglyceride and adenosine triphosphate (ATP)-γ^{32}P were incubated with the enzyme. The phosphatidic acid which was formed was isolated by column chromatography, and the radioactivity incorporated was determined in a liquid scintillation counter. Both enzymes require Mg^{++} for optimal activity and are inhibited by high concentrations of ethylenediaminetetraacetate (EDTA). Other divalent cations are ineffective or inhibitory. Sulfhydryl reagents (2-mercaptoethanol, reduced glutathione) stimulate the enzymes and reverse the inhibitory effects of parachloromercuribenzoate. Phosphatidic acid phosphatase has a pH optimum of 7.4 in Tris-HCl buffer and a temperature optimum of 37°C. The reaction rate is linear for 3 hr and is dependent on protein concentration although the curve is not strictly linear above 0.6 mg/ml. The K_m for phosphatidic acid is 0.6 mmole/liter. Monovalent cations and albumin do not increase the enzyme activity. Detergents and potassium fluoride inhibit the enzyme. The rate of phosphatidic acid conversion to diglyceride is 2.0 ±0.5 mμmoles/mg protein per min in platelets from seven normal subjects. (Supported by PHS Grant No. 5 S01 RR 05409-09.)

56. Connective Tissue Activation: the Effect of Metabolic Inhibitors and Antirheumatic Drugs. C. WILLIAM CASTOR, Ann Arbor, Mich.

A polypeptide extractable from human cells induced metabolic hyperactivity (activation) in cultured human synovial cells resembling that seen in chronic rheumatoid synovitis. Increased metabolic activity included overproduction of hyaluronic acid, lactic acid, and increased glucose consumption. Inhibition of DNA synthesis with cytosine arabinoside (20–100 μg/ml) did not block the activation process. Actinomycin D (0.25–0.5 μg/ml), chromomycin A_3 (100 μg/ml), and acridine orange (0.03 μmole/ml) effectively blocked the synovial response to activator peptide. In addition, α-amanitin (3.0 μg/ml) suppressed the activation process. Inhibitors of protein synthesis, including puromycin (1.25 μg/ml), cycloheximide (10 μg/ml), and acetoxycycloheximide (0.25 μg/ml) blocked the activation process when added to synovial cultures simultaneously with activator peptide. Delayed addition of actinomycin D (4 hr) and cycloheximide (15 hr) abolished the capacity of these agents to inhibit activation. Synovial cells activated under nitrogen exhibited a substantial increase in hyaluronate synthesis, but failed to develop a maximal response to activator peptide. 2,4-Dinitrophenol (4×10^{-5} mole/liter) prevented hyperformation of hyaluronate by synovial cells after an effective activator stimulus. Sodium fluoride (2×10^{-3} mole/liter) completely abolished the activation response. Among the antirheumatic drugs tested, cortisol (1.0 μg/ml), acetylsalicylic acid (150 μg/ml), indomethacin (15 μg/ml), and phenylbutazone (75 μg/ml) were most effective in blocking activation, whereas chloroquine diphosphate (2.0 μg/ml), hydroxychloroquine sulfate (2.0 μg/ml), and sodium salicylate (150 μg/ml) were less effective. Serum decreases the capacity of antirheumatic drugs to block activation, but does not change their relative efficacy. We suggest that activation of human synovial cells by an exogenous activator peptide does not require DNA synthesis, but does require DNA-mediated RNA synthesis and protein synthesis. It is of interest, however, that hyaluronic acid is formed in the face of inhibition of protein synthesis. The energy requirements for activation are substantially met by glycolysis, although oxygen appears necessary for maximal activation. Several antirheumatic drugs, employed at clinically used concentrations, were

partially effective in blocking the activation response of synovial cells.

57. Gonadotropin Binding by Rat Testis Receptors. KEVIN J. CATT,* MARIA L. DUFAU,* AND TSUNEO TSURUHARA,* Bethesda, Md. (introduced by Mortimer B. Lipsett**).

The binding of labeled gonadotropins to the receptors in the interstitial cell fraction of the rat testis has been utilized to develop a sensitive radioligand assay system for luteinizing hormone (LH) and chorionic gonadotropin (CG), by use of radioiodinated human LH or CG as tracer. ^{125}I-labeled hormones of specific activity 20–50 μCi/μg were prepared by the chloramine-T method and purified by elution from powdered cellulose. Such human CG–^{125}I migrated identically with unlabeled human CG during analytical polyacrylamide-gel electrophoresis and on electrofocussing. The labeled gonadotropins were rapidly bound by the interstitial cell fraction during incubation in vitro at 37°C, reaching a plateau at 10–12% of the added radioactivity within 2 hr. The presence of excess unlabeled hormone reduced the uptake of labeled human LH and human CG to the low levels observed during incubation with interstitial cells at 4°C, or with dispersed testis tubule cells at 37°C. The uptake of tracer hormones was not influenced by the presence of glucose or puromycin in the incubation medium, or by homogenization of the interstitial fraction. Binding was progressively inhibited by the addition of increasing amounts of ovine or human LH, and human or monkey CG, but not by human FSH. The assay system is sensitive to 5 mU LH and human CG, and provides a new approach for measurement of the biological activity of gonadotropins in tissue extracts and body fluids, and for study of gonadotropin-receptor interaction. The fully desialated forms of human LH and human CG reduced tracer binding as effectively as the intact molecules, indicating that the sialic acid residues of gonadotropins are not essential for combination with the receptor sites.

58. Hemoglobin Rampah (α95 (G2) Pro → Ser): Disruption of a Critical Intramolecular Contact? SAMUEL CHARACHE, LINDA L. SMITH,* AND TITUS H. J. HUISMAN,* Baltimore, Md., and Augusta, Ga.

Hemoglobin Rampah was first discovered by de Jong et al. in India. Identification of the variant in an elderly white woman of English ancestry permitted study of some of its clinical effects and physicochemical properties. The patient was slightly anemic (hematocrit 33%), as were some other heterozygotes in her family. Heinz bodies were not present in their red cells, and were not produced by incubation with brilliant cresyl blue. The amino acid substitution does not involve a change in electrostatic charge, but the abnormal hemoglobin could be separated by electrophoresis as a slow component, indicating that it had undergone a change in conformation. It was somewhat unstable in vitro, but ^{3}H and ^{14}C studies indicated that significant denaturation did not occur in vivo. Blood oxygen affinity did not differ significantly from normal, but affinity of purified hemoglobin Rampah was moderately increased. A Bohr effect was observed, but heme-heme interaction was decreased ($n \sim 1.6$). Measurements of sedimentation velocity and viscosity suggested that the oxy-form exists as dimers ($s_{20,w} = 3.07$) in 0.1 M NaCl at pH 7.0, while the deoxy-form is present as tetramers. Hemoglobin Rampah is produced by a substitution near the corner between helices F and G of the α-chain. Normally, this corner dovetails with the CD corner of the adjacent β-chain. Contact between proline G2α and tryptophane C3β is normally maintained even during the structural changes accompanying oxygenation. Loss of proline G2α may disrupt this contact, for it alters conformation, stability, oxygen affinity, heme-heme interaction, and subunit dissociation. Despite these abnormalities, clinical effects produced by amino acid substitution at this important site were minimal in the patient and her affected relatives. (Research supported by Grants HE-02799, RR-35, HE-10591, HE-05168 from NIH.)

59. Dissociation of Ketonuria and Ammoniagenesis during Starvation. J. T. CHENG,* D. G. SAPIR,* O. E. OWEN,* AND W. G. WALKER, Baltimore, Md., and Philadelphia, Pa.

In starvation increases in urinary ammonia and ketone bodies are closely correlated ($NH_4 = 43.6 + 0.82 \times$ ketones, $r = 0.89$, $P < 0.001$). Ingestion of 7.5 g CHO (five subjects) reduces both in parallel (mean $\Delta\ U_{NH_4}V = -57.1$ mmoles/day ($P < 0.05$), mean $\Delta\ U_{ketones}V = -54.7$ mmoles/day, $P < 0.01$), and further reduction follows increase in CHO to 15.0 g daily ($NH_4 = 28.1 + 0.9 \times$ ketones, $r = 0.84$ $P < 0.001$). This reduction occurs in the absence of changes in urine pH, venous ketones, insulin, glucose FFA concentration, or bicarbonate and a rise in venous pH of 0.03 U. This implies that the reduction of $U_{NH_4}V$ is caused by reduced ammonia production. The close correlation between $U_{NH_4}V$ and $U_{ketone}V$ during development and regression of starvation ketosis further suggests that ammoniagenesis and ketogenesis may be linked metabolically. However, evidence that the rate of ammonia production is not directly dependent upon ketone excretion was obtained by giving two fasting patients 30 mmoles $KHCO_3$ daily for 13–16 days. With 30 mmoles $KHCO_3$, mean change in daily $U_{NH_4}V$ was -28.6 ($P < 0.02$) and -8.6 ($P < 0.02$) mmoles, and mean change in daily potassium excretion was $+27.4$ ($P < 0.02$) and $+5.0$ mmoles respectively. There was no change in daily ketone excretion or urine pH. Blood pH rose from 7.39 to 7.41. We conclude that in starvation ammonia excretion can be dissociated from ketonuria; that potassium can supplant ammonia during ketonuria in the absence of urine pH changes and thereby conserve nitrogen during starvation. The observation that 7.5 g of ingested CHO substantially reduced $U_{NH_4}V$, while not inconsistent with the hypothesis linking gluconeogenesis and ammoniagenesis, does, however, require that renal gluconeogeneis respond to remarkably small quantities of ingested CHO. (Supported by Grants H 3303 and 5 M01 RR349 from NIH.)

60. Comparison of the Number and Morphology of Granulocytic and Mononuclear Cell Colonies Grown In Vitro from Human Blood and Marrow. P. A. CHERVENICK,* Pittsburgh, Pa. (introduced by Jessica Lewis**).

Previous studies from our laboratory have demonstrated that colonies of granulocytes and mononuclear cells can be grown in vitro from human blood and marrow. This report

compares the number and morphology of colonies arising from these sources in normal individuals as well as in patients with various diseases. Blood was collected by venipuncture and marrow aspirated from the sternum or iliac crest. After sedimentation, the cell-rich plasma was suspended in 1.8% methyl cellulose mixed with McCoy's 5A tissue culture medium. 1 ml of the medium containing either 5×10^5 nucleated blood cells or 2×10^5 marrow cells was pipetted into tissue culture plates and incubated at 37°C in 7.5% CO_2. Colony cell growth was stimulated by conditioned media prepared from peripheral leukocytes. Blood leukocytes from normal subjects gave rise to 4–50 colonies per 10^6 cells. In patients with various diseases including lymphoma and leukemia, 0–44 colonies were observed per 10^6 cells. Marrow from these patients gave rise to 0–64 colonies per 2×10^5 cells. In several patients with acute myelocytic leukemia a greater number of small abortive colonies was observed arising from blood and marrow. Except for several of the leukemic cell populations, growth characteristics were similar for cells grown from the blood or marrow. Colonies began to appear between days 6 and 10 and grew to a maximum size of 200–1500 cells by days 18–20. Morphologically, these colonies were either eosinophilic, neutrophilic, or monocytic when examined before 4 wk. Colonies examined after 4 wk contained predominantly macrophages. Studies with tritiated thymidine revealed that all colonies examined contained labeled cells and mitoses were evident on Wright's stained smears. This report indicates that colonies of varied cell type can be grown in vitro from blood and marrow of normal and diseased individuals and suggests that the cell giving rise to such colonies circulates freely.

61. Cholesterol Metabolism in the Diabetic Hamster. ARAM V. CHOBANIAN, GEORGE C. GERRITSEN,* AND FRANCISCO MANZUR,* Boston, Mass., and Kalamazoo, Mich.

The relationship between diabetes and hypercholesterolemia remains to be clarified. This problem has been examined in Chinese hamsters with genetically determined spontaneous diabetes which appears relatively similar to the human disease. Studies of plasma lipids, blood glucose, plasma immunoreactive insulin, and body cholesterol metabolism (calculated from plasma disappearance rates of injected cholesterol-^{3}H) have been performed over a 5 wk period in 10 nonketotic diabetic animals and their matched controls. Plasma cholesterol and triglyceride levels were significantly greater in the diabetic than in the control group. The respective concentrations of plasma cholesterol and triglycerides (nonfasting) averaged 253 and 541 mg/100 ml in the diabetic hamsters as compared with 163 and 195 mg/100 ml in the control animals. Blood glucose ranged from 192 to 420 mg/100 ml in the diabetic group as compared with 82–113 mg/100 ml in the normal hamsters. Fasting and postprandial plasma insulin concentrations did not differ significantly between the two groups. The turnover of plasma cholesterol most closely conformed to a theoretical two pool model. The $t_{\frac{1}{2}}$ of cholesterol in the rapidly exchanging compartment averaged 3.0 days in the diabetic and 2.4 days in the normal hamsters. The $t_{\frac{1}{2}}$ in the slowly exchanging pool averaged 15 days in the diabetic and 10 days in the normal animals. The pool size of the rapidly exchanging compartment of the diabetic hamsters generally exceeded that of the control group. The results indicate that the nonketotic diabetic hamster exhibits spontaneous hypercholesterolemia which is associated with a diminished body turnover rate of cholesterol. The findings suggest that the hyperlipidemia in diabetes is not related directly to abnormal plasma insulin levels. The Chinese hamster appears to be a useful animal model for studies of the relationship between diabetes and abnormal lipid metabolism.

62. Plasma Renin Activity in Diabetics with Orthostatic Hypotension. A. RICHARD CHRISTLIEB* AND C. MUNICHOODAPPA,* Boston, Mass. (introduced by Alexander Marble**).

Plasma renin activity (PRA) in the supine (S), ½ hr upright (½U), and 4 hr upright (4U) position was studied in six patients with diabetes mellitus who had orthostatic hypotension (DOH) and in five diabetic matched controls without orthostatic hypotension (D). DOH had a mean age of 44 yr and mean duration of diabetes 15½ yr; D 42 yr and 18 yr respectively. All DOH but no D had symptoms of autonomic dysfunction. Neither serum electrolytes nor 24-hr urine electrolyte excretions differed between the groups. The mean creatinine clearance in DOH was 41 ml/min, in D 89 ml/min ($P < 0.05$). Four patients with DOH but none of D had elevated supine BP. Mean BP in DOH decreased from 109 to 73 mm Hg ($P < 0.001$), in D mean BP increased from 87 to 94 mm Hg (not significant). The pulse increased in DOH from 79 to 85/min (not significant), in D from 75 to 95 ($P < 0.05$). Mean S(PRA) in DOH was 213 ng/100 ml, in D 463 ng/100 ml (not significant). Mean ½U(PRA) in DOH was 277 ng/100 ml, in D 774 ng/100 ml ($P < 0.02$). Mean 4U(PRA) was 319 ng/100 ml in DOH, 915 ng/100 ml in D ($P < 0.05$). One patient with DOH received infusions of norepinephrine (NE) for ½ hr on 2 successive days with no change in the PRA. These results contrast with those in four of five reported cases of nondiabetic orthostatic hypotension (OH) whose PRA increased appropriately with upright posture. The obvious explanation for unresponsive PRA in patients with diabetic orthostatic hypotension would appear to be defective catechol stimulation of renin release. However, the associated nephropathy suggests the additional possibility of defective renal renin stores. This possibility is supported by the failure of PRA to respond to NE infusion in the one case so studied here which is unlike the findings in three reported cases of nondiabetic OH. (Research supported by NIH Grants HE13368-01 and HE11306-03.)

63. Abnormal Carbohydrate Metabolism Associated with Drug Abuse. B. P. CITRON,* M. HALPERN,* L. MILLER,* AND B. J. HAVERBACK, Los Angeles, Calif.

The existence of an abnormality in carbohydrate metabolism associated with drug abuse and with the characteristics of diabetes mellitus has not been previously described. 41 young people aged 17–40 yr who are in the study, had used narcotics, hallucinogens, stimulants, and sedatives. 25 patients had used methamphetamine alone or in combination with other drugs, usually heroin or *d*-lysergic acid diethylamide. The remaining 16 patients, who denied the use of methamphetamine, had used heroin or barbiturates. A family history of diabetes mellitus was absent in all patients. After a 3 day

300 g carbohydrate diet, a 4 hr glucose tolerance test was performed. Serum glucose was determined by the cyanide reduction technique. 23 of the 25 patients who had used large doses of methamphetamine had abnormal glucose tolerance curves. The patterns of abnormality were divided into two groups; the first pattern, that of a diabetic curve with the 2-hr postprandial serum glucose values ranging from 150 to 385 mg/100 ml (average of 208 mg/100 ml); the second pattern revealed the 3- and 4-hr serum glucose values to be less than 60 mg/100 ml. Insulin assays using the double antibody technique of Morgan and Lazarow correlated with the glucose abnormalities. In the 16 patients without a history of methamphetamine abuse, glucose tolerance curves and insulin assays were normal. The occurrence of abnormal glucose tolerance and insulin assays in 23 of 25 patients who have taken methamphetamine supports the concept that this drug may produce chemical diabetes.

64. Primary Phosphaturia and Parathyroid Hormone Unresponsiveness: Two New Disorders of Renal Phosphate Transport. FREDRIC L. COE,* JANET M. CANTERBURY,* AND ERIC REISS,** Chicago, Ill. (introduced by Louis N. Katz**).

A systematic study has been made of glomerular filtration rate (GFR), phosphorus excretion, and immunoassayable parathyroid hormone (PTH) concentration in 100 patients with nephrolithiasis and/or selective renal acidification defects. The expected relationships between GFR, PTH, and tubular reabsorption of phosphate (TRP) were documented in 73 patients: diminished GRF regularly resulted in increased PTH and phosphaturia, whereas phosphaturia did not occur in patients with normal serum PTH. Disruption of these normal relationships was documented in the remaining 27 patients. In 16 patients with decreased GFR, 10 had the expected elevation of PTH, but phosphaturia was either absent or very slight. The remaining 6 patients failed to display the appropriate elevation of PTH but nevertheless had definite phosphaturia. In 11 patients with normal GFR, 9 had high PTH without phosphaturia; the remaining 2 had phosphaturia with normal PTH. These data indicate that phosphaturia can occur without elevation of PTH in patients with normal or decreased GFR. None of these patients had any stigmata of familial or acquired hypophosphatemic rickets. This apparently represents a new type of tubular transport defect. Since recent data indicate that phosphate retention is an important initiating factor in the genesis of renal hyperparathyroidism, primary phosphaturia, unrelated to PTH, probably prevented increased serum PTH in the patients with low GFR. The data also indicate the existence of a second kind of tubular defect that blunts the normal phosphaturic action of PTH so that phosphaturia does not occur despite elevated PTH. None of the patients with this defect had the usual stigmata of pseudohypoparathyroidism. (Supported by NIH Grant AM-08572.)

65. Heme Proteins in Heart and in Fetal, Neonatal, Adult Normal, and Dystrophic Skeletal Muscle. LOUIS COHEN,* JANE MUTTI,* CYNTHIA JELINEK,* AND JULIET MORGAN,* Chicago, Ill. (introduced by William Barclay**).

The present studies were undertaken to (*a*) identify all the heme proteins, extractable from muscle with aqueous buffers, which were detectable by benzidine staining after starch-gel electrophoresis; (*b*) study these muscle heme proteins through fetal and neonatal stages of development into adulthood; and (*c*) determine if there are qualitative differences in these heme proteins, particularly in the myoglobin of normal and dystrophic muscle. Extracts were prepared from 10 fetal, 20 neonatal, 51 adult skeletal, and 10 adult cardiac muscle samples. Muscle samples from five persons with Duchenne dystrophy, and four with myotonic dystrophy were also studied. These samples were obtained at autopsy or by biopsy. Five heme proteins were identified in these extracts by electrophoretic, chromatographic, immunological, and spectrophotometric means. Hemealbumin, hemoglobins A and A_2, myoglobin, and cytochrome *c* were found in all but the fetal muscle samples. Hemoglobin F was not found earlier than in the 12-wk-old fetus, and was present up to at least 1 wk after birth; it is the major heme protein in fetal muscle extracts. Both hemoglobins F and A were seen in extracts of neonatal muscle. Myoglobin was not seen before 40 wk gestation. The heme proteins in adult skeletal and cardiac muscle were identical, qualitatively. Similarly, no qualitative differences were found in the heme proteins of normal and dystrophic muscle, and the persistence of a unique fetal heme protein in dystrophic muscle, identified by others, could not be confirmed.

66. Gastrin Supersensitivity in the Pathogenesis of Lower Esophageal Sphincter Hypertension in Achalasia. SIDNEY COHEN,* WILLIAM LIPSHUTZ,* AND WILLAIM HUGHES,* Philadelphia, Pa. (introduced by Arnold S. Relman**).

In achalasia, hypertension of the lower esophageal sphincter (LES) is a major factor in the impairment of esophageal emptying. This study defines the role of gastrin in the pathogenesis of LES hypertension. Quantitation of LES pressure, using an open-tipped perfused system, was carried out in 20 untreated patients with achalasia and 20 normals. LES pressure, in achalasia, was 50.5 $\pm$4.6 mm Hg (mean $\pm$SE), as compared to 19.4 $\pm$1.3 mm Hg in normals ($P < 0.001$). Upon suppression of endogeneous gastrin release by gastric acidification (0.1 N HCl), reduction of LES pressure occurred in both groups, but was more marked in achalasia than in normals, 86.9 $\pm$2.2% and 68.4 $\pm$3.1% respectively ($P < 0.001$). These findings, in achalasia, suggested either elevated endogenous levels of gastrin or an increased sensitivity to gastrin. Immunoassay for serum gastrin, in achalasia, was 81.9 $\pm$7.6 $\mu\mu$g/ml, as compared to 76.4 $\pm$6.6 $\mu\mu$g/ml in normal patients ($P > 0.05$). Dose response curves of LES pressure to intravenous pulses of synthetic gastrin I were constructed for both groups. In achalasia, the curve had a parallel shift to the left indicating that the sensitivity to gastrin I was more than twice that of normals. This gastrin supersensitivity in achalasia persisted after pneumatic dilatation, although LES pressure was now reduced to a normal level of 18.3 $\pm$3.0 mm Hg. These studies show that LES pressure in achalasia is (*a*) elevated, (*b*) lowered by gastric acidification, and (*c*) supersensitive to exogenous gastrin I. We conclude that supersensitivity to endogenous gastrin may be responsible for sphincteric hypertension and impaired esophageal emptying in achalasia. (Research supported by grants from VA and NIH.)

67. Binding of Fibrin Monomer by Lambda Myeloma Proteins. MORTON COLEMAN,* MARC WEKSLER,* AND RALPH NACHMAN, New York.

Plasmas from seven patients with lambda myeloma globulins displayed the "gelation" phenomenon. This phenomenon is characterized by bulky, gelatinous clots, impaired clot retraction, and prolonged thrombin times reflecting inhibition of fibrinogen-fibrin conversion. This latter finding results from circulating anticoagulant activity inhibiting fibrin monomer polymerization. Anticoagulant activity was displayed by the isolated myeloma proteins and was identical with the inhibitors previously described as "antithrombin V." The mechanisms of inhibition by these isolated myeloma proteins and their structural subunits were investigated utilizing thrombin clotting times and a spectrophotometric system measuring fibrin monomer polymerization. The addition of calcium partially corrected the prolonged thrombin times in plasma and fibrinogen clotting systems containing the myeloma proteins. Increasing concentrations of inhibitor myeloma proteins produced progressively greater elevations of the thrombin time despite excess calcium. All five of the IgG^L myelomas were of the $\gamma G1$ subgroup, whereas both IgA^L myelomas were $\gamma A1$. Low concentration (1.5 mg/ml) of the isolated IgG proteins inhibited polymerization. The IgA^L proteins did not demonstrate this activity at low concentration but were active at concentrations comparable to in vivo levels. Fab and $F(ab')_2$ fragments produced by papain and pepsin digestion, respectively, displayed full inhibitory activity of the native IgG^L proteins. Fc fragments and isolated light and heavy polypeptide chains did not possess any measurable inhibitory activity. Washed clots produced in the presence of radioactively labeled inhibitor myeloma proteins revealed a sixfold increase in percentage binding of radioactivity compared to binding with clots in the presence of noninhibitor myeloma proteins. These studies suggest that the Fab sites of certain lambda myeloma proteins have specific binding properties responsible for fibrin polymerization inhibition. The possibility must be considered that these anticoagulant myeloma proteins are antibodies to sterically unavailable determinants of plasma fibrinogen which becomes accessible during the formation of fibrin monomer. (Research supported by grants from the NIH and ACS.)

68. Fate of Stress Reticulocytes: Hemolysis or Fragmentation? STEVEN E. COME,* STEPHEN B. SHOHET,* AND STEPHEN H. ROBINSON, Boston, Mass.

Stress reticulocytes (SR) produced in response to acute anemia are macrocytic and appear to be hemolyzed prematurely. Conversely, it has been proposed that these cells survive normally but are reduced in size by the gradual removal of superficial cell fragments. Complete hemolysis would result in proportionate elimination of both cell membrane and cytoplasmic hemoglobin, whereas surface fragmentation would result in a disproportionate loss of membrane. To evaluate these two possibilities SR were induced in rats by repeated bleeding. Animals were then given simultaneously $^{32}P_i$ and glycine-2-^{14}C to label membrane phospholipid and hemoglobin, respectively. Labeled cells were harvested 1 day later and transfused into normal littermates. Recipient blood was sampled periodically for radioassay of chromatographically isolated membrane phosphatidylethanolamine (PE) and crystallized hemoglobin heme. PE-^{32}P was shown to be a stable and nonexchangeable membrane component which, like hemoglobin-^{14}C, is synthesized almost entirely by young erythrocytes. In rats transfused with SR the mean specific activity of membrane PE fell 72% in 72 hr, as compared to only 16% for hemoglobin heme. In animals given labeled reticulocytes from normal donor rats the changes in specific activity were more nearly concordant: PE fell 9%, hemoglobin 7%. With labeled reticulocytes heated to 52°C to induce irreversible damage to whole cells, there was rapid and symmetrical loss of both membrane and hemoglobin labels. The magnitude of loss of cellular constituents is overestimated by these changes in specific activity, since the animals were not in a steady state. However, the comparative changes in membrane and hemoglobin labels indicate that the apparent disappearance of SR is due largely to a process of superficial fragmentation, rather than to hemolysis of whole cells. (Supported by NIH Grants AM 09834 and HD 02777 and a Medical Foundation fellowship.)

69. Prophylactic Use of Gamma Globulin to Prevent Endemic Viral Hepatitis. MARCEL CONRAD, ALLEN YOUNG,* PATRICIA CONRAD,* RICHARD PARK,* WILLIAM BANCROFT,* AND GEORGE BERNIER,* Washington, D. C.

The prophylactic administration of human serum gamma globulin to U. S. soldiers assigned in Asia from 1964 to 1967 did not seem to reduce the incidence of viral hepatitis. Thus a prospective study was performed in 107,803 men to ascertain if gamma globulin prepared from U. S.–donated blood protected U. S. soldiers stationed in Korea. Each soldier was injected intramuscularly with 10 ml of either gamma globulin or an albumin solution upon arrival in Korea and 65% received a second injection 5–7 months later. The test solutions were obtained from a commercial source and showed no IgG in the albumin solution and less than 5% of the gamma globulin was fragmented. Three-fifths of the soldiers were injected with either 2 ml, 5 ml, or 10 ml of 16% serum gamma globulin and two-fifths received the placebo solution. Selection of the test injection for each soldier was random. There were 467 documented cases of icteric viral hepatitis with an incidence of 5.67 cases per thousand among the control subjects who received albumin and 3.39 (10 ml), 2.90 (5 ml), and 4.04 (2 ml) cases per thousand among soldiers injected with various amounts of gamma globulin. Australia antigen (HAA) was identified in serum from 12% of patients by complement fixation testing. The gamma globulin seemed to provide similar protection against both HAA-positive and -negative endemic hepatitis. Patients who received gamma globulin had a diminished history of chills, fever, and icterus, decreased albuminuria, and lower peak mean serum bilirubin values than control subjects. Other comparisons to quantify severity showed no significant difference between groups. Prophylactic injections of gamma globulin did not significantly alter the incidence of other commonplace infectious diseases observed in the soldiers of this study. (U. S. Army.)

70. Metabolism of Isotopically Labeled Sulfur Amino Acids in a Patient Excreting β-Mercaptolactate–Cysteine Disulfide. J. C. CRAWHALL, P. PURKISS,* AND J. B. STANBURY,** Montreal, Canada and Boston, Mass.

A patient who was mentally retarded was found to excrete β-mercaptolactate–cysteine disulfide. Sulfate and thiosulfate excretion were in the normal range. Oral administration of cysteine but not of methionine increased the urinary excretion of the mixed disulfide. Excretion of sulfate was increased after cystine and methionine administration but taurine only increased after cysteine administration. 50 μCi L-cystine-^{14}C was injected intravenously and blood and urine collected at intervals during the next 6 hr. An aliquot of each urine was applied to a Dowex 50(H^+) ion-exchange column which was washed with water and the amino acids eluted with 4 N aqueous ammonia. The eluate was concentrated and the amino acids separated on a Beckman 120C amino acid analyser. Eluate fractions were collected and assayed for radioactivity. Cystine-^{14}C was incorporated into the mixed disulfide within the 1st half hr and no other significant radioactivity was observed. 3 months later 40 μCi L-methionine-^{35}S was injected intravenously. Radioactivity was incorporated into the acidic metabolites of cystine within the 1st half hr but no radioactivity was found in the mixed disulfide region in the 6 hr of collection. These results can only be explained if the injected cystine is used preferentially for mixed disulfide formation whilst the injected methionine can be converted to acid metabolites of cystine without becoming available for mixed disulfide formation. The acidic metabolites formed appear to be taurine and cysteic acid but further confirmation of their structure is still in progress. (Supported by grants from the Canadian MRC and USPHS.)

71. Cholelithiasis in Experimental and Human Protoporphyria. DEREK J. CRIPPS* AND EDGAR S. GORDON,** Madison, Wis.

Albino mice 8-wk-old, made protoporphyric (P. mice) with 1% griseofulvin feed, developed gallstones and hepatic changes that were compared with human protoporphyria (EPP) with 6% incidence of cholelithiasis and cholecystitis. Quantitative porphyrins and liver biopsies in 10 patients and gallstones in 2 patients with EPP were compared with (57) P. mice sacrificed at intervals. Livers of 10-wk-old normal and P. mice were cultured in Eagle's minimum essential media. Fluorescence of porphyrins in tissue was evaluated by microfluorospectrophotometry (MFSP). In EPP liver, three had periportal fibrosis and eight showed deposits of protoporphyrin (PP) appearing as brown pigment in liver and Kupffer cells particularly around bile ducts. Similar deposits of PP were seen in P. mice livers which in addition showed a remarkable increase in size, after 10 days feed; the ratio of mean normal to P. mice liver weights was 1:2.4, after 20 days 1:2.9, and at 46 days 1:4.2. In tissue culture P. mice liver cells survived well and subcultures at 3 wk were viable at 10 wk, with a high quantity of precipitated PP in the cell cytoplasm identified by MFSP. PP in gallstones in micrograms per gram wet weight in nine human controls was <9.7, in a 19-yr EPP was 232–479, in a 57-yr EPP cholesterol stone was 43, and pooled small stones from 4-wk P. mice showed a high value of 2315. PP is comparatively insoluble and could be a factor in liver changes and formation of gallstones in human and experimental protoporphyria. (Research supported by NIH Grant AM 0.9995-05.)

72. Action Spectrum and Experimental Immunoflorescence in Lupus Erythematosus (LE). DEREK J. CRIPPS* AND JOHN RANKIN,** MADISON, Wis.

Subacute disseminated discoid lupus erythematosus (SDLE) is a form of LE most aggravated by sunlight. Four patients with SDLE (negative LE and antinuclear factor) were studied to determine action spectrum, ie. minimal erythema of measured intensity with wavelengths (λ) 250–330 nm using a high intensity prism-grating monochromator, and compared with 19 controls. In addition reproducibility of LE with various monochromatic λ was attempted by maintaining gross erythema for 10 days (total intensity $<10^6$ microwaves/sec per cm^2). Direct fluorescent antibody technique was determined in SDLE on involved, uninvolved, and irradiated sites (5 mm^2). The action spectrum results in SDLE were within the standard deviation but slightly lower than the mean of 19 controls. The major difference was the development of erythema at 330 nm (<320 nm in controls); persistence of minimal erythema at 270–305 nm up to 7 days in contrast to controls. The selected monochromatic radiation sites with λ 270–305 nm in which palpable erythema was maintained for 10 days was observed for several months and became indistinguishable from LE. Positive immunoflorescence with IgG and IgM at the junction of dermis and epidermis was noted in LE, but not uninvolved skin, and IgG in selected radiated sites was detectable only after 2–6 months. In conclusion LE can be produced by any λ capable of producing sunburn erythema. (Research supported by Grant AM 0.9995-05 from NIH.)

73. Insulin-Receptor Interactions in Adipose Cells. PEDRO CUATRECASAS,* Baltimore, Md. (introduced by Victor A. McKusick**).

A sensitive method has been devised for measuring directly the physical interaction of insulin with the specific receptors of intact, isolated fat cells. Free monoinsulin-^{125}I is separated from that bound to cells by membrane filtration procedures. The dependence of binding on insulin-^{125}I concentration parallels the dependence of glucose transport on the concentration of insulin-^{125}I or native insulin. The pattern of displacement of the insulin-^{125}I–receptor complex by native insulin indicates identical behavior of the two molecules. Various kinetic parameters (24°) of the insulin-receptor interaction, determined independently, reflect a simple, reversible, bimolecular process with a homogenous binding site. The rate constant of association is 1.5×10^7 M^{-1} sec^{-1}, nearly that of a diffusion controlled process. The measured rate constant of dissociation (strictly first order) is 7.4×10^{-4} sec^{-1}; the half-life is 16 min. The dissociation constant is thus 5.0×10^{-11} M. An adipose tissue cell can bind specifically a maximum of 11,000 molecules of insulin. Insulin antibodies dissociate the receptor-insulin complex. Insulin molecules bound to the receptor can be recovered free of receptor and restudied. Their behavior is indistinguishable from that of native insulin. The

interaction of insulin with its receptor does not involve covalent-bond changes, and no significant chemical alterations of insulin result from its biological action. It is now possible to study insulin-receptor interactions in cells by means not dependent on metabolic processes. Enzymatic and chemical modifications of cell surface structures demonstrate that the receptor-binding and biological functions can be studied separately. The results of these studies permit an assessment of the possible role of carbohydrate, protein, and lipid components of the cell membrane in the mechanism of insulin action.

74. Role of Carbonic Anhydrase in Maintaining a High pH at the Calcifying Site of Epiphyseal Plates. L. A. CUERVO,* D. S. HOWELL,** AND J. C. PITA,* Miami, Fla.

An alkaline pH at calcifying sites in cartilage has long been postulated, and the first direct measurements confirmed this view (Howell et al. 1968. *J. Clin. Invest.* **47**: 1121). The effect of pH on the deposition of Ca-P mineral on various in vitro systems is substantial in the range of 7.3–7.7. The extracellular fluid (Cfl) at the calcifying site of rat tibial epiphyseal plates collected by micropuncture techniques revealed a pH of 7.56 ±0.05 and a P_{CO_2} of 41–45 mm Hg. Acetazolamide, a carbonic anhydrase (CA) inhibitor, lowered the blood pH to 7.20 ±0.09 and the Cfl pH to 7.18 ±0.10. In contrast, NH_4Cl reduced blood pH to 7.23 ±0.11 and Cfl pH only to 7.33 ±0.08. A CA assay micromethod was developed with a sensitivity of 0.5 Maren units. The hypertrophic cell and provisional calcification zones of the growth plate showed only CA activity which was computed to result from blood contamination. A 200 A wide band of epiphyseal and metaphyseal bony tissue, contiguous with the epiphyseal plate, both contained 45–53 Maren units/g dry blood-free tissue. It is concluded that a carbonic anhydrase–dependent bicarbonate secretion from a structure such as the penetrating capillary complexes and associated bone cells is involved in the maintenance of the high Cfl pH in epiphyseal cartilage.

75. Evaluation of Cellular Immunity and Blocking Antibody Activity in Patients with Malignant Tumors. F. J. CUMMINGS,* G. H. HEPPNER,* AND PAUL CALABRESI, Providence, R. I.

Cellular immunity inhibits growth of malignant neoplasms. Humoral (blocking) antibody, however, may interfere with this protective response. In order to assess the relative importance of these opposing immunological factors, cells from human tumor specimens were obtained at surgery and cultured in petri dishes containing agar, Waymouth's medium, and irradiated P-815Y cells. After 1–3 wk, the cells were harvested for a colony-inhibition test. Lymphocytes from these patients (autochthonous), from patients with similar tumors (cross-reacting), and from nontumor patients (control) were incubated for 1 hr with 2.5×10^3 cultured cells, at ratios of 100/1, 10/1, and 1/1. Aliquots containing 500 tumor cells were then cultured in Waymouth's medium and semisoft agar and incubated for 7–10 days. Percentage inhibition of colony formation was determined by comparison of the number of colonies present after incubation with either autochthonous or cross-reacting vs. control lymphocytes. Serum blocking factors, presumably antibody, were detected by the ability of test sera to interfere with the inhibition of colony formation by immune lymphocytes. Patients with breast carcinoma, malignant melanoma, and three of five with colon carcinoma had, respectively, 18.4, 38.2, 36.9, 57.8, and 18.9% inhibition by autochthonous lymphocytes. Cross-reacting lymphocytes inhibited by 34.6% cells from a lung carcinoma patient. Patients with breast carcinoma, lung carcinoma, malignant melanoma, and two of eight with colon carcinoma had, respectively, 32.2, 51.8, 32.8, 42.3, and 32.2% blocking antibody activity in autochthonous sera. These quantitative measurements afford the opportunity to perform sequential determinations of a patient's immunological reactivity to his tumor in order to correlate the clinical implications of these responses during the course of the disease.

76. Hormonal Stimulation of Parathyroid Hormone Secretion in Man. WILLIAM G. CUSHARD, JR.,* MARGARET BERCOVITZ,* JANET M. CANTERBURY,* AND ERIC REISS,** Chicago, Ill.

The only known regulator of parathyroid hormone (PTH) secretion is serum concentration of divalent cations. To explore the possibility that other regulators of PTH secretion exist, we have initiated a systematic study of the effects of various hormones on serum PTH, measured by radioimmunoassay. All studies were performed in normal volunteers. Test hormones were administered intravenously in 120 ml physiologic saline over 60 min. Glucagon (GN) was studied because it has slight hypocalcemic action and is believed to enhance calcitonin release. Subjects were given 2–3 mg GN. Serum PTH increased 100–200% above base line values. Despite sampling every 10 min, serum calcium did not change in these experiments. Adrenocorticotrophic hormone (ACTH) was studied as a prototype of pituitary trophic hormones. In each of four subjects studied, PTH rose briskly during infusion of 80 U ACTH. Peak values, 100–300% above base line values were reached 15 min after completion of the infusions, with prompt decrease to preinfusion levels within 60 min. Small decreases in serum calcium (0.2–0.4 mg/100 ml) were noted in two subjects but these were not correlated with increases in PTH levels. Human growth hormone (HGH) was suspected as a mediator of PTH secretion because of disordered calcium metabolism in acromegaly and, in particular, because of the nocturnal spike in secretion of both hormones. Four subjects were given 2–5 mg HGH. No changes resulted in PTH levels or serum calcium during or after the infusion. Interpretation of these unexpected findings is unclear. However, potent stimulation of PTH secretion by GN and ACTH casts doubt upon the traditional view that divalent cation concentration is the sole regulator of PTH secretion. (Supported by NIH Grant AM-08572.)

77. Evidence for an Intramuscular Lipid Pool in the Human Forearm. G. R. DAGENAIS,* R. G. TANCREDI,* AND K. L. ZIERLER,** Baltimore, Md.

Free fatty acids (FFA) are the major substrates for oxidative metabolism in resting skeletal muscle of postabsorptive subjects. Although FFA are removed from plasma in amounn sufficient to account for all of the oxygen uptake, it has beets suggested that plasma FFA enter a lipid (triglyceride) pool in

muscle fibers in exchange for other FFA which are formed by hydrolysis of triglyceride and immediately oxidized. We tested this hypothesis in resting forearms of seven postabsorptive subjects by measuring deep venous (DV) $^{14}CO_2$ activity in response to constant infusion of oleic-1-^{14}C acid into the brachial artery. Extraction of infused oleic-1-^{14}C acid becomes constant in 10 min, but DV $^{14}CO_2$ activity was not detectable for 30 min and was very low even after 1 hr. Intra-arterial infusion of HCO_3-^{14}C showed transit times through the forearm CO_2 pool of less than 5 min. Therefore, oleic acid extracted from plasma by skeletal muscle is not oxidized immediately but must enter an intramuscular lipid pool. That this pool is heterogeneous is suggested by the fact that the apparent plateau in DV $^{14}CO_2$ activity, reached after 3 hr of infusion, accounted for only 14% of the extracted oleic-1-^{14}C acid. We conclude that FFA taken up by muscle from plasma go first to a lipid pool, that this lipid pool is the immediate source of the oxidized substrate, and that the pool may be heterogeneous. (Research supported by Grants 1F03-HE-44504 and AM-13568 from NIH and a grant from MDA.)

78. Altered Bile Acid Metabolism in Patients with Cholesterol Cholelithiasis. RUDY G. DANZINGER,* ALAN F. HOFMANN, LESLIE J. SCHOENFIELD, AND JOHNSON L. THISTLE,* Rochester, Minn.

Human gallstones are composed predominantly of cholesterol; bile acids and lecithin are necessary to solubilize cholesterol in bile. Patients with cholesterol cholelithiasis have altered bile composition, since the concentration of bile acids plus lecithin is decreased in relation to that of cholesterol. To determine whether this abnormality in bile composition is associated with a decrease in the amount of circulating bile acids, we determined pool size and synthesis rates of the two primary bile acids in five women with gallstones and six healthy women matched for age and weight. Chenodeoxycholic acid-^{3}H and cholic acid-^{14}C were administered intravenously; fasting bile was obtained by duodenal drainage on days 1, 2, 3, 5, and 7, and the specific activity of individual bile acids determined. Gallstone patients had greatly decreased chenodeoxycholic acid pools (mg/kg body weight, mean ±SEM: patients, 2.7 ±0.6; controls, 10.4 ±1.2, $P < 0.001$); yet synthesis was only slightly lower than that of control subjects (patients, 75 ±12 mg/day; controls 104 ±14). No consistent differences between patients and controls were demonstrated for cholic acid. Bile analyses confirmed a decreased bile acid plus lecithin to cholesterol ratio in gallstone patients, and the decreased ratio was highly correlated ($r = 0.80$) with the decreased chenodeoxycholic acid pool in individual patients. Oral chenodeoxycholic acid administration has been shown to restore bile composition to normal in women with cholelithiasis. In initial results, we have now found that the administration of chenodeoxycholic acid for 1 yr was without toxicity, increased the chenodeoxycholic acid pool to normal, and decreased gallstone size by 70%. These experiments confirm an abnormality of bile acid metabolism in patients with cholesterol cholelithiasis, as previously reported by Vlahcevic, indicate a method for correction, and suggest that such correction could lead to reduction in gallstone size. (Research supported in part by NIH Grant AM-6908 and the McLaughlin Foundation.)

79. Mechanism of Inhibition of Proximal Sodium Reabsorption in Volume-Expanded Rats. T. M. DAUGHARTY,* I. UEKI,* D. NICHOLAS,* AND B. M. BRENNER,* San Francisco, Calif. (introduced by T. B. Bradley, Jr.).

We examined the influence of postglomerular vascular [protein] on proximal fluid reabsorption during comparable degrees of vascular volume expansion using either rat plasma (2.5% body weight) or colloid-free Ringer's (10% body weight). Free-flow recollection micropuncture estimates of nephron GFR and absolute and fractional proximal reabsorption and efferent arteriolar [protein] were obtained before and after volume expansion. Initial hydropenic values and induced changes in these measures in 17 rats after plasma averaged 41.7 nl/min ±1.6 SE (n = 55) and +58.0% ±5.4 (n = 55) ($P < 0.001$), 22.0 nl/min ±1.2 (n = 55) and +6.1% ±4.2 (n = 55) ($P > 0.1$), 0.55 ±0.02 (n = 55) and −33.2% ±2.0 (n = 55) ($P < 0.001$), and 9.0 g/100 ml ±0.2 (n = 15) and −1.2% ±2.0 (n = 14) ($P > 0.5$), respectively. Changes from comparable hydropenic values after Ringer's in 14 rats averaged +47.0% ±6.6 (n = 31) ($P < 0.001$), −24.0% ±7.1 (n = 31) ($P < 0.005$), −51.0% ±3.2 (n = 31) ($P < 0.001$), and −31.0% ±2.5 (n = 9) ($P < 0.001$). Using peritubular capillary microperfusion technics in eight other comparably Ringer's-loaded rats we next selectively restored postglomerular [protein] to normal preexpansion levels using $NaHCO_3$-Ringer's perfusate containing 9–10 g/100 ml albumin. Despite continued volume expansion and unchanged values for nephron GFR (mean = − 4%) absolute and fractional reabsorption rose by 25% or more in 9/11 and 10/11 tubules respectively and on average were restored to within 80% of hydropenic values. Control microperfusion (6–7 g/100 ml albumin) yielded no significant mean changes (< 4%) in reabsorption. We conclude that the inhibition in absolute proximal reabsorption after Ringer's is mediated predominately by the resultant changes in postglomerular [protein]. While this inhibition together with the measured increase in filtered load results in the observed depression in fractional reabsorption after Ringer's, increased load alone accounts for the lesser depression after plasma (plasma vs. Ringer's $P < 0.001$). (Supported by NIH and VA.)

80. Ascorbic Acid and Phagocytosis: a New Biochemical Mechanism. LARRY R. DECHATELET,* M. ROBERT COOPER,* AND CHARLES E. MCCALL,* Winston-Salem, N. C. (introduced by Manson Meads**).

A new mechanism is proposed to explain the biochemical concomitants of phagocytosis. I. Ascorbate + O_2 → Dehydroascorbate + H_2O_2. II. Ascorbate + H_2O_2 → Dehydroascorbate + H_2O. III. Dehydroascorbate + GSH → Ascorbate + GSSG. IV. GSSG + NADPH → GSH + NADP. V. Glucose-6-P + NADP → 6-PGA + NADPH. The first reaction in the sequence has been described in the chemical literature for over 20 yr. The second reaction has been shown to be bactericidal in vitro (T. E. Miller. 1969. *J. Bacteriol.* **98**: 949) and might contribute substantially to the bactericidal activity of the leukocyte. Evidence for the over-all scheme is as follows. (*a*) The addition of ascorbate to resting cells results in a marked stimulation (5- to 10-fold) of the hexose monophosphate shunt (HMS), as measured by the conversion of glucose-1-^{14}C to $^{14}CO_2$. The addition of dehydroascorbate

results in still greater stimulation (15- to 20-fold) of the HMS. (*b*) Reactions II and III can be directly observed in vitro by following the absorbance due to ascorbic acid at 260 nm. (*c*) The addition of DHA, GSH, and NADPH to a sonic extract of leukocytes results in a 40-fold stimulation of the HMS. This stimulation is dependent upon the presence of all three compounds, and ascorbate will not substitute for DHA in the system, although GSSG will substitute for the combination of GSH and DHA. This scheme can explain the biochemical events accompanying phagocytosis and invokes a bactericidal mechanism which is independent of myeloperoxidase-halide-H_2O_2 interactions. (Supported by NIH Grants RR5404 and AI09169 and a Forsyth Cancer Service Grant.)

81. In Vitro Demonstration of an Isolated Iodide-Trapping Defect in Benign and Malignant "Cold" Thyroid Nodules. FRED DE RUBERTIS,* KAMEJIRO YAMASHITA,* ANDREW DEKKER,* REED LARSEN,* AND JAMES B. FIELD, Pittsburgh, Pa.

"Cold" thyroid nodules do not accumulate $^{131}I^-$ in vivo before or after thyroid-stimulating hormone (TSH) administration. To examine this phenomenon, slices or homogenates of 17 such nodules (15 benign, 2 papillary carcinomas) and surrounding normal thyroid obtained at surgery were studied for basal and TSH-stimulated $^{131}I^-$ trapping, organification, colloid droplet formation, adenyl cyclase activity, cyclic adenosine monophosphate (cAMP) concentration, glucose-1-^{14}C oxidation and ^{32}P incorporation into phospholipids. Responses in benign and malignant nodules were similar. Slices from nodules did not concentrate $^{131}I^-$ (T/M 0.8 $\pm$0.2 compared to 9 $\pm$2 in normal). Since iodide trapping and control of thyroid function appear to involve cAMP, the adenyl cyclase system was compared in nodules and normals. Adenyl cyclase activity was greater in nodules (42 $\pm$8 cpm/mg) than in normals (17 $\pm$3). TSH increased activity threefold in both tissues. cAMP was similar in nodules (4.5 $\pm$1.5 mμmoles/g) and in normals (2.7 $\pm$0.5) and increased fourfold in both with TSH. Nodules demonstrated significantly greater glucose-1-^{14}C oxidation and ^{32}P incorporation but similar TSH responsiveness. Colloid droplets increased equally in both tissues after TSH. Despite the iodide-trapping defect in nodules, basal and TSH-stimulated (50%) ^{131}I organification was similar to the normal. ^{131}I was incorporated in vitro into monoiodotyrosine (MIT), diiodotyrosine (DIT), and thyroxine in both tissues. The trapping defect was not due to differences in Na^+-K^+– or Mg^{++}-activated adenosine triphosphatases (ATPases) between nodules and normal. Thus "cold" benign and malignant nodules have a specific iodide-trapping defect unrelated to activation of adenyl cyclase–cAMP or its TSH responsiveness. This defect could be identical with that in goitrous cretins with defective trapping. (Research supported by a grant from NIH.)

82. Complement- and Leukocyte-Dependent Acute Immunologic Injury in the Rabbit Knee Joint. CLAUDE V. DESHAZO,* La Jolla, Calif. (introduced by John H. Vaughan**).

An in vivo model has been studied to determine the role of complement components and neutrophils in acute immunologic injury. The knee joint of rabbits served as an injection site for 300 μg rabbit anti-BSA purified by DEAE-cellulose chromatography. Antigen (BSA) given intravenously produced a reversed passive Arthus (RPA) injury in the synovial tissue. The combining of Ab and Ag at the vessel wall caused perivenular accumulation of polymorphonuclear (PMN) leukocytes. Loss of circulating RSA-^{125}I and thyroglobulin-^{131}I markers (15–25 $\times$ controls) and serum proteins (2–3 $\times$ controls) into the joint space was quantitated by a standardized irrigation method. Increased permeability in the RPA injury was detectable at 30 min, peaked at 2–3 hr, then declined at 5–6 hr. At 8–9 hr a second rise in permeability occurred, this accompanying a lesion histologically identical with that induced by intra-articular injection of preformed immune complexes in other experiments. After 12–24 hr the RPA injury virtually disappeared. The importance of PMN neutrophils in this experimental lesion was shown by (*a*) abolition of both early and late permeability in rabbits depleted of PMN's with nitrogen mustard, and (*b*) reinstitution of the lesion by injection of antibody with a PMN suspension into the joints of granulocytopenic animals. When a standard RPA injury was produced in rabbits genetically deficient in C-6 the parameters studied, along with the histologic picture of neutrophil accumulation, was retarded 3 hr. This RPA lesion in normal rabbits 90% depleted of C-3 by cobra venom factor showed an even more marked retardation with a 5 hr delay in vascular permeability. (Research supported in part by NIH Grant 5-TO1-GM 01733.)

83. The Site of Airways Obstruction in Asthma. PAUL DESPAS,* MICHELE LEROUX,* AND PETER T. MACKLEM, Montreal, Canada.

The maximum expiratory flow volume curve and inspiratory pulmonary resistance (R_L) during tidal breathing were measured in 25 asthmatics. Measurements were made while the patients were seated in a volume-displacement plethysmograph and breathed air and while they breathed a gas mixture with a lower density (helium 80%, oxygen 20%). He-O_2 caused an increase in maximum expiratory flow (MEF) measured at 50% vital capacity ($\dot{V}_{max_{50}}$) in 11 patients. This increase was similar to that predicted for normal subjects in whom $\dot{V}_{max_{50}}$ is inversely related to the square root of the density of the gas breathed (Wood and Bryan. 1969. *J. Appl. Physiol.* **27**: 4–8). In 14 patients either $\dot{V}_{max_{50}}$ did not change or else the change was considerably less than predicted. R_L fell significantly (> 20%) during He-O_2 breathing in 13 patients. In general, when R_L did not change there was little or no increase in $\dot{V}_{max_{50}}$. There was no relation between the severity of the airways obstruction at the time of study and the effects of He-O_2 on $\dot{V}_{max_{50}}$ or R_L. According to the equal pressure point (EPP) theory of Mead et al. (1967. *J. Appl. Physiol.* **22**: 95–168) MEF will be density dependent when the EPP's are in central airways and there is turbulent flow in airways between EPP's and the alveoli. EPP's will be in central airways in asthmatics only if the site of obstruction is in these airways. This was the case in 11 patients. When EPP's are in peripheral airways (due to peripheral obstruction) MEF will be independent of density and this was the case in 14 patients. We conclude that the site of airways obstruction in asthmatics may be in large or small airways. (Supported by a grant from the MRC of Canada.)

84. Computer-Oriented Analysis of Translocations in Human Chromosomes. RUSSELL W. DICKERSON,* NGUYEN-HUU XUONG,* AND OLIVER W. JONES,* La Jolla, Calif. (introduced by William L. Nyhan).

Lubs and Ruddle have recently shown that translocation mutants in human chromosomes occur at a frequency nearly twice that of other chromosome defects in man. Many of these are difficult to detect by usual visual interpretation of chromosome patterns. We have developed a computer-oriented program for chromosome analysis which has provided increased speed, accuracy, and sensitivity in detection of translocation mutants and carriers of balanced translocations. Characterization of chromosomes is based upon the following criteria: (*a*) arm ratio vs. the total length/sum of total lengths; (*b*) DNA density ratio vs. the DNA density sum/summation of DNA density sums; and (*c*) short arm center mass ratio vs. total center mass. These criteria have resulted in a rapid, reproducible classification which is consistent with pairing based upon standard visual interpretation. In cases of chromosome translocation, this method results in a more precise definition of the translocation exchange. Several families with unusual translocation defects have been studied. In one case, the translocation defect is obvious to visual as well as computer analysis. However, the computer-developed analysis established that a completely balanced translocation was present whereas this could not be concluded by visual examination alone. In all other cases studied, the translocation was either missed visually or only suspected. After analysis by criteria we have defined, the translocation and specific exchange were identified. This approach may be especially helpful in large screening surveys and in prenatal detection of certain chromosome mutations.

85. Examination of a Hypothesis for the Origin of Tubular Proteinuria. MARTIN J. DILLARD,* AMADEO J. PESCE,* AND VICTOR E. POLLAK, Chicago, Ill.

In patients with tubular proteinuria it is generally assumed that glomerular function is intact, and that the capacity of the damaged tubules to reabsorb the filtered proteins is decreased. Proteins of molecular size smaller than albumin are presumed to be filtered by the normal glomerulus more readily than is albumin. In tubular diseases, therefore, the decreased reabsorptive capacity of the tubules should be reflected in proteinuria characterized by an unusually high proportion of low molecular weight serum proteins. To test this hypothesis, the urinary proteins of two patients with Fanconi syndrome and of three patients in the early diuretic phase of acute tubular necrosis were studied by both Sephadex gel filtration and quantitative immunochemical analysis. By Sephadex G-75 gel filtration the ratio of the 40,000–60,000 mol wt peak/albumin peak was 1.7 (range 0.5–2.8). In proteinuria associated with glomerular damage published observations using immunochemical methods (Bienenstock, J., and J. Poortmans. 1970. *J. Lab. Clin. Med.* **75**: 297) indicate that the ratio of the low molecular weight proteins/albumin is 0.14. In the five patients with renal tubular disease, albumin and eight plasma proteins of mol wt 40,000–61,000 were measured by radial immunodiffusion. The ratio of the low molecular weight proteins/albumin was 1.2 (range 0.7–2.2). After recovery from tubular necrosis the ratio was 0.3 (range 0.2–0.5). These data are consistent with the hypothesis that tubular proteinuria results from a relative failure of absorption of low molecular weight proteins normally filtered by glomerulus. (Research supported by NIH Grants AM10314 and AM12330.)

86. Quantitation of Absorptive Capacity for Vitamin B_{12}, Ileal Absorptive Surface Area, and Total Number of Intrinsic Factor–Bound Vitamin B_{12} (IF-B_{12}) Receptor Sites in the Hamster. R. M. DONALDSON, S. R. ROBINS,* D. M. SMALL, AND V. I. MATHAN,* Boston, Mass.

The ileum efficiently absorbs small amounts of intrinsic factor–bound vitamin B_{12} (IF-B_{12}), but the absorptive capacity for IF-B_{12} is remarkably limited (10^{-9} moles in man). Since IF-B_{12} attaches to specific receptors on hamster ileal microvillous membranes (MVM), we wished to relate the total number of these surface receptors to the hamster's absorptive capacity for IF-B_{12}. Hamsters fed increasing B_{12} doses absorbed a maximum of 3.6×10^{-12} moles. In vitro uptake of IF-B_{12} by MVM was determined over a 40-fold range of IF-B_{12} concentrations. Analysis of binding data by Scatchard plot and Hill equation indicated that maximal MVM uptake of IF-B_{12} was 4.5×10^{-13} moles/mg MVM lipid and that IF-B_{12} binds to uniform membrane receptors ($K_d = 0.3 \times 10^{-9}$M) with each IF-B_{12} molecule binding to a separate receptor site. The surface area occupied by a monolayer of ileal MVM lipid measured in a Langmuir trough was 3920 cm²/mg. Assuming that lipid exists in MVM as a bilayer, we calculated that each cm² of ileal absorptive surface contains 1.4×10^8 IF-B_{12} receptor sites or one site per microvillus. Measurements of maltase and lipid in entire ileum and in purified ileal MVM allowed estimation of total ileal MVM lipid from which total ileal surface area was calculated. Thus hamster ileal absorptive surface totalled 6100 cm² and contained 8.5×10^{11} receptor sites which bind 8.5×10^{11} molecules (1.4×10^{-12} mole) of IF-B_{12}, a value approximating the in vivo absorptive capacity of the hamster (3.6×10^{-12} mole). These findings indicate that (*a*) IF-B_{12} complex attaches to a uniform species of receptor on hamster MVM, (*b*) each receptor site binds one molecule of IF-B_{12} complex, and (*c*) the limited number of these receptor sites per surface area of ileum accounts for the restricted capacity of the hamster to absorb vitamin B_{12}.

87. Tritiated Thymidine Labeling of *Mycobacterium leprae*. DAVID J. DRUTZ* AND MARTIN J. CLINE, San Francisco, Calif.

Lepromatous leprosy (LL) is characterized by continuous bacillemia. Untreated patients have 10^4–10^5 bacilli per ml of blood, largely within monocytes, and their blood is infective for the mouse footpad. Peripheral blood monocytes from bacillemic LL patients were cultivated in Leighton tubes containing 30% normal human serum in McCoy's medium at 31°C or 37°C for 1–3 wk. Resulting macrophages, many of which harbored *M. leprae*, were variously exposed to tritiated thymidine of high specific activity and radioautographs of cover slips were prepared. Nuclear labeling of macrophages was negligible. Single acid-fast bacilli (AFB) were seldom labeled, but those in bundles (globi) were consistently labeled.

Labeling was apparent within 6 days of macrophage cultivation and was heavier and more frequent at 31°C than 37°C. Although numerical increases in AFB were not demonstrated, bacillary elongation was obvious by 3 wk. Preliminary studies employing normal human macrophages inoculated with footpad-derived *M. leprae* suggest that thymidine labeling of AFB also occurs in this system. *M. leprae* has never been successfully cultivated in vitro; replication in the mouse footpad is the sole available method for evaluating its viability and requires 4–6 months. These studies demonstrate for the first time an approach to the evaluation of viability of *M. leprae* in tissue culture and support previous speculation that *M. leprae* has a preference for temperatures below 37°C. (Supported by NCI Grant CA 11067, the CDW Research Fund, and other University of California Research Funds.)

88. Extraction of Water and Electrolytes from Canine Legs with Osmotic Transients. RICHARD M. EFFROS,* New York (introduced by Francis P. Chinard**).

A new "background dilution" technique has been used to quantitate the extraction of water and electrolytes from resting canine hind limbs by hypertonic injections. 1.0 ml of hypertonic urea, glycerol, fructose, or sucrose was rapidly injected into the femoral artery of intact or skinned legs of anesthetized dogs and femoral venous blood was collected in serial samples at $\frac{3}{4}$ sec intervals. Hemoglobin, Na^+, and K^+ concentrations were measured in the collected blood. Extraction of fluid from the leg by osmotic transients reduced whole blood concentrations of hemoglobin, Na^+, and K^+. After urea injections, fractional reductions in Na^+ and K^+ averaged 0.93 ± 0.15 SD and 0.95 ± 0.20 SD ($n = 7$) of fractional reductions in hemoglobin. After sucrose injections, fractional reductions in Na^+ and K^+ were significantly less than with urea (Na^+, $P < 0.005$; K^+, $P < 0.05$) averaging 0.58 ± 0.13 SD and 0.75 ± 0.15 SD ($n = 6$) of fractional reductions in hemoglobin. These data indicate that fluid extracted by sucrose contains higher concentrations of these electrolytes than that extracted by urea. Intermediate values were obtained for glycerol and fructose. Data obtained in these and earlier studies indicate that muscle capillaries are significantly more permeable to small molecules such as urea than to large molecules such as sucrose. The present experiments suggest that urea rapidly crosses capillary walls and establishes an osmotic gradient across cell membranes, producing predominantly cellular dehydration. The relative absence of electrolytes in the extracted fluid probably reflects the low permeability of cell membranes to electrolytes. Sucrose remains largely intravascular in a single circulation and exerts an osmotic gradient across capillary walls, depleting the interstitial volume more than urea. The greater abundance of electrolytes in fluid extracted by sucrose is attributed to the ease with which they traverse capillary walls. (Supported by LIMRF and NIH grants.)

89. Dissociation of Sodium-Retaining and Kaliuretic Effects of Mineralocorticoids in Pregnancy. EDWARD N. EHRLICH* AND MARSHALL D. LINDHEIMER,* Chicago, Ill. (introduced by Richard L. Landau**).

The role of strikingly increased aldosterone secretion during normal pregnancy is poorly understood. Claims that aldosterone secretion exceeds physiologic requirements are based on reports that administered mineralocorticoids or adrenocorticotrophic hormone (ACTH) fail to induce sodium retention, i.e., pregnant women have escaped from the renal effects of aldosterone. Mineralocorticoid activity was increased or decreased experimentally during metabolic balance studies performed on third trimester volunteers. Sodium content of constant diets ranged from 72 to 340 mEq/day. In 14 studies deoxycorticosterone acetate (DOCA), 9α-fluorocortisol, or ACTH was administered for 4–12 days. Sodium retention averaging 265 mEq/study and suppression of aldosterone consistently occurred. Discontinuance of treatment was followed by natriuresis. When heparinoid, R01-8307, an inhibitor of aldosterone secretion, was administered to three gravidae, substantial urinary sodium wasting occurred, while aldosterone excretion was still greater than normal nonpregnant values. Potassium excretion was unaffected by the above experimental maneuvers. Mineralocorticoid-induced sodium retention was not accompanied by kaliuresis, and heparinoid inhibition of aldosterone secretion was not associated with potassium retention. For instance, no kaliuresis occurred when a subject's sodium intake was increased from 93 to 195 mEq/day during mineralocorticoid administration. In a preliminary study, DOCA "escape" was induced in a normal man and kaliuresis occurred. While continuing DOCA, addition of progesterone 100 mg/day, did not alter sodium but decreased potassium excretion to control values mimicking observations in pregnancy. We conclude that increased aldosterone secretion during pregnancy is not excessive but is required for volume homeostasis. Our results contrast with previous reports that pregnant women have escaped from sodium-retaining effects of administered mineralocorticoids but indicate that the renal tubule is unresponsive to their kaliuretic action. This apparent dissociation may be due to progesterone.

90. Guinea Pig Leukemia L_2C: Immunoprotection and Immunotherapy. LEONARD ELLMAN* AND IRA GREEN, Bethesda, Md.

Guinea pig leukemia L_2C, a lymphatic leukemia which arose spontaneously in an inbred strain 2 guinea pig, has been serially transplanted in this strain for over 15 yr. Immunization of strain 2 guinea pigs with large numbers of irradiated L_2C cells intradermally or emulsified in complete Freund's adjuvant and injected in the footpads protects almost all animals against subsequent lethal leukemic challenge. Animals similarly immunized with normal strain 2 lymphocytes or a chemically induced syngeneic hepatoma were not protected. Thus, the L_2C leukemia possesses a tumor-specific transplantation antigen. When specifically immunized animals are challenged intradermally with L_2C cells, a delayed hypersensitivity skin reaction occurs. Cellular immunity is also demonstrated in vitro in a one-way mixed lymphocyte reaction. Adoptive transfer of lymph node and spleen cells from immunized animals into normal recipients afforded complete protection against leukemic challenge. Unlike familiar murine leukemia models, serum antibodies to L_2C cells in immunized animals could not be detected by a variety of in vivo and in vitro assays. Cellular immunity thus appears to be the major factor in the protection observed against L_2C

leukemia. Immunotherapy of the leukemia by active immunization or adoptive transfer was also shown to be successful when initiated early in the course of the disease. Our studies in guinea pigs suggest that vigorous immunization of patients with leukemia with autologous tumor cells during periods of remission may achieve successful immunotherapy. Our findings also demonstrate that certain immunization techniques may be more likely to promote development of cellular immunity with little if any humoral and potentially enhancing antibody.

91. Effect of Glucose and Fructose on Hepatic Triglyceride Synthesis and Serum Triglyceride. HAROLD J. FALLON AND MARION B. WADDELL,* Chapel Hill, N. C.

The mechanism of carbohydrate induction of hypertriglyceridemia was studied in male rats. Triglyceride (TG) synthesis from glycerophosphate-^{14}C (GP) was measured in liver homogenates from rats fed 74% glucose or fructose and isocaloric control diets for 12 hr to 6 days. The rate of glycerol-^{14}C incorporation into hepatic and serum glycerides was measured under the same conditions, in vivo. Hepatic GP and serum glycerol were unchanged but hepatic TG level was increased 2-fold by glucose and 5-fold by fructose diets. Serum triglyceride was increased less than 25% by 74% glucose but rises over twice normal after 5 days of 74% fructose. Both diets produce 2- to 4-fold increases in TG formation from GP-^{14}C by liver homogenates. This rise is maximum at 2 days with glucose and by 5 days with fructose. Increased diglyceride (DG) formation also occurs and these changes are not accompanied by a rise in hepatic acylthiokinase, glycerokinase, or diglyceride acyltransferase activities. The increased DG and TG formation is accompanied by a 2-fold increase in glycerol-^{14}C incorporation into hepatic DG and TG in vivo. After triton administration to block TG uptake, glycerol-^{14}C incorporation into serum TG was increased 2-fold by both glucose and fructose. The results suggest that high glucose and fructose diets enhance hepatic TG synthesis and release by accelerating the conversion of GP to TG. The higher serum levels of TG in rats fed fructose presumably results from slower TG removal than in rats fed glucose. (Supported by grants from NIH.)

92. Treatment of Wegener's Granulomatosis with Cyclophosphamide. ANTHONY S. FAUCI* and SHELDON M. WOLFF, Bethesda, Md.

The outcome of generalized Wegener's granulomatosis (GWG) is inevitably fatal, although a few remissions induced by various cytotoxic agents have been reported. 11 patients, 8 males and 3 females, ages 24–65 yr, with biopsy-proven Wegener's granulomatosis were studied. Two had localized pulmonary Wegener's granulomatosis (LWG) and the others had renal biopsies consistent with the diagnosis. Most of the patients were admitted to the study while on corticosteroid therapy, which was thereafter discontinued. Three patients, all of whom received corticosteroids, died before (less than 1 wk) adequate treatment with cyclophosphamide was begun. In the remaining eight, cyclophosphamide was begun in doses of 1 mg/kg by mouth except in one with rapidly advancing renal failure who initially received intravenous therapy. All the patients have been maintained on oral therapy. The patients with GWG all had lowering of the BUN, rise in creatinine clearance, clearing of urinary sediment, and in four out of six disappearance of proteinuria. One patient, with an initial BUN of 117 mg/100 ml and a creatinine clearance of 2 ml/min, responded to 6 months of cyclophosphamide with a fall in BUN to 32 mg/100 ml and a rise in creatinine clearance to 63 ml/min. However, he died (age 66) of an acute myocardial infarction. All the others are alive with a mean duration of illness of 34 months. In the five remaining patients with renal disease serial renal biopsies showed total disappearance of disease activity and scarring consistent with previously active disease. The two patients with LWG are asymptomatic with marked roentgenographic improvement. This study clearly demonstrates that striking and long-term remissions of Wegener's granulomatosis are attainable with cyclophosphamide therapy.

93. Stimulation of Hepatic Collagen Formation by Ethanol Consumption. LAWRENCE FEINMAN* AND CHARLES S. LIEBER, New York.

Because of the clinical association between cirrhosis and alcoholism, the effect of ethanol on collagen formation was investigated. 16 baboons were pair fed protein-restricted diets (7% of total calories) with 36% of calories either as ethanol or carbohydrate (controls) for 7 months. Livers from ethanol-fed animals had a 2.5-fold ($P < 0.01$) increase in hydroxyproline concentration, indicative of collagen accumulation. To determine whether ethanol consumption can produce collagen accumulation even when dietary protein is adequate, 16 additional baboons were pair fed with diets containing 20% of calories as proteins and 36% of calories either as ethanol or carbohydrate for 7 months. In addition, rat littermates were pair fed nutritionally adequate liquid diets with 18% of total calories as protein and 36% either as ethanol or carbohydrate. In primates fed ethanol, hydroxyproline concentration doubled ($P < 0.02$) after 7 months; in rats, it increased by 69% after 7 months (435 vs. 258 mg/g; eight pairs; $P < 0.001$) and 107% after 14 months (552 vs. 266 mg/g; five pairs; $P < 0.05$). To determine whether enhanced synthesis of collagen participates in its accumulation, proline-^{14}C incorporation into hepatic collagen hydroxyproline was measured in liver slices: in ethanol-fed rats, incorporation significantly increased by 50% at 7 months. To study the mechanism of the increased collagen formation, rat and primate livers were assayed for collagen proline hydroxylase activity, which is necessary for collagen synthesis and represents the earliest indicator of hepatic fibrosis. Enzyme activity rose 50% (nine pairs; $P < 0.01$) in rats fed ethanol 1 month and 100% (seven pairs; $P < 0.01$) after 6 months: it increased 44% in primates after 7 months (12 pairs; $P < 0.05$). In conclusion, feeding ethanol with adequate or protein-restricted diets results in hepatic collagen accumulation, enhanced collagen formation, and increased hepatic collagen proline hydroxylase activity. (Research supported by USPHS Grants MH 15558 and AM 12511, the Veterans Administration and Laboratory for Experimental Medicine and Surgery in Primates, N. Y.)

94. Effects of Bile Salts and Detergents on Ion Transport of Absorbing Jejunum. DANIEL S. FELDMAN,* SHELLY RABINOVITCH,* AND ELAINE B. FELDMAN,* Brooklyn, N. Y. (introduced by David M. Kydd**).

Active glucose transport by rat jejunal mucosa generates a transmural potential difference (E_{tmp}) augmenting the resting potential. When E_{tmp} is reduced to zero by an external current source, current flow (I_{sc}) represents the ion mass (mostly sodium) undergoing active transport. I_{sc} varies linearly with E_{tmp}; tissue resistance (R) is constant. Bile salts, not actively transported by jejunum, affect E_{tmp}, I_{sc}, and R in opposite ways from anionic and neutral detergents, but resemble cationic detergents. Bile salts (deoxycholate, taurocholate, taurodeoxycholate, glycocholate) in solution bathing jejunal mucosa in an Ussing chamber increased E_{tmp}, 42.6 ±7.8% (mean ±SEM, n = 14). I_{sc} changed minimally, −9.5 ±6.8%; R increased significantly, 93.9 ±20.3%. Nonionic detergents increased E_{tmp}, 9.6 ±11.2% (n = 17) significantly increasing I_{sc}, 50.9 ±17.3%, leaving R unchanged, −3.9 ±12.9%; anionic detergents yielded comparable results. Cationic detergents were toxic, decreasing E_{tmp}, −15.5 ±4.2%, but resembled bile salts in increasing R, 21.9 ±6.4%, and decreasing I_{sc}, −28.4 ±3.9%. When control I_{sc} was below 27 $\mu A/cm^2$, reflecting reduced tissue viability, bile salts resembled anionic and neutral detergents: E_{tmp} increased, 24.3 ±6.6%; I_{sc}, 41.1 ±12.0%; R was unchanged, −3.8 ±11.0% (n = 20). Conjugated bile salt effects, quantitatively unchanged at all concentrations above the critical micellar concentration (4–14 mmoles/liter), were specific for mucosal application. These data suggest (*a*) constraints on ion mobility (R) are factors in E_{tmp}; (*b*) specific bile salt effects on ion transport in the intestine exist independent of detergency properties; (*c*) steric and charge distribution factors determine detergent effects on mucosa; (*d*) molecular species of bile salts are active; and (*e*) bile salt effects are influenced by mucosal vitality. (Supported by NIH Grant HE 10742.)

95. Starvation in Human Pregnancy: Placental-Maternal and Hormone-Substrate Interactions. PHILIP FELIG,* YOUNG J. KIM,* AND VINCENT LYNCH,* New Haven, Conn. (introduced by P. K. Bondy**).

Human placental lactogen (HPL) has been proposed as the anticatabolic hormone promoting maternal protein conservation in late pregnancy. Its physiologic role has not been established, however, since acute alterations in circulating metabolic fuels (glucose, amino acids) fail to alter its secretion. We have now shown that in prolonged fasting in mid-pregnancy, during which maternal catabolic gluconeogenic mechanisms compete for available amino acids with placental transport processes necessary for fetal protein synthesis, HPL secretion is increased. Seven nonpregnant women (NON-PREG) and 14 physically healthy pregnant women (PREG) undergoing therapeutic abortion for psychiatric reasons during wk 16–22 of pregnancy, were fasted for 84 hr. Plasma glucose was significantly lower in PREG reaching its nadir in 36 hr and falling to 47 ±3 mg/100 ml vs. 61 ±3 mg/100 ml in NON-PREG. Plasma insulin fell to 4.1 ±0.4 μU/ml in PREG, 50% below NON-PREG levels. Maternal hepatic gluconeogenesis, as reflected in urinary urea excretion, was not increased in PREG. Plasma levels of alanine, the primary hepatic gluconeogenic substrate, were reduced by 20% in PREG ($P < 0.001$) and fell by 15–30% during the fast. However, alanine levels in amniotic fluid were 30–40% higher than in maternal plasma in both the fed and fasted state. Administration of alanine at the termination of the fast resulted in a 50% increase in blood glucose. Plasma HPL in PREG was 360 ±35 mμg/ml in the basal state, and remained unchanged for the first 36 hr, but increased 30–40% ($P < 0.001$) after 60 hr of fasting. We conclude that substrate presentation is the prime regulatory factor in maternal hepatic gluconeogenesis. Transfer of amino acids to the conceptus limits their availability for maternal gluconeogenesis. Prolonged fasting is a potent stimulus of secretion of HPL which may retard maternal catabolism by enhancing substrate transfer to the fetus. (Supported by NIH Grants AM 13,526 and RR-00125.)

96. Collagen Metabolism in Zinc Deficiency. FELIX FERNANDEZ-MADRID,* ANANDA S. PRASAD, AND DONALD OBERLEAS,* Detroit, Mich.

Impaired wound healing and growth retardation as a result of zinc deficiency in man has been reported recently. This suggested the possibility that zinc may have a functional role in the metabolism of collagen. Experiments, therefore, were designed to study biosynthesis and aggregation of collagen in zinc-deficient animals and their pair-fed controls. After 5 wk on a zinc-deficient diet, rats were implanted with polyvinyl sponges subcutaneously. After 6 days sponges were removed and capsules from zinc-deficient and pair-fed animals were incubated in vitro in Krebs-Ringer phosphate pH 7.4, with 0.07 μmole of each of 18 amino acids, 1 mg/ml *d*-dextrose, and the addition of proline-^{3}H. After varying periods of incubation, tissues were homogenized in a hypotonic buffer. Ribosomes isolated by differential centrifugation were further fractionated by zonal centrifugation through sucrose gradients. Collagen was sequentially extracted in 0.15 M NaCl, 1.0 M NaCl, and 0.5 M acetic acid. Other parameters included in our studies were wet and dry weights of the tissues and their content of total nitrogen, hydroxyproline, DNA, and RNA. RNA/DNA ratio was significantly lower in zinc-deficient tissues as compared to their pair-fed controls (1.1 ±0.14 vs. 1.5 ±0.14, $P < 0.001$). Total collagen was also significantly decreased in deficient tissues (57.0 μg vs. 105.5 μg collagen per mg tissue, $P < 0.001$), but extractable collagen was significantly greater (38.5 vs. 23.3% soluble collagen, $P < 0.01$) in comparison to pair-fed controls. In addition, zinc-deficient tissues showed a greater proportion of monosomes and less polyribosomes formation, with a shift of collagen-synthesizing polyribosomes to a smaller aggregate size. It is concluded that in zinc deficiency there is a selective abnormality in the biosynthesis and aggregation of collagen.

97. The Effect of Uterine Blood Flow upon Uterine Renin Secretion. THOMAS F. FERRIS,* CLARK W. DISTELHORST,* RACHEL W. ABERNETHY,* AND JAY H. STEIN,* Columbus, Ohio (introduced by James V. Warren**).

To evaluate the effect of variation in uterine blood flow upon uterine renin secretion experiments were carried out in

nephrectomized pregnant rabbits. Cardiac output was measured by dye dilution and uterine blood flow measured by the distribution to uterus and placenta of a known number of radioactive microspheres injected into the left ventricle. 24 hr postnephrectomy uterine vein plasma renin activity (PRA) was significantly higher than aortic: uterine vein 731 ±120 mμg/100 ml per hr, aortic 580 ±86 ($P < 0.01$). Reduction in uterine blood flow induced by partial ligation of the uterine arteries caused a significant increase in uterine vein PRA from 2825 ±1033 to 5600 ±1423 ($P < 0.01$), which represented an increase in absolute renin secretion since aortic PRA rose similarly from 1660 ±700 to 2840 ±700 ($P < 0.01$). Angiotensin (10 mμg/kg per min) caused a 5–10 mm Hg rise in blood pressure with no significant change in cardiac output (control 141 ±7 ml/kg per min, angiotensin 143 ±8 ml/kg per min), but caused an increase in percentage of cardiac output to uterus 2.5 ±0.5% to 3.98 ±0.7% and placenta 1.76 ±0.5% to 2.98 ±0.6%. Accompanying this increase in uterine and placental blood flow there was a significant fall in both uterine vein PRA, 731 ±125 to 491 ±124 ($P < 0.01$), and aortic PRA, 580 ±86 to 391 ±91 ($P < 0.01$). These findings suggest that uterine renin might be involved in the regulation of uterine blood flow, secretion being increased in response to a reduction in flow and the resultant rise in systemic angiotensin causing an increase in uterine blood flow.

98. Control of Aldosterone in the Inappropriate Antidiuretic Hormone (ADH) Syndrome (SIADH). MARSHAL FICHMAN,* ANDREW MICHELAKIS,* AND RICHARD HORTON, Los Angeles, Calif., and Nashville, Tenn.

Plasma renin (PRA) and aldosterone (A) by double isotope or immunoassay was measured in four patients with SIADH and in three normals given antidiuretic hormone and H_2O (ADH-Normals), serum Na < 120 mEq/liter. Despite an increase in total body water (^3H_2O) and extracellular water (^{82}Br), supine A on 150 mEq Na and 80 mEq K diet was 2–7 mμg/100 ml (normal 5.2 ±1.8 SD mμg/100 ml). Standing for 1 hr increased A 4- to 6-fold in both SIADH and ADH-Normal. Pressor angiotensin or ACTH also normally increased A 4- to 8-fold and fludrocortisone suppressed A. Despite these normal A responses, supine PRA was undetectable or very low ≤ 100 mμg (normal 180 ±30 mμg/100 ml) in both SIADH or ADH-Normal. Standing did not increase PRA in SIADH but it did increase to 150 mμg/100 ml in ADH-Normal (normal standing PRA 400 ±180 SD mμg). On 10 mEq Na, 80 mEq K diet, supine A increased to 22 mμg/100 ml, while PRA remained undetectable. Only the combination of 10 mEq Na diet and standing caused a rise (subnormal) in PRA while A increased normally. H_2O restriction in SIADH markedly increased both PRA and A. We concluded that (*a*) despite H_2O excess and high urine sodium, plasma A and the A response to endogenous and exogenous stimuli are normal in both SIADH and ADH-Normals; (*b*) plasma renin (PRA) is suppressed unless there is H_2O restriction; and (*c*) this study reveals for the first time in man a dissociation between PRA and A. Acute increases in aldosterone without change in renin activity, Na_s, or K_s indicates the presence of another control mechanism for aldosterone in man. (Research supported by a NIH grant.)

99. Actions of Human Growth Hormone upon Peripheral Tissues: a Reconsideration. S. EDWIN FINEBERG* AND THOMAS J. MERIMEE,* Boston, Mass. (introduced by Robert W. Wilkins**).

Human growth hormone (HGH) at physiologic concentrations has not been definitely shown to have acute effects upon the metabolism of peripheral fat or muscle of humans. During HGH perfusion of the forearm, measurements were made of blood flow and of arteriovenous differences (A-V) for plasma potassium, free fatty acids (FFA), and glucose across peripheral muscle and adipose tissue. After a 1 hr control period, a 30 min perfusion of HGH (0.02 μg/kg of body weight) resulted in a measured mean venous HGH concentration of 29.7 ±2.4 mμg/ml in both the deep vein (DV) which drains mostly muscle and in the superficial vein (SV) which drains mostly adipose tissue. Proper venous sampling was assured by the significant differences in A-V for FFA and potassium between the deep and superficial beds. In contrast to previous findings using pharmacologic perfusions of HGH, no increases in muscle uptake or adipose tissue output of FFA were discernible during the 30 min HGH perfusion or during the 90 min recovery period. Neither were there increases of potassium uptake across fatty tissue. Glucose uptake as reflected by (A-V) decreased promptly during the perfusion. Deep vein glucose difference (A-DV) was 0.42 ±0.03 μmole/ml basally and decreased to 0.21 ±0.04 μmole/ml during the perfusion. Simultaneously, superficial vein glucose difference (A-SV) decreased from 0.38 ±0.05 μmole/ml to 0.20 ±0.05 μmole/ml. The significant depression of glucose uptake continued in the deep vein for 45 min after the perfusion. These data indicate that the lipolytic and insulin-like activity of HGH in man cannot be accounted for by actions of physiologic concentrations of HGH upon peripheral muscle or fat metabolism, because at such concentrations HGH acutely depressed glucose uptake across peripheral muscle and adipose tissue without altering lipolysis. No insulin-like activities of HGH upon glucose or FFA were noted.

100. Human Small Bowel Propulsive Force. JIMMY G. FINLEY* AND CHARLES E. POPE II,* Seattle, Wash. (introduced by Wade Volwiler**).

A force transducer was constructed by attaching a 10 mm sphere to a mercury-in-sialastic strain gauge. Propulsive force exerted on the sphere causes elongation of the gauge and change in electrical resistance. Three infused (17 μl/sec) catheters whose openings were 1.5, 4, and 12 cm from the sphere were attached to the transducer. 10 normal volunteers were studied with this assembly under fluoroscopic control. As the transducer passed from antrum to duodenum, an increase in base line force values ranging from 80 to 300 g was recorded. A comparable fall in base line force values was indicated when the transducer was withdrawn from duodenum to antrum. At the ligament of Treitz, two types of transducer output were observed. Single monophasic waves of 2–4 sec duration and 2.6 g mean amplitude (range 3–100 g) were recorded, associated with pressure spikes sensed by the nearby pressure catheters. Increases and decreases in base line force values, averaging 29.2 g (range 13.7–55 g) were also observed.

eseh T were not necessarily related to pressure events occurring nearby. Rarely, monophasic force waves were superimposed on base line force changes. This was most often seen during bursts of pressure spikes occurring at 11/min. Marked variation in force patterns were observed from minute to minute in any one subject and between different normal subjects. It is concluded that a steady aboral propulsive force is exerted by normal small bowel. Force values provide an assessment of small bowel muscle activity not detectable by intraluminal pressure measurements. Force values can be used for prolonged monitoring of small bowel activity. (Research supported by grants from VA Part I, and NIH 5-T01-AM05099-14.)

101. False Neurochemical Transmitters in Hepatic Failure. JOSEF E. FISCHER* AND J. HOWARD JAMES,* Boston, Mass. (introduced by W. Gerald Austen).

The following studies relate to a hypothesis which suggests that accumulation of false adrenergic neurochemical transmitters (FNT) may be one mechanism in the causation of hepatic coma. Catecholamines, particularly norepinephrine (NE) and dopamine, play an important role in normal brain transmission. In the peripheral sympathetic nervous system, where NE is the putative transmitter, other sympathomimetic amines, such as octopamine (OCT), may replace NE and act as FNT. FNT precursors, such as tyramine, are produced in the gut and, once absorbed, usually catabolized by the liver. When hepatic function is impaired and/or blood shunted around the liver, these amines may accumulate, replace normal transmitters, and result in asterixis, hepatic coma, and the high output state of hepatic failure. β-Hydroxylated phenylethanolamines were assayed by enzymatically labeling amines with a methyl-^{14}C group, selective extraction, and chromatography. 2 months after portacaval (PC) shunt in rats, brain OCT was elevated threefold (sham 2.88 $\pm$1.06 ng/g, shunt 9.3 $\pm$1.8 ng/g, n = 13, $P < 0.01$). In "acute hepatic coma," produced by ligation of the hepatic artery 24 hr after PC shunt, brain OCT was elevated in rats who appeared "somnolent" (sham 4.9 $\pm$0.7; "coma" 13.3 $\pm$0.9, n = 4, $P < 0.05$). As "coma" progressed, brain OCT was markedly elevated (sham 4.6 $\pm$0.8; "coma" 34.1 $\pm$4.2, $P < 0.01$, n = 4). In patients in hepatic coma urinary excretion of OCT was elevated (controls 1.81 $\pm$0.35 μg/24 hr; coma 4.73 $\pm$0.41, n = 6, $P < 0.05$). This hypothesis may explain the efficacy of L-dihydroxyphenylalanine (L-DOPA) in the treatment of hepatic coma, and the use of systemic aramine or dopamine infused into the renal artery in treatment of the hepatorenal syndrome. L-DOPA, a precursor of NE and dopamine, may replenish normal transmitter stores. Case reports illustrating the effect of L-DOPA on cardiovascular parameters and mental status in hepatic coma, and the effects of aramine in the hepatorenal syndrome will be presented. (Supported in part by NIH Grant FR-05486-08.)

102. Thyroidal Thyronine and Nonthyronine Iodine Secretion in Euthyroid Subjects. D. A. FISHER,* J. H. DUSSAULT,* AND T. H. ODDIE,* Torrance, Calif., and Little Rock, Ark. (introduced by J. W. St. Geme, Jr.).

Thyroxine (T_4) iodine turnover (H_4), triiodothyronine (T_3) iodine turnover (H_3), and thyroidal iodine accumulation (A) have been quantified simultaneously in 22 euthyroid adult subjects. T_4 and T_3 kinetic studies were conducted after simultaneous doses of T_3-^{131}I and T_4-^{125}I. T_3 kinetics were assessed simultaneously by urine, plasma, and whole body counting for three independent estimates of degradation rate. Serum T_4 was measured using a protein-binding assay and serum T_3 by a double-column assay procedure which completely separates T_3 and T_4 and includes a correction for T_4 conversion to T_3. Thyroidal nonthyronine iodine secretion was calculated as $A-H_4-H_3$. Mean iodine intake was 556 μg/day and mean A was 126 μg/day. Mean values for H_4 and H_3 were 57 and 19 μg/day or 58 and 11 μg iodine per day respectively. The T_4/T_3 turnover ratio approximated 4/1. Mean thyroidal nonthyronine iodine secretion was 57 μg daily. These data suggest that at a mean iodine intake of 500–600 μg daily, T_4 is the major thyronine secreted by the thyroid gland. If hormone turnover studies reflect tissue utilization, then T_3, which is 2–4 times more potent than T_4, may account for $\frac{1}{3}$ to $\frac{1}{2}$ of the total effect of secreted thyronines. At this level of iodine intake, nonthyronine iodine secretion (presumably iodide) approximates T_4 iodine secretion and exceeds T_3 iodine secretion 5 times. This is the first quantitative estimate of thyroidal nonthyronine iodine secretion in man. (Supported by USPHS Grant HD-04270.)

103. On the Structure and Function of Y Protein. G. FLEISCHNER,* S. MISHKIN,* H. REYES,* J. ROBBINS,* A. J. LEVI,* Z. GATMAITAN,* AND I. M. ARIAS, New York.

We have described a protein (Y protein) in liver cytoplasm which binds various organic anions including bilirubin, dyes, drugs, cholecystographic agents, and steroids. Y protein has been purified from rat liver as judged by acrylamide-gel electrophoresis and electrofocusing, and a monospecific antibody has been produced. Y is a basic protein of mol wt 36,000. Chemical, physical, and immunologic studies show that the Y protein, the hepatic cortisol metabolite-binding protein of Litwack, and the azocarcinogen-binding protein of Ketterer appear identical. Quantitative immunoprecipitation reveals Y to constitute 5% of hepatic, 1% of small intestinal, and 1% of kidney cytoplasmic proteins. Immunofluorescent studies reveal localization of Y in parenchymal liver cells, renal tubules, and small intestinal mucosal cells. Administration of phenobarbital (0.2–15 mg/100 g per day for 10 days) progressively increased Y concentration to 230% of control values. This was associated with significant increase in plasma disappearance rates and hepatic content of Bromsulphalein (BSP), indocyanine green, and bilirubin. Phenobarbital (8 mg/100 g per day for 4 days) also increased hepatic relative storage of BSP without changing biliary Tm BSP. Using a double isotope (^{3}H- and ^{14}C-labeled leucine) technique (1969. *J. Biol. Chem.* **244**: 3303), in normal and phenobarbital-treated rats, we have demonstrated that the increased concentration of Y results from enhanced synthesis (induction) and not decreased degradation (stabilization). These observations support our hypothesis that Y protein plays an important role in the selective transfer of various organic anions from plasma into the liver. (Research supported by grants from NIH.)

104. Determinants of Systolic Time Intervals in the Human Heart. ATHANASIOS P. FLESSAS,* JUAN C. POMPOSIELLO,* GILBERT P. CONNELLY,* AND THOMAS J. RYAN,* Boston, Mass. (introduced by L. E. Braverman).

To analyze the determinants of the systolic time intervals, a beat-to-beat analysis of end-diastolic volume (EDV), stroke volume (SV), ejection fraction (EF), velocity of fiber shortening (Vcf), and aortic pressure (Ao) was correlated with simultaneously measured preejection period (PEP) and left ventricular ejection time (LVET) in 13 patients with atrial fibrillation who underwent LV cineangiography. Four to six consecutive beats demonstrating a wide variation in EDV (range 20–200%) and Ao pressure were studied in each patient. PEP varied inversely with EDV ($r = -0.92$), Vcf ($r = -0.67$), and EF ($r = -0.94$), whereas there was a direct relationship to Ao diastolic pressure ($r = 0.76$). In the failing heart with depressed Vcf, PEP was in normal range for the beats with large EDV. For the group as a whole, PEP alone could not discriminate normal from abnormal ventricular function since for any given PEP there was a wide variation in EDV and Vcf. LVET was directly related to SV ($r = 0.94$) and, for a given SV, was inversely related to EDV and Vcf. The ratio PEP/LVET was inversely related to EF ($r = -0.94$) but not to Vcf when normalized for end-diastolic length (circumference/sec, $r = -0.39$). Patients with mitral regurgitation and those with small EDV had higher ratios. These data suggest that the systolic time intervals are determined by EDV and Ao diastolic pressure as well as by the inotropic state of the heart. Accordingly, their application as an index of ventricular function requires a concurrent estimation of both the preload and afterload under which the heart is operating.

105. Mannitol Reversal of "No-Reflow" after Renal Ischemia. JORGE FLORES-CALLE,* CLYDE H. BECK, JR.,* DONALD R. DI BONA,* CARLOS MARCILIO,* AND ALEXANDER LEAF,** Boston, Mass.

Hypoxia interferes with the energy supply for extrusion of sodium from cells. The resulting cell swelling after transient occlusion of the major blood supply to an organ may prevent reflow of blood to part or all of the parenchyma. To test this hypothesis, ischemia was produced in heparinized rats by clamping renal arteries for variable periods. The effect of increasing blood osmolality on the vascular pattern and functional impairment of the ischemic kidney was studied. After 2 hr of clamping of renal arteries and 10 min of reflow, silicone rubber injections of the vasculature revealed nonrefilling of vessels primarily in the subcortical regions of the kidney. This postclamping vascular obstruction was largely prevented by the intravenous injection of 1 ml of 25% mannitol per 100 g of rat during the last 10–20 min of the ischemic period. Serum osmolality rose from a mean of 304 mOsm/kg before mannitol infusion to 360 mOsm/kg 5 min after mannitol infusion ended. The BUN of 12 control ischemic animals and 10 mannitol-treated ischemic animals 24 hr after ischemia ended was 193 ± 9 mg/100 ml and 140 ± 8 mg/100 ml ($P < 0.001$), respectively. The mannitol-induced rise in serum osmolality may reduce the degree of ischemia-induced azotemia and vascular abnormality by prevention of cellular swelling.

106. Diagnostic Effectiveness of High-Frequency Electrocardiography. NANCY C. FLOWERS* AND LEO G. HORAN, Augusta, Ga.

Over a 10 month period 92 patients referred for cardiologic evaluation were studied for the incidence of QRS notching with high-frequency, high-speed electrocardiograms. The cardiac status of the same patients was also evaluated radiographically with barium swallow, and hemodynamically at cardiac catheterization. Based on these studies the patients were placed into groups without ventricular enlargement (VE), isolated right ventricular enlargement (RVE), isolated left ventricular enlargement (LVE), and biventricular enlargement (BVE), In no instance did an individual with a normal sized heart have more than six notches in three mutually orthogonal leads combined, and the group had a mean notch count of 2.9. The etiologies of their heart disease were varied, but valvular heart disease of rheumatic and congenital origins predominated. Regardless of etiology, the subjects with no VE could be separated from those with BVE ($P < 0.001$) and from the groups with isolated VE ($P < 0.005$) on the basis of notch count alone. Groups with BVE were also easily separable from groups with isolated VE ($P < 0.001$). We concluded that results of evaluation of the QRS complex for the occurrence of high-frequency notching correlate well with the dynamic evidence of isolated for combined VE in a living population. This could potentially provide a simple, safe, noninvasive means of screening large populations for the presence of heart disease. When combined with other indices such as those from a screening phonocardiogram, the criterion of high-frequency notch counting may provide the additional jump in diagnostic effectiveness necessary for a high level of screening accuracy. (VA, NHLI, AHA.)

107. Lithium Polyuria: an Example of Reversible Nephrogenic Diabetes Insipidus. JOHN N. FORREST, JR.,* ALLAN D. COHEN,* JORGE TORRETTI,* AND FRANKLIN H. EPSTEIN,** New Haven, Conn.

About one-third of patients given lithium for treatment of manic-depressive disorders complain of polydipsia and polyuria which occasionally reach levels expected in diabetes insipidus. This is especially interesting since lithium is concentrated in both the hypothalamus and the renal medulla and might therefore interfere selectively with molecular mechanisms concerned with the release or the action of vasopressin. The phenomenon can be reproduced in 200-g rats by giving 3–4 mEq Li^+/kg intraperitoneally daily. Massive polyuria (75–100 ml/day) develops, with Uosm below 160 mOsm/kg, but as long as water intake is not limited the BUN does not rise. The polyuria and polydipsia disappear when lithium is stopped. Polyuria is not due to hypothalamic diabetes insipidus since the hyposthenuria is resistant to repeated injections of 1 U of vasopressin in oil q 4 hr $\times$ 3, a dose that produces a concentrated urine in control rats in which "physiological diabetes insipidus" and comparable polyuria is evoked by adding glucose to their drinking water. Lithium is said to inhibit adenyl cyclase in vitro so it was important to test the effect of cyclic adenosine monophosphate (AMP). 1–5 mg of dibutyryl cyclic AMP given intravenously transiently interrupts water diuresis in normal rats anesthetized with Inactin, raising Uosm from less than 160 mOsm/kg

to 450–700 mOsm/kg. During lithium polyuria with Uosm under 160, similar doses of dibutyryl cyclic AMP failed to increase Uosm above plasma osmolality; large doses of aqueous pitressin were also ineffective in concentrating the urine. Lithium appears to produce reversible nephrogenic diabetes insipidus, perhaps by interfering with the action of vasopressin at a chemical step beyond the formation of cyclic AMP. (Supported by Grants HE-00834 and AM 5015 from NIH.)

108. Dependence of Tension in Heart Muscle on the Resting Potential. HARRY FOZZARD* AND RAY GIBBONS,* Chicago, Ill. (introduced by Leif B. Sorensen).

While the cardiac action potential acts as the normal trigger for cardiac muscle contraction, it also plays a role in regulating the magnitude of the contraction. This relationship between membrane voltage and isometric tension has been difficult to measure until recently, when a satisfactory voltage-clamp technique became available. We have studied systematically the relationship in sheep cardiac Purkinje fibers, and found activation, relaxation, and recovery of contraction to be a complex function of voltage and time. Contraction obtained in response to stepwise depolarizations of membrane voltage depended on the duration of the depolarization and on the voltage established by the depolarizing step. There was also a strong relationship between the size of the tension response and the resting or holding potential of the fiber before the clamp. If clamps were made from a holding potential more negative than −75 mv, the contraction obtained on depolarization to a voltage near that of the normal action potential plateau was maximal. For holding potentials more positive than − 75 mv, less contraction was obtained, and for potentials more positive than − 20 mv, no tension response could be obtained. The curve relating contraction to steady membrane potential was sigmoid, with a V_{half} of − 46 mv and a slope of 6–7. Threshold voltage for tension development was also a nonlinear function of voltage between − 75 and − 20 mv resting potential. From these results we would anticipate that depolarization of cardiac muscle from any cause would directly decrease isometric contraction, independent of changes in excitability of the fiber. (Supported in part by USPHS Grant HE 11665.)

109. Common Structural and Immunologic Characteristics of Homogeneous γM Rheumatoid Factors. EDWARD C. FRANKLIN AND BLAS FRANGIONE,* New York.

Chemical and immunological studies have demonstrated similarities in the Fd fragments of two paraproteins produced by the same individual, and have also shown certain structural features characteristic of a given type of antibody in the case of human cold agglutinins, human γG rheumatoid factors (RF), mouse myelomas with anti-phosphoryl choline specificity, and homogeneous rabbit antibodies to streptococci. Whether these similarities reflect properties of the combining site or variable region subclasses remains unknown. Studies of a serum (Don) with a γMk-mixed cryoglobulin RF and an inert γAk myeloma protein suggest that both are involved. Don γA (inert), Don γM RF, and two other γM RF's (Furn-Wys) had a peptide on diagonal map which was absent in another γM RF (Bal). The peptide appeared to be in the heavy (H) chain and in one instance had the composition: Leu(Cys-Thr-Ser-Gyl-Val-Leu). Don γA and γM had PCA as the N-terminus of the H chain while Furn had Glu. Two antisera to inert Don γA absorbed with NHS, γA, and γM globulins reacted with eight of nine mixed cryo γM RF, and about ⅓ of ordinary RF. Antisera to γM RF Don and γM RF Furn reacted with eight of nine γM-mixed cryo RF, about ⅓ of ordinary RF, but not with γA DON or inert macroglobulins. The presence of the same unusual peptide in RF and inert proteins as well as the ability of antisera to inert γA Don to recognize many RF proteins indicate involvement of sites other than the antibody combining site. In contrast, the reactivity of antisera to γM RF with RF's regardless of the N-terminus and the presence or absence of the peptide supports the possibility that the antisera recognize the combining site and that the observed chemical variability may be related to the complexity of the antigens. (Research supported by grants from NIH, HRC, and N. Y. Chapter A.F.)

110. A Function for Ribosomal Protease as an Initiation Factor in Hemoglobin Synthesis. MICHAEL L. FREEDMAN,* DOROTHY FRIEDBERG,* STEVEN ROFFMAN,* JITKA MUCHA,* AND WALTER TROLL,* New York (introduced by Robert Silber).

One of the initiation steps of hemoglobin synthesis in rabbit reticulocytes is the dissociation of an 80S ribosome free from mRNA into the 40S and 60S subunits. Bacterial studies suggest that this phase of initiation is mediated by a protein, designated "dissociation factor." Previous work from this laboratory has shown that an exogenously added proteolytic enzyme, pronase, selectively dissociates rabbit reticulocyte 80S ribosomes not attached to mRNA into subunits. This system, then, might serve as a model for the function of an intracellular proteolytic enzyme in mammalian cells. The present study describes the isolation and characterization of an endogenous ribosomal proteolytic enzyme and presents evidence for its function as a "dissociation factor." Reticulocyte 80S ribosomes spontaneously dissociated into subunits in a 0.02 M Tris–0.05 M KCl, 0.01 M magnesium acetate buffer (buffer A). In a similar buffer with lower concentrations of salt (buffer B), no dissociation occurred. Endogenous proteolytic activity, assayed by following the hydrolysis of radioactive tosyl arginine methyl-ester at pH 5, was demonstrable only in buffer A. A specific protease inhibitor, tosyl lysine chloromethyl ketone (TLCK), prevented cell-free ribosomal dissociation in buffer A. TLCK also inhibited the endogenous ribosomal proteolytic enzyme. Furthermore, TLCK is a potent inhibitor of hemoglobin synthesis in intact reticulocytes, acting at the site of polyribosome formation. Proteolytic activity is demonstrable, therefore, under conditions where endogenous ribosomal dissociation occurs. This suggests that this enzyme may function as a "dissociation factor" necessary for initiation of hemoglobin synthesis. (Supported by NIH Grants AM 13532-01 and Core Grant BSS-ES 0014 NCI-CA 06989.)

111. Studies on Mechanism of the Neural Lesion of Pernicious Anemia. EUGENE P. FRENKEL,* Dallas, Tex. (introduced by Elias Strauss**).

In man, vitamin B_{12} is indispensable in only one biochemical reaction, that of propionic acid (Pro) metabolism (Pro →

Pro CoA → methylmalonyl [MMA] CoA → succinyl CoA) in which B_{12} is a coenzyme for the final conversion of MMA CoA to succinyl CoA. Since excess Pro and MMA occur in pernicious anemia (PA), this pathway was evaluated as a potential biochemical mechanism for the neural changes in PA. Sural nerve biopsy slices from six PA's and four normals were incubated in propionate-^{14}C or acetate-^{14}C, saponified, and the methyl esters prepared for fatty acid analysis by gas-liquid chromatography (GCL) with isotopic counting of the separated peaks. Labeled propionate was incorporated into both normal and PA nerves as efficiently as acetate-^{14}C. In the normal the Pro-^{14}C was found primarily in short-chain (C12 and C14) fatty acids. The PA nerve differed from the normal by evidence of a cold peak between myristic (C14·0) and palmitic (C16·0) acids and between palmitoleic (C16·1) and stearic (C18·0) acids. The predominant radioactivity was also identified in these two peaks which on chromatography with appropriate standards proved to be anteiso-C15 (branched chain) and C17 (odd chain) fatty acids. Catalytic reduction and rechromatography failed to alter the peaks; thus they are not unsaturated fatty acids. Thin-layer chromatography demonstrated that these two qualitatively abnormal fatty acids are in highly polar lipids. Since the lipid composition of the myelin and the integrity of the Schwann cell are critical for normal neural function, the presence of abnormal fatty acids provides a possible explanation for structural alteration in the PA nerve with resultant Schwann cell injury, dysmyelinization, and neural loss.

112. First Step in Insulin Action: Direct Study of Binding to Liver Receptors. PIERRE FREYCHET,* JESSE ROTH, AND DAVID NEVILLE,* Bethesda, Md.

The first step in insulin action is binding to specific receptors on the plasma membrane of target cells. For direct study of this step, iodoinsulin was prepared by iodinating at a low iodide to insulin ratio and separating iodoinsulin from uniodinated insulin by chromatography on DEAE-cellulose. Pure monoiodoinsulin-^{127}I, whose composition was verified by spectral titration, retained full bioactivity (25 U/mg) measured as stimulation of glucose oxidation in isolated fat cells. Insulin-^{125}I of high specific radioactivity prepared by this method bound to plasma membranes purified from rat liver. 20% of the bound insulin-^{125}I was displaced by unlabeled insulin at 6 ng/ml (10^{-9} mole/liter), equivalent to basal insulin concentrations in portal blood. Displacement was 80% with 10^{-7} mole/liter. Insulins with biological potencies that differed over a 100-fold range (pork, human, beef, fish, and guinea pig insulin, porcine proinsulin, desalanine-desasparagine, and deoctapeptide-insulin) displaced insulin-^{125}I from liver receptors in proportion to their ability to stimulate glucose oxidation in fat cells. Insulin chains, inactive in fat cells, produced no displacement of labeled insulin. Glucagon, adrenocorticotrophic hormone (ACTH), and human growth hormone (HGH) were also without effect. The amount of insulin bound per milligram of liver protein increased 20-fold as plasma membrane was purified from crude homogenate. Treatment of plasma membrane with trypsin destroyed its ability to bind insulin. Binding was more rapid and more complete at 30°C than at 0°C. Addition of excess insulin produced rapid dissociation of insulin-^{125}I. The rapid reversible binding of biologically active insulin-^{125}I, its displacement by unlabeled insulins and derivatives in minute quantities and in proportion to their bioactivity, and copurification of receptor with plasma membrane, indicate that this system reflects closely the first physiological event by which insulin acts on a target tissue. (Supported in part by USPHS International Postdoctoral Research Fellowship 1 F05 TW01512-01.)

113. Extrarenal Erythropoietin (Ep) Production in Male and Female Rats. W. FRIED, W. H. KNOSPE,* AND F. E. TROBAUGH, JR.,* Chicago, Ill.

Extrarenal sites of Ep production in rats respond to hypoxia, cobalt, and bleeding. Testosterone propionate in doses sufficient to increase renal Ep production is, however, unable to increase that by extrarenal sites. Ep production of males exceeds that of females, probably because of their elevated androgen titers. These studies compare the response of renal and extrarenal sites of Ep production of males and females to hypoxia. Plasma Ep levels of unoperated and nephrectomized male and female rats were measured after exposure to 0.5, 0.465, 0.435, or 0.4 atmospheres for 8 hr. Those of nephrectomized males were 0.2 U/ml* at 0.5 atmospheres and 0.3 U/ml at 0.465 atmospheres. They did not increase further with increased hypoxia. Those of nephrectomized females rose progressively with increasing hypoxia from 0.04 U/ml at 0.5 atmospheres to 0.3 U/ml at 0.4 atmospheres. The findings in unoperated rats were comparable, but Ep levels were tenfold greater than in nephrectomized animals at each level of hypoxia. We conclude that female kidneys can produce as much Ep as those of males. Female extrarenal sites can also produce as much Ep as those of males and about 10% as much as the kidneys. However, male renal and extrarenal sites are more sensitive to hypoxia than are those of females and are stimulated to maximum production with less hypoxia. The increased sensitivity of male sites of Ep production to hypoxia probably results from being exposed to higher androgenic steriod levels. Inability of extrarenal sites to respond to testosterone injections suggests that they may be less sensitive to androgens than the kidneys and may require either more prolonged exposure or exposure to higher titers of a specific metabolite of testosterone. (These studies were supported by NIH Grant AM 12936-02.)

114. Glutathione Reductase (GSSG-R): Diaphorase Function and Isozyme Transformation by Stromal NADPase. H. FRISCHER,* C. NOYES,* AND R. NELSON,* Chicago, Ill. (introduced by P. Carson).

Stroma-free dialyzed and undialyzed hemolysates, incubated with or without purified stromata (1 hr, 37°C or 45°C), were electrophoresed for 17 hr, 4°C, in a vertical polyacrylamide Tris-borate system (pH 8.2.) Gels were stained with NADPH (or NADH), dichlorophenolindophenol, MTT tetrazolium, 0.15 M Tris-HCl (pH 8.2) with or without one of the following substrates: GSSG, dehydrolipoate, arsenite, dehydrocholesterol, dehydroascorbate, menadione, primaquine. Gels containing dialyzed hemolysates incubated without stromata and developed with each substrate, NADPH, or

NADH, revealed two systems of bands: system A, three or four bands (principal band, R_f 0.36 relative to HbA); system B, one to three slower bands (R_f 0.10). Substrate-free stains revealed band with undialyzed but not with dialyzed hemolysates. They were located only in fractions containing GSSG-R activity after elution by electrodialysis, intensified or weakened if the hemolysate was preincubated with flavin-adenine dinucleotide or 6-phosphogluconate respectively, faint in hemolysates with decreased GSSG-R activity. In hemolysates incubated with stromata or exposed to EDTA, GSSG-R system A stained more intensely, system B disappeared. Neurospora NADase did not absolish system B. Nicotinamide and NADPH incubated with stromata prevented its disappearance; NADPH but not nicotinamide added after exposure to stromata before electrophoresis resulted in its reappearance. We conclude that (*a*) hemolysate contains slow GSSG-R isozymes (system B), only detectable without EDTA; (*b*) stromal NADPase converts GSSG-R system B into system A; (*c*) GSSG-R can not only reduce GSSG and dehydrolipoate, but is also an NADPH or NADH diaphorase with arsenite, dehydrocholesterol, dehydroascorbate, menadione, and primaquine; and (*d*) undialyzed hemolysates may contain such substrates; interpretation of data on diaphorase should take into account this function of GSSG-R. (Supported by USAMRDC Contract DA-49-193-MD-2413 and USPHS Grant HE-06078.)

115. Genetic Regulation of Enzyme Induction in Somatic Cell Heterokaryons. THOMAS D. GELEHRTER* AND E. BRAD THOMPSON,* New Haven, Conn., and Bethesda, Md. (introduced by Leon E. Rosenberg).

The production of somatic cell heterokaryons allows one to study the interaction of different genomes within a common cytoplasm, and thus the genetic regulation of specific differentiated functions. The control of an inducible enzyme, hepatic tyrosine aminotransferase (TAT), has been investigated in heterokaryons between two rat cell lines, HTC and BRL-62. HTC cells are an established tissue culture line derived from a rat hepatoma, in which glucocorticoid hormones induce a tenfold increase in the synthesis of TAT. BRL-62 cells, derived from normal rat liver, have little or no basal TAT and the enzyme is not induced by steroids. Heterokaryons were prepared by mixing the parental HTC and BRL-62 cells with UV-inactivated Sendai virus, after first labeling one parental line with thymidine-^{3}H. After incubation of the fused cells with hormone, heterokaryons (defined as cells containing at least one thymidine-labeled and one unlabeled nucleus) were identified by radioautography, and assayed for TAT by a histochemical technique. In all cases, heterokaryons lacked inducible TAT; whereas homokaryons containing only HTC nuclei were always TAT positive. BRL-62 cells do not contain an inhibitor of TAT, nor do they inactivate the inducing steroid. Furthermore, over-all protein and RNA synthesis in multinucleate cells appeared normal as judged by radioautography. Therefore, the suppression of TAT in heterokaryons appears to be specific. Since the full DNA complement of both parental cells is present in the heterokaryons, it appears that the TAT-negative genome is capable of repressing induced synthesis of this enzyme by the TAT-positive HTC cell genome.

116. Plasma Renin Activity (PRA) in Renal Failure with Hypertension. G. G. GEYSKES,* J. D. SAPIRA,* H. V. MURDAUGH, S. J. GALLA,* AND A. P. SHAPIRO,** Pittsburgh, Pa.

In 39 patients with renal failure and severe hypertension (grade III-IV retinopathy), PRA was measured repeatedly to investigate correlation with cause of renal disease, blood pressure (BP), acceleration of hypertension, hydration as judged from weight, and [Na^+]. Patients were divided into three groups: (1) primary renal parenchymal disease (chronic glomerulo- and pyelonephritis, polycystic disease); (2) primary hypertension with arteriolar nephrosclerosis; (3) periarteritis nodosa and scleroderma. PRA was high with renal arteriolar disease (groups 2 and 3); medians were 2930 and 1613 ng/100 ml, respectively. PRA did not correlate with hydration, [Na^+], or decreasing retinopathy. PRA was significantly lower in group 1 (median 420) and correlated inversely with hydration but not with [Na^+]. Balance studies were performed on four patients in group 1 and two in group 2. After dialysis to clinically dry state, volume was increased by 2 liters of saline intravenously. Thereafter they were ultrafiltrated back to dry weight. In both states, PRA was measured recumbent and after mobilization along with BP's and plasma catecholamines. Dry and wet states were documented by dilution volumes of Evans blue, H_3O, and ^{22}Na. After hydration blood volume increased from 8.1 to 9% body weight; H_3O space from 63.5 to 66.4%; ^{22}Na from 52.9 to 54.6 mEq/kg body weight. In group 1 PRA increased after mobilization and in the dry state and decreased during the wet period; BP's correlated inversely with PRA. When BP fell on upright position, plasma norepinephrine tended to rise, epinephrine did not. In group 2, PRA and BP remained high and did not correlate with posture and hydration. The balance studies confirm the over-all data and indicate that a primary determinant of PRA and BP in renal parenchymal disease is state of hydration, while in primary hypertension, arteriolar nephrosclerosis may override physiologic influences on renin release. (Supported by grants from NIH and AHA.)

117. Human Placental Alkaline Phosphatase, an Inhibitor of Hemagglutination by PR8 Influenza A Virus. N. K. GHOSH,* A. RUCKENSTEIN,* C. LABOWSKY,* AND R. P. COX, New York.

Human placental alkaline phosphatase (E.C.3.1.3.1), a sialoglycoenzyme, inhibits hemagglutination (HA) by PR8 influenza A virus. Enzyme purified 700-fold exhibits a hemagglutination-inhibition (HA-I) titer of 130,000 U/mg of protein. Heavy molecular weight isozymes as determined by starch-gel electrophoresis and gel filtration on Sephadex G-200 showed a significantly higher titer than the lower molecular weight species. Gel filtration profiles of alkaline phosphatase activity, sialic acid content, and HA-I titer were nearly superimposable on one another. Viral HA-I potency of the enzyme preparation was dependent on pH, time, and virus concentration but was not altered appreciably by variation in temperature. Placental alkaline phosphatase is equally effective in inhibiting PR8 virus HA of human and chicken erythrocytes. The HA-I titer of antigen-antibody complexes of placental alkaline phosphatase precipitated by specific antiserum was lower than that of the enzyme alone. Removal of sialic acid residues from

the enzyme or by treatment with neuraminidase (E.C.3.2.1.18) or by oxidation of carbohydrate moieties with periodate caused a substantial reduction of HA-I titer. This study suggests that a mammalian glycoprotein possesses dual biological specificities: alkaline phosphatase activity and the capacity to inhibit myxovirus-induced HA. The HA-I activity of alkaline phosphatase presumably is determined by the availability of the sialic acid residues of the carbohydrate moieties, and these are more accessible in the high molecular weight isozymes.

118. Increased Proximal Tubular Reabsorption of Sodium Caused by Renal Alpha Adrenergic Stimulation. J. R. GILL, JR.,* AND A. G. T. CASPER,* Bethesda, Md. (introduced by Frederic C. Bartter**).

Beta adrenergic stimulation decreases proximal tubular sodium reabsorption, probably through the mediation of cyclic adenosine monophosphate (AMP) which has a similar effect on the tubule. Conversely, previous studies with an alpha adrenergic blocking agent suggest that renal alpha adrenergic stimulation increases the proximal tubular reabsorption of sodium. The present studies support this suggestion. Propranolol, 0.17 μg/kg per min, was infused into the left renal artery of hypophysectomized, cortisol-treated dogs and urine was collected from each kidney by ureteral catheters. Water diuresis was produced by infusion of 2.5% dextrose. After three clearance periods at stable urine flow, norepinephrine (NE), 0.009 μg/kg per min, was added to renal artery infusate to stimulate alpha receptors for four periods; four postcontrol periods were obtained. Control C_{In}, V/100 ml GFR, and C_{H_2O}/100 ml GFR were 33 $\pm$3 (SE), 7.0 $\pm$0.5, and 5.5 $\pm$0.5 ml/min, respectively (right), and 30 $\pm$2, 7.8 $\pm$0.7, and 6.2 $\pm$0.7 ml/min, respectively (left); with NE the right was 32 $\pm$2, 7.0 $\pm$0.6, and 5.5 $\pm$0.7 ml/min, respectively, but the left was 30 $\pm$2, 6.3 $\pm$0.5 ($P < 0.01$), and 5.0 $\pm$6 ($P < 0.01$) ml/min, respectively; during postcontrol the right was 33 $\pm$2, 6.9 $\pm$0.6, and 5.5 $\pm$0.7 ml/min, respectively, and the left was 32 $\pm$2, 7.3 $\pm$0.6, and 5.9 $\pm$0.8 ml/min, respectively. The fraction of delivered sodium reabsorbed in the loop of Henle ($C_{H_2O}/(C_{Na} + C_{H_2O}) = 0.97$) did not increase with NE (0.97). With NE, C_{PAH} decreased 9 ml/min (right) and 11 ml/min (left); filtration fraction was similar in the two kidneys (0.46 right; 0.48 left). The results suggest that renal alpha adrenergic stimulation increased proximal but not distal tubular reabsorption of sodium. It probably did so partly by direct tubular effects, as hemodynamics in the two kidneys were similar, possibly through the mediation of cyclic gaunosine 3',5'-monophosphate which appears to increase the tubular reabsorption of sodium when infused in a renal artery.

119. Amyloidosis: a Disease Associated with Tissue Deposition of Fragments of Homogeneous Immunoglobulins. G. G. GLENNER,* W. D. TERRY,* C. ISERSKY,* D. PAGE,* AND M. HARADA,* Bethesda, Md. (introduced by John L. Fahey**).

Amyloidosis is a condition characterized by the accumulation in tissues of a unique fibrillar glycoprotein. Several investigators have hypothesized that amyloid protein is derived from immunoglobulin proteins and that immunoglobulins are directly involved in the pathogenesis of this disease. Amyloid fibrils derived from postmortem tissues of six patients were analyzed with immunochemical and chemical techniques. Concentrates of amyloid fibrils identified by staining and electron microscopic methods were obtained by differential centrifugation techniques. Purification of these concentrates was accomplished by a series of gel filtrations with removal of approximately 30% of minor constituents. The purified protein components from these six patients each showed a single band on acrylamide-gel electrophoresis and had apparent monomeric molecular weights ranging from 5000 to 18,000 as determined from calibrated Sephadex G-100 columns and SDS polyacrylamide disc-gel electrophoresis. Additional analytical studies were performed on amyloid proteins from two patients (amyloid VIII and X). Antigenic similarity between these two proteins was demonstrated by immunochemical tests in which antibody prepared against each protein gave a reaction of partial identity with the other in gel diffusion. These antisera also precipitated certain κ–Bence Jones proteins. Amino acid sequences of the amino-terminal 35 (VIII) and 36 (X) residues were determined with a Beckman protein sequencer. A unique sequence was obtained for each protein and in both cases they were homologous to the sequence expected for the amino-terminus of a kappa light chain of the $V_{\kappa I}$ subgroup. Amyloidosis is, therefore, a disease that is associated with the tissue deposition of fragments of a homogeneous immunoglobulin. These data support the hypothesis that immunoglobulins are the source of the fibrillar component of amyloid deposits.

120. Effects of the Metabolism of Labeled Hormone (Cortisol) during Distribution upon the Study of Endogenous Hormone Kinetics. NORMAN I. GOLD* AND JOHN F. CRIGLER, JR.,* Boston, Mass. (introduced by Roger B. Hickler).

Radioactively labeled cortisol (F) and cortisone (E) were combined, administered intravenously to human subjects, and the urinary metabolites analyzed. 10–60% more of the E-isotope than the F-isotope in 3α,17,21-trihydroxy-5β-pregnane-11, 20-dione (THE) than in 3α, 11β, 17, 21-tetrahydroxy-5β-pregnan-20-one (THF) indicated that a portion of the labeled E was reduced to metabolites *without entering the F pool.* F-^{14}C and E-^{3}H combined were given intravenously over 30 sec. Urine was collected each 15 min. In the first sample ^{3}H/^{14}C in THE was 260% above the equilibrium value confirming formation of labeled THE *before* complete interconversion of F-^{14}C and E-^{3}H. ^{3}H/^{14}C of THF was *constant from the outset* indicating complete interconversion before significant labeled THF formed. Thus, a portion of labeled E formed from injected labeled F before distribution of the latter always tends to produce a *higher specific activity* (SA) for THE than THF in the total urinary metabolities and always results in a portion of administered labeled F being irreversibly lost as THE before complete mixing with the F pool. Serum protein binding was shown to have only a minor affect on the transformation of labeled F before distribution. The role of the *site* of administration was demonstrated when orally administered labeled F gave rise to much larger increases in THE-SA relative to THF than did rapid intravenous injection. The importance of the *mode* of administration was evident when a 4 hr infusion of labeled F showed greater increases in THE-SA relative to THF than did rapid intravenous administration. The irreversible metabolism of administered labeled hormone

during mixing with its pool, capable of influencing significantly the calculations of production rates and other kinetic parameters by all isotope dilution procedures, emphasizes the need to understand pathways and rates of metabolism of biological substances when analyzing kinetic data. (Research supported by USPH Service Grant RR-00128 and General Research Support Funds of The Children's Hospital Medical Center.)

121. Mechanism of Analgesic Abuse Nephropathy. M. GOLDBERG, C. L. MYERS,* W. PESHEL,* D. MCCARRON,* AND A. B. MORRISON,* Philadelphia, Pa.

Whether renal medullary lesions from excessive analgesics are due to phenacetin (P), aspirin (A), or P + A is unknown even though hydropenia increases papillary accumulation of P metabolite. To evaluate this problem, as well as the role of fluid intake, histological and physiological studies were performed in three experimental groups of rats, each containing eight triads. In each triad, rats were trio fed: one rat received a restricted water intake to maintain stable hydropenia and either P (0.5 g/kg) (group I), A (0.2 g/kg) (group II), or A + P (group III); one rat received same drug but was fed glucose/water to maintain water diuresis; a third rat was on restricted water intake and no drug. By 2–3 months, hydropenic-drug rats of group III only, manifested renal concentrating defect ($P < 0.005$). Kidneys histologically showed marked characteristic papillary changes in these animals which were graded significantly higher than the milder changes in hydropenic-drug rats of group I ($P < 0.005$) and minimal changes in water diuretic-drug rats of group III ($P < 0.001$). Abnormalities consisted of thickening of basement membranes of all tubules and deposition of periodic acid–Schiff (PAS)- and trichrome-positive granular interstitial material in increasing intensity toward papillary tip. In severely involved kidneys, intracellular PAS-positive granules and architectural disruption were present. No other rat showed these changes. Since A + P + hydropenia are required for papillary lesions, a mechanism similar to analgesic damage to red cells may be involved. Hexose monophosphate shunt enzyme activity (HMP) was assayed in rat's kidney homogenates and found to be present in a papilla/cortex ratio of 1.04/1.60. Enzyme kinetic studies using NADP substrate with and without A (20n moles/liter) revealed competitive and reversible inhibition of HMP by A. Therefore A + P may act synergistically in renal papilla if A inhibits the defense against oxidative damage (HMP), whereas P and metabolites act as oxidants which are concentrated best during hydropenia.

122. Difference Between Acute and Chronic Suppressibility of Parathyroid Function during Chronic Hemodialysis. RALPH S. GOLDSMITH,* JACOB FURSZYFER,* WILLIAM J. JOHNSON,* AND CLAUDE D. ARNAUD,* Rochester, Minn. (introduced by Randall G. Sprague**).

Autonomy of parathyroid function in chronic renal insufficiency has been defined as failure of plasma immunoreactive parathyroid hormone (IPTH) to decrease significantly during hypercalcemia induced by calcium infusion. We observed that IPTH did not decrease more than 20% (error of assay = ±16%) from control values in 14 of 15 patients on chronic hemodialysis during 8-hr calcium infusions (4 mg/kg per hr, mean maximal serum calcium, 15 mg/100 ml). In contrast, long-term dialysis (3–6 months) of the same patients against a dialysate calcium concentration of 7–8 mg/100 ml resulted in a significant and progressive decrease in IPTH in all patients (−47 to −92%) in spite of a maximal plasma calcium during dialysis of less than 11.0 mg/100 ml. In this regimen plasma phosphate was decreased below 6 mg/100 ml with oral aluminum hydroxide. A dialysate calcium concentration of 7–8 mg/100 ml was shown in separate studies to produce a net gain to the patient of 600 mg of calcium per dialysis whereas the standard dialysate calcium concentration of 5–6 mg/100 ml produced a net gain of only 100 mg/dialysis. These studies show that (*a*) it is incorrect to equate lack of acute suppression of plasma IPTH during calcium infusion with autonomy of parathyroid function, and (*b*) the excessive secretion of PTH in the majority of patients on hemodialysis may be responsive to a chronic suppressive regimen such as the one outlined. (Research supported by Grants 69-2168 and AM 12302 from NIH.)

123. Effect of Radiation on Pulmonary Bactericidal Function. E. GOLDSTEIN,* J. P. LEWIS,* F. HAHN,* C. EAGLE,* AND P. D. HOEPRICH,** Davis, Calif.

The enhanced susceptibility to pulmonary infection that follows whole body irradiation is poorly understood. Since intrinsic antibacterial mechanisms normally keep the lungs bacteria free, quantitative assessment was made in mice of the impact of sublethal and lethal X-irradiation of the killing of inhaled staphylococci. Genetically mixed mice were sublethally irradiated with 500 R (GSI). Syngeneic mice were lethally irradiated with 850 R (SLI). Half of these mice received transplants of 5×10^6 bone narrow cells postirradiation (TSLI). Mice from each group were exposed to ^{32}P-labeled *S. aureus* 3, 10, 14, 21, and 31 or 35 days postirradiation. Measurements of pulmonary bacterial and ^{32}P concentrations, made 4 hr after aerosol exposure, allowed calculation of rates of intrapulmonary bacterial killing. In GSI mice, bactericidal function was decreased slightly on day 14, more markedly on day 21 ($P < 0.01$), and unimpaired at other times. Both SLI and TSLI mice had small decreases in bactericidal function 3 and 10 days postirradiation; only TSLI mice survived to manifest severe defects 21 and 35 days after irradiation. There was a leukopenia on days 3 and 10 with normal values thereafter in irradiated mice. Since bone marrow transplantation did not prevent impairment of pulmonary bactericidal function but insured survival, whole body irradiation appeared to (*a*) affect cellular defenses of the lung, and (*b*) lead to death through extrapulmonary effects. The delayed and transient nature of the defect is consistent with a radiation effect on precursor cells. (Supported by grants from Project Clean Air of California, NIH, ATS.)

124. Rescue of Senescent Human Fibroblasts by Hybridization with Cultured Hamster Cells. SAMUEL GOLDSTEIN* AND CHYI-CHYANG LIN,* Hamilton, Canada (introduced by John W. Littlefield).

Human diploid fibroblasts cultured in vitro provide an excellent model for the study of aging; after a period of growth,

they inevitably lose their ability to divide and eventually die. To explore the basis of this nonmitotic state, senescent fibroblasts from an adult diabetic were fused with a permanent line of azaguanine-resistant, trypsin-sensitive, Syrian hamster fibroblasts, using inactivated Sendai virus to stimulate fusion. Presumptive hybrids were selected in hypoxanthine-amethopterin-thymidine medium. Two clones survived manipulations and were analyzed with identical results. Karyotypes revealed 90 chromosomes, the sum of diploid human and modal hamster complements. Marker chromosomes specific for each parent were readily visualized. Definite proof of hybridization was obtained from starch-gel electrophoresis which identified both parental bands of glucose-6-phosphate dehydrogenase plus an intermediate band; however, examination for 6-phosphogluconate dehydrogenase (6-PGDase) revealed only the hamster band. The absence of human 6-PGDase activity in hybrid cells suggests extinction by regulatory hamster factors, or less likely, unnoticed shedding of all or a portion of the same autosome in both clones simultaneously. Hybrids, unlike hamster cells, were not killed by trypsin indicating recessivity of a possible "membrane mutant." These data show that senescent human fibroblasts can be rescued by viable cells, as has been shown by Davidson and Ephrussi in mouse × mouse hybrids. This rules out the presence in senescent cells of dominant aging factors, generalized irreparable DNA damage, permanent DNA cross-linking, and irreversible histone binding. Our results are consistent with the hypothesis that aging in vitro, and perhaps in vivo, is a quasi-differentiated state which could be amenable to modification. (Supported by grants from the MRC of Canada and the Canadian Diabetic Association Foundation Fund during the tenure of a MRC Scholarship [SG].)

125. Prostaglandins as Mediators of Cutaneous Vascular Reactivity in Dog and Man. MARC GOLDYNE* AND R. K. WINKELMANN,* Rochester, Minn. (introduced by Ward S. Fowler**).

Arteries (200–400 μ O.D.) from dog's paw and skin of back and ear and from skin of human breast and finger were dissected into continuous helical strips and doughnut-shaped pieces and studied for changes in tension induced by potassium chloride, catecholamines, and prostaglandins (PGE_2 and PGA_2). Of 95 dog skin vessel preparations, 46 responded to prostaglandin E_2 (PGE_2) with contractile response. Only 7 of 40 ear vessel preparations responded, but 32 of 45 paw vessels gave contractions. PGE_2 potentiated catecholamine response, and application of subthreshold PGE_2 followed by subthreshold catecholamine increased reactivity and lowered threshold response to epinephrine and norepinephrine. Prostaglandin added after catecholamine augmented the response. PGE_2 potentiated epinephrine responses in 32 of 40 ear vessels and 36 of 40 paw vessels. Only 4 of 40 vessel strips from human breast skin and 1 of 8 from fingers responded to PGE_2; 21 of 37 strips of breast skin showed potentiation by PGE_2 of catecholamine response and 4 of 8 finger skin vessels showed potentiation. Preliminary studies with PGA_2 gave similar results. Alpha blockade with Regitine did not block prostaglandin activity in responsive vessel preparations. Beta blockade with propranolol did not influence the effect of prostaglandin. Results show that cutaneous vascular smooth muscle of both dogs and humans contains a prostaglandin receptor that can augment the alpha receptor activity. Prostaglandins may be important mediators of cutaneous vascular activity by augmenting catecholamine responses.

126. Possible Role of Cyclic Guanosine Monophosphate in the Response of the Kidney to Metabolic Acidosis. A. DAVID GOODMAN,* ALTON L. STEINER,* AND ANTHONY S. PAGLIARA,* Albany, N. Y., and St. Louis, Mo. (introduced by Stuart Bondurant).

In metabolic acidosis there is an increase in renal production of ammonia from glutamine. We have shown previously that cyclic guanosine monophosphate (cGMP) suppresses, and cyclic adenosine monophosphate (cAMP) stimulates renal cortical production of ammonia from glutamine in vitro. In the present study we have used specific radioimmunoassay techniques to measure these cyclic nucleotides in rat renal cortex in metabolic acidosis. cGMP concentration in renal cortex of the normal rat was approximately 1.8×10^{-8} moles/kg wet weight, and cAMP 140×10^{-8}. 3 hr after a single dose of NH_4Cl (14 mmoles/kg), cortical cGMP fell 40% ($P < 0.001$) but cAMP did not change. When the same study was repeated with the addition that all of the rats were given theophylline (30 mg/kg) intraperitoneally 2 hr before renal biposy, cortical cGMP was decreased 54% in the NH_4Cl group ($P < 0.001$), whereas cAMP was unaltered. In rats fed NH_4Cl for 2 days (20 mmoles/kg per day), cortical cGMP fell 49% ($P < 0.001$) but cAMP did not change. In the latter study mean plasma cGMP, which was 0.87×10^{-8} moles/liter in the control group, fell 30% in the NH_4Cl group ($P < 0.01$); this decrease was not sufficient to account for the observed fall in the renal cortical level. We conclude that (*a*) in metabolic acidosis renal cortical cGMP is decreased, and (*b*) as cyclic GMP is an inhibitor of cortical ammoniagenesis, the increase in renal ammonia production in metabolic acidosis may be mediated by the fall in renal cortical cGMP. (Supported by NIH Grant AM-9232.)

127. Aldosterone and the Fatty Acid Composition of Toad Bladder Phospholipids. DAVID B. P. GOODMAN,* JAMES E. ALLEN,* AND HOWARD RASMUSSEN, Philadelphia, Pa.

Aldosterone stimulates the active transport of sodium across the epithelial cell layer of the isolated amphibian urinary bladder. In addition, aldosterone (*a*) increases the sensitivity of the bladder to oxygen poisoning; (*b*) increases the susceptibility of the sodium transport system to inhibition by ouabain; and (*c*) increases the response of this tissue to a standard dose of vasopressin as measured by either bulk water flow or Na^+ transport. All these changes raised the possibility that aldosterone altered the composition of the cell membranes. Consequently we undertook an investigation of the effects of aldosterone upon phospholipid metabolism. The hormone did not significantly alter the amounts of turnover of the various classes of phospholipids in the tissue. However, it increased glucose-1-^{14}C decarboxylation within the first hour after its addition and increased incorporation of labeled glucose and pyruvate into the fatty acids of the phospholipids. Analysis of these fatty acids from phospholipids by gas-liquid chromotography revealed that the hormone specifically and signifi-

cantly increased the weight percentage of several long-chain polyunsaturated fatty acids. In further experiments it was found that after aldosterone treatment phospholipase A caused a 4-fold greater release of labeled fatty acids from lipid extracts of bladder tissue. Phospholipase A catalyzes specifically the hydrolyses of fatty acids from the 2-position of phospholipids which is the predominant locus of unsaturated fatty acids in these molecules. These results indicate that aldosterone alters the fatty acid composition of membrane phospholipids. These findings may provide an explanation for the diverse effects of aldosterone upon membrane function in the toad bladder. They also raise the possibility that other steroid hormones act by altering the lipid composition of target cell membranes. (Supported by grants from the NIH and ONR.)

128. Studies of Hypothalamic Metabolism In Vitro as an Approach to Chemoreceptor Mechanisms. CHARLES J. GOODNER AND JAMES T. OGILVIE,* Seattle, Wash.

The characteristics of hypothalamic systems for regulating appetite and other aspects of metabolism must be reflected in the metabolism of hypothalamic cells. Accordingly, we have studied slices prepared from discrete areas of rat hypothalamus and compared their in vitro metabolism with slices of amygdala, cerebral cortex, and anterior pituitary (AP). No major differences were found among brain tissues in the rate or pattern of glucose metabolism tested at glucose concentrations from 20 to 300 mg/100 ml. Addition of insulin failed to increase glucose metabolism in hypothalamic slices taken from the region of the ventral medial nucleus or in any brain tissue. All brain tissues exhibited measurable capacity to oxidize free glycerol and incorporate glycerol into phospholipid. Hypothalamic slices were twice as active as cerebral cortex while AP was even more active. Direct assay of gylcerokinase confirmed these quantitative relationships. Hypothalamic tissues also oxidized palmitate more actively than cerebral cortical slices. In hypothalamus, AP, and muscle, but not in cerebral cortex fatty acid oxidation was inversely related to media glucose concentration. Thus, hypothalamic metabolism differed most clearly from cerebral cortical metabolism in total capacity to metabolize glycerol and fatty acids and in metabolic control of fatty acid oxidation by glucose. However, after an hour's infusion in vivo, incorporation of glycerol-^{14}C or palmitate-^{14}C into hypothalamic lipids was small compared to muscle and AP, suggesting poor penetration of the blood barrier. We conclude that glycerol and fatty acids derived from lipolysis in peripheral fat depots could serve as chemical signals to the hypothalamus. Such a role might ultimately depend upon the ability of these substances to penetrate the blood brain barrier in specific areas of the hypothalamus. (Research supported by grants from NIH.)

129. Inotropic and Antiarrhythmic Effects of Hypercalcemia during Myocardial Ischemia. K. GOPINATHAN,* M. U. JESRANI,* M. I. KHAN,* H. A. OLDEWURTEL,* AND T. J. REGAN,** Newark, N. J.

Diminished contractility of ischemic ventricle has been related to reduced availability of calcium for regulating troponin inhibition of actomyosin. To determine if enhanced plasma calcium can influence the course of ischemia, intact anesthetized dogs were studied during acute thrombus formation in the anterior descending coronary artery via a catheter electrode. Group I consisted of untreated animals surviving 75 min. Group II received a sustained systemic infusion of calcium chloride after 15 min of ischemia, sufficient to double plasma concentrations for 60 min. In groups I and II coronary blood flow (^{86}K injected distal to thrombus) decreased to approximately 25% of control without significant change in heart rate or aortic pressure. Injury potential and size of ischemic areas at termination were comparable. Left ventricular stroke volume, ejection fraction, and dp/dt maximum were increased by calcium to preischemic levels without additional rise of end-diastolic pressure and volume (indicator dilution method) above the initial ischemic response. Group I had a reduction of ejection fraction from 0.20 ± 0.02 to 0.12 ± 0.01, which in group II was restored from 0.22 ± 0.03 to 0.21 ± 0.06 despite persistent reduction of coronary flow. Intracoronary calcium infusion also restored dp/dt and end-diastolic pressure to control levels. Seven of an original 21 in group I had ventricular fibrillation; in group II the incidence was one of 12. Analysis of ischemic muscle revealed that group II had a smaller loss of K^+ and gain of Na than in group I, consistent with the arrhythmia incidence. Thus, enhanced extracellular calcium can improve function of ischemic as well as nonischemic muscle, associated with a reduction of K^+ loss and diminished ventricular arrhythmias.

130. Transport and Net Removal of Galactose by the Intact Liver. CARL A. GORESKY AND BRITA E. NADEAU,* Montreal, Canada.

D-Galactose, a monosaccharide rapidly phosphorylated within liver cells, is irreversibly removed from the portal circulation. We have studied the kinetic relations between the hepatic cell entry process and the metabolic sequestration process, by means of the multiple indicator dilution technique. Labeled red cells (a vascular indicator), labeled sucrose (an extracellular reference), and labeled galactose were rapidly injected into the portal vein, and from rapidly sampled hepatic venous blood, normalized outflow-time patterns were secured. The labeled red cell curve rises to the highest and earliest peak, and decays rapidly; and that for labeled sucrose rises to a later and lower peak. Its extrapolated recovery is equivalent to that of the labeled red cells. At low blood galacotse concentrations, the labeled galactose appears at the outflow with labeled sucrose, but is much reduced in magnitude, and exhibits a long tailing. Its outflow recovery is much reduced. At high blood galactose concentrations, the initial part of the profile increases towards that for labeled sucrose, the tailing becomes much larger in magnitude, and the outflow recovery becomes almost complete. We have modeled the uptake of labeled galactose, and find two parts to the predicted outflow pattern, corresponding to our experimental observations: throughput material, which sweeps past the cell surface in the extracellular space, and returning material, which has entered the cells but escaped the sequestration process. Analysis of the data by use of this model provides estimates of both transmembrane fluxes and rates of sequestration. The capacity of the process subserving cell entry is found to be 50 times that for phosphorylation; and, whereas the K_m value

for sequestration is less than 15 mg/100 ml, that for entry is approximately 350 mg/100 ml.

131. Specific Chemical-Enzyme Mediation of Tobacco Smoke Toxicity for Pulmonary Alveolar Macrophages (PAM). G. M. GREEN, G. M. POWELL,* AND T. G. MORRIS,* Burlington, Vt.

Although agents such as cigarette smoke are chemically complex, single, or few, components may cause *the* significant defect in the appropriately susceptible cell. The sulfhydryl nature of tobacco smoke toxicity for PAM was used as a label to (*a*) identify metabolic characteristics of cell damage; (*b*) identify the most susceptible enzyme; and (*c*) identify the most active chemical in the cigarette smoke. Cigarette smoke was freshly drawn through a Cambridge filter to isolate the filtered gaseous phase (FGP). 30 ml of FGP was fractionated by gas chromatography on a column of Porapak Q. The subfractions were tested for phagocytosis inhibition in an alveolar macrophage staphylococcus in vitro system, and for enzyme inhibition in histochemical preparations, by enzyme kinetic studies with crystalline enzymes and whole cell preparations, and by measurements of 1-^{14}C and 6-^{14}C labeled glucose metabolism. Whole FGP, fraction 5A (containing a mixture of acrolein and propionaldehyde), and pure acrolein (but not propionaldehyde) caused equivalent dose-response suppression of (*a*) bacterial uptake and (*b*) glyceraldehyde-3-phosphate dehydrogenase activity that was largely prevented in the presence of cysteine. Fraction 5 and acrolein, but not FGP, inhibited O_2-6-^{14}C production from specifically labeled glucose. Whole FGP stimulated the production of both O_2-6-^{14}C and O_2-1-^{14}C production despite the inhibition of glyceraldehyde-3-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase was not inhibited by FGP, fractions, or acrolein. Lactic dehydrogenase was also not inhibited. The results are interpreted as showing that the most sensitive specific toxicity of tobacco smoke for alveolar macrophage bacterial phagocytosis in vitro is caused by the action of acrolein on glyceraldehyde-3-phosphate dehydrogenase with suppression of glycolysis via the Emden-Myerhof pathway and suppression of the energy production necessary for particle uptake. (Supported by grants from NIH (AI 08900) and AMA-ERF.)

132. Immunoglobulin on the Surface of Human Lymphocytes: Distribution on Lymphocytes from Normal, Hypogammaglobulinemic, and Chronic Lymphatic Leukemic Individuals. HOWARD M. GREY,* ENRIQUE RABELLINO,* BERNARD PIROFSKY,* AND EMIL UNANUE,* Denver, Colo., Portland, Ore., and Boston, Mass. (introduced by R. S. Farr**).

Immunoglobulins (Ig) can be detected on the surface of 30–60% of human peripheral blood lymphocytes from normal individuals by immunofluorescence. Of the immunoglobulin-positive cells, 35–70% contain IgG, 8–30% IgA, 20–55% IgM, 60–90% kappa light chains, and 10–40% lambda chains. Studies in chickens and mice clearly indicate that Ig-containing lymphocytes are bone marrow or bursa derived (B), whereas Ig-negative cells are thymus derived (T). The lymphocytes from four individuals with acquired hypogammaglobulinemia and nine with chronic lymphatic leukemia (CLL) were examined for the presence of surface Ig. Less than 1% of the lymphocytes obtained from the hypogammaglobulinemic patients contained surface Ig, whereas 50–100% of the lymphocytes from CLL patients contained surface Ig. Moreover, only a single immunoglobulin light and/or heavy chain class was found in any individual patient. The cells from three patients contained only light chain determinant. All of the remaining six patients contained IgM (three kappa, three lambda) on the lymphocyte surface. These results suggest that the lymphocytes from CLL patients are monoclonal in the same sense that myeloma cells are, i.e., only a single immunoglobulin class is associated with them. Why IgM or isolated light chains are the only Ig expressed with other Ig's excluded is unknown. The results with hypogammaglobulinemic individuals are in keeping with the hypothesis that the basic defect in these patients resides in the B cell population. (Supported by grants from NIH and AHA.)

133. Splenic Transplantation in Gaucher's Disease. C. G. GROTH,* R. BLOMSTRAND,* S. DREBORG,* L. HAGENFELDT,* P. A. ÖCKERMAN,* K. SAMUELSSON,* L. SVENNERHOLM,* AND B. WERNER,* Lund, Gothenburg, and Stockholm, Sweden (introduced by Joseph Canary).

The primary defect in Gaucher's disease is a deficiency of glucocerebrosidase. Since splenic tissue contains a high concentration of this enzyme, grafting of a healthy spleen has been suggested as a means of its supplementation. A 24-yr-old male suffering from juvenile Gaucher's disease received a splenic homograft from an unrelated living donor on 4 January 1971. The recipient's previous history included splenomegaly, for which a splenectomy had been performed in childhood; later on, skeletal deformities, progressive mental retardation, and frequent generalized convulsions developed. Gaucher cells were abundant in the bone marrow. The transplantation was performed after administration of antilymphacytic globulin, prednisolone, and azathioprine to the recipient; before insertion the graft was irradiated. The same immunosuppressive measures were continued postoperatively. Technetium-99M scans have shown good but somewhat diminishing isotope uptake by the graft. Postoperative problems include severe trombocytopenia and hemolysis, requiring transfusions of 5500 ml of fresh whole blood over 2 wk. Plasma cerebrosides were extracted, and separated by thin-layer chromatography. Before treatment, the level of cerebroside measured as total lipid hexose was 128–142 μmoles/liter (normal less than 60); after the preoperative course of immunosuppression it fell to 83–97. During the first 2 postoperative wk the level dropped to normal with a minimum of 32 μmoles/liter. Plasma glucocerebroside and ceramide assays by gas chromatography and mass spectrometry, and measurement of β-glucosidase, β-galactosidase, and α-mannosidase in plasma and urine are in progress.

134. Increased Incidence of Two Leukocyte Antigen Specificities in Systemic Lupus Erythematosus (SLE). F. CARL GRUMET,* ANN COUKELL,* JULIA G. BODMER,* WALTER F. BODMER,* AND HUGH O. MCDEVITT, Stanford, Calif.

Lilly has shown that susceptibility to leukemogenesis by the Gross murine leukemia virus is linked to the major histo-

compatibility (H-2) locus in the ninth mouse linkage group. Resistance is dominant in the F_1 and this gene is linked to the right-hand or "K" region of the H-2 locus. Single genes controlling specific immune responses of inbred mice to synthetic polypeptide and protein antigens are also linked to the H-2 locus. High antibody response is dominant in the F_1, and one of these genes, immune response-1 (Ir-1), is also linked to the right-hand part of H-2. Previous studies in man have demonstrated an association between Hodgkin's disease and the human histocompatibility antigen HL-A5. In search of further evidence of association between disease susceptibility and histocompatibility (HL-A) type, we have studied the HL-A antigens of patients with systemic lupus erythematosus (SLE), a disease characterized by several immunologic abnormalities. 40 patients with SLE were HL-A typed by a standard lymphocyte cytotoxicity test. Specificity HL-A8 was present in 33% and the W15 component of LND was present in 40% of the patients, compared to control population frequencies of 16% ($P < 0.025$) and 10% ($P < 0.0005$), respectively. 19 other HL-A antigens did not differ significantly between SLE and control populations, and no unusually frequent phenotypes were observed. It is suggested that the association between histocompatibility loci and susceptibility to certain diseases may be mediated by histocompatibility-linked immune response genes analogous to Ir-1. Three possible mechanisms for this association will be presented. (Research supported by grants from NIH.)

135. Phonoangiography: a New Noninvasive Method for Diagnosis of Occlusive Arterial Disease. NELSON GURLL,* C. FORBES DEWEY, JR.,* AND ROBERT S. LEES,* Cambridge, Mass. (introduced by Irving M. London**).

We have observed, from sound spectral analysis of human carotid and femoral arterial bruits (phonoangiography), that the extent of arterial occlusion can be related to the amplitude and frequency spectrum of the arterial sound. Theoretical analysis of arterial blood flow suggested to us that the power spectral amplitude of an arterial bruit (P^2) should be proportional to $V^3 \cdot (D/d)^8$, where V is linear flow velocity and D/d is the ratio of unoccluded to occluded vessel diameter. To test these relationships experimentally, stenoses were produced in dog carotid and femoral arteries by the application of aluminum bands of known internal diameter. Bruits produced by this technique were qualitatively and quantitatively similar to spontaneous human arterial bruits. These sounds were stable with time for at least 3 months. With 25 bruits produced by stenoses varying from 0 to 85% occlusion of the cross-sectional area of the vessel, P^2 was linearly proportional to $(D/d)^8$ ($r = 0.96$, $P < 0.001$). The relationship between P^2 and V^3 was examined for 19 bruits; it, too, was linear ($r = 0.95$, $P < 0.001$). The constant association of a given sound spectrum with a given extent of stenosis and the predictability of the relationship between the characteristics of the sound, the velocity of blood flow, and the extent of stenosis give promise that phonoangiography will have value as a simple noninvasive method for assessment of occlusive arterial disease. (Research supported by grants from the NIH, the National Dairy Council, the Lillia Babbitt Hyde Foundation, and the Charles A. King Trust.)

136. A Radioimmunoassay for Human Prolactin. HARVEY GUYDA,* PETER HWANG,* AND HENRY FRIESEN,* Montreal, Canada (introduced by J. M. McKenzie).

The sensitivity of the radioimmunoassay using monkey prolactin-^{131}I and antiserum to human prolactin was equivalent to 50 ng/ml of our laboratory standard. Human growth hormone and human placental lactogen showed no significant cross-reaction in the assay. Random sera from 92 normal subjects of both sexes (aged 16–85 yr) and from 11 acromegalics contained less than 150 ng/ml. Elevated serum prolactin levels (250–500 ng/ml) were observed in 12 of 13 patients with galactorrhea. Of 12 normal subjects (six male and six female) subjected to an insulin tolerance test only three (two females and one male) showed a significant increase in serum prolactin, but of six subjects infused with arginine none showed an increase in serum prolactin whereas in both tests the expected increase in human growth hormone was found. In pregnancy, serum prolactin was detected as early as the 6th wk, increasing from 200 ±90 ng/ml in the first trimester to 900 ±250 ng/ml at term. The serum prolactin concentration declined to first trimester levels within 96 hr postpartum but remained elevated during the 1st wk after delivery. In subjects who were breast feeding, the decline was slower. In the newborn, serum prolactin concentrations were similar to those found in the mother. This specific radioimmunoassay will greatly facilitate studies of the physiologic role of prolactin in man. (Research supported by grants from MRC and NIH.)

137. Evidence for Abnormal Hypothalamic Function in Acromegaly. T. C. HAGEN,* L. KIRSTEINS,* AND A. M. LAWRENCE,* Chicago, Ill. (introduced by Leon O. Jacobson**).

In acromegaly, studies have demonstrated virtual absence of endocrine autonomy of growth hormone (GH) release, but relative insensitivity of hypothalamic-pituitary axis to usual inhibitory titers of GH. These observations and demonstration of an increased capacity of acromegalic serum to promote monkey pituitary GH release suggests that in acromegaly there may be chronic excessive release of hypothalamic growth hormone releasing factor (GHRF). In normal subjects, by raising GH levels above normal, it was possible to effectively inhibit GH release to known provocative stimuli such as arginine or exercise. In these studies GH levels were raised by exogenous administration of HGH or by promoting endogenous release of GH with a prior stimulus. In active acromegaly a similar effect was observed indicating that although the hypothalamic-pituitary axis is apparently intact, the locus for secretion of GHRF is relatively insensitive to the negative feedback effect of raised GH levels. In acromegaly, with clinically quiescent disease and normal basal GH after external radiation therapy, a different pattern was observed. Here a second peak GH rise could be elicited with a provocative stress administered at a time when GH levels had been raised by a prior exercise stimulus. When GH was infused simulating a level present during the active disease, a second GH response to the stimulus in tandem was abolished. Incubation studies demonstrated that acromegalic serum promoted a greater release of GH from monkey pituitaries than did normal serum. These preliminary studies suggest that in acromegaly there is (*a*) an intact hypothalamic-pituitary GH axis associated with

a GHRF center relatively insensitive to usual inhibitory titers of GH, and (*b*) increased quantity of a circulating mediator of GH release, possibly hypothalamic GHRF.

138. Effective Renal Plasma Flow and Glomerular Filtration Rate in Recipients Compared with Their Donors. WILLIAM M. HAMBY,* CLARENCE L. GANTT,* OLGA M. JONASSON,* AND ROGER D. SMITH,* Hines and Chicago, Ill. (introduced by Malcolm M. Stanley**).

Recognition of rejection in renal allografts depends upon urine and serum creatinine and urea concentrations, urinary protein excretion, blood cell counts, renal biopsy, and changes in body temperature and blood pressure. These values are usually adequate to identify acute rejection but frequently fail to identify slowly progressive chronic rejection. Comparison of the function of the kidney in the recipient with that of the one remaining in the donor might be a way to identify chronic rejection. We performed measurements of function (urea clearance, Cu; creatinine clearance, Ccr, inulin clearance, GFR; PAH clearance, ERPF; and tubular maximal excretory capacity, Tm_{PAH}) pre- and posttransplant in donors and recipients. To date we have followed 10 patients and their respective donors for a minimum of 1 yr. Unilateral renal function studies were performed on prospective donors using the technique of external ureteral compression. These studies were all within normal limits. Initial follow-up studies were performed as soon as feasible and routinely at 6-month intervals. Studies were performed more frequently when indicated. Results reveal increased Cu, Ccr, GFR, ERPF, and Tm_{PAH} in all successful transplants. These data parallel the results obtained from their donors. In two cases unsuspected progressive rejection was identified and treated successfully. The diagnosis was based upon a marked decrease in recipient renal function compared to his donor. Rejection was identified in spite of normal values for GFR, ERPF, and Tm_{PAH}. These values improved after treatment. In the absence of chronic progressive rejection we have been able to decrease immunosuppressive therapy to minimal levels using these comparative results as one of the indices for evaluation.

139. Canine Renal Allograft Survival as a Function of Soluble Ribonucleic Acid Dose and Perfusion Time. WILLIAM M. HAMBY,* DORIS M. SCHAAFF,* CASIMIR F. FIRLIT,* AND JOHN R. CANNING,* Hines, Ill. (introduced by John T. Sharp).

Increased survival of canine renal allografts after treatment with soluble ribonucleic acid (sRNA) has been reported. Using extracorporeal perfusion we studied the effects of dose and perfusion time on allograft survival. The perfusate contained lactated Ringer's, low molecular weight dextran, bentonite-treated, pooled dog plasma, procaine, epinephrine, and syngeneic sRNA. Perfusate flow averaged 280 ml/min. Perfusion time ranged from 20 to 85 min. Soluble RNA was extracted from the recipient's spleen using a hot phenol technique. The dose of sRNA ranged from 0 (control) to 32.18 mg. Mongrel dogs weighing between 17 and 27 kg were studied in randomly selected pairs. Cross-transplantation was accomplished after perfusion and contralateral nephrectomy. Maximum ischemia time was 40 min. Treated animals survived an average of 12 days which was significantly greater ($P < 0.01$) than that of controls. Survival in the control group was decreased by increasing perfusion time. Survival time for treated animals was lengthened by increasing the dose of sRNA or by increasing the time for perfusion. Thus, prolonged perfusion (85 min) with low dose (2.5 mg) of sRNA or short perfusion (20 min) with high dose (32.18 mg) of sRNA increased survival significantly ($P < 0.05$). Therefore increased survival is a function of exposure to the sRNA. This supports the conclusion that the sRNA enters the cell, acts as a virus, and takes over the activity of the polyribosome. This results in formation of specific proteins which are of the recipient rather than of the donor allotype. Consequently, the kidney is accepted as self. This hypothesis is supported by other data which will be reported from this laboratory.

140. Defective Fibrin Cross-Linkages: a Genetic and Biochemical Study of Three Families. JAMES W. HAMPTON,* ROBERT O. MORTON,* DAVID BANNERJEE,* AND EKRAM KALMAZ,* Oklahoma City, Okla. (introduced by Stewart Wolf**).

Fibrin cross-linkage results from a chemical interaction between monomer molecules which are bound together by covalent bonds involving the epsilon amino groups of lysine residues and the γ-carboxyamide group of specific glutamines. An enzyme, plasma transglutaminase, which is activated by thrombin, cross-links the monomeric units. Three families with excessive bleeding were studied for fibrin cross-linkage defects. In one family a functional deficiency of transglutaminase was demonstrated in a homozygote and four heterozygotes. The defect is inherited as an autosomal and recessive trait. A second family has normal plasma transglutaminase but a qualitatively abnormal fibrinogen substrate (fibrinogen Oklahoma). In addition to reduced recovery of the clottable protein when fractionation methods are used, there is an abnormal electrophoretic pattern of the chains and a missing peptide on two-dimensional chromatography. The inheritance of this defect would appear to be autosomal and dominant. A third family has an abnormal euglobulin lysis time, reduced plasma transglutaminase, and fibrin split products in the serum with an autosomal dominant inheritance. All three defects appear to result from an abnormality of the cross-linkage interaction between substrate and enzyme and are genetically independent. Only the enzyme deficiency results in a severe bleeding disorder and it can be managed by prophylactic plasma transfusions. (Research supported by Grants T01AM05107 and HE12316 from NIH.)

141. Effects of Aldosterone, Vasopressin, Ouabain, and Amiloride on an Epithelial Cell Preparation from the Toad Urinary Bladder. JOSEPH S. HANDLER, AGNES S. PRESTON,* AND JACK ORLOFF,** Bethesda, Md.

The effects of four agents that alter the rate of active sodium transport by the intact toad urinary bladder were examined on a preparation of sheets of epithelial cells scraped from collagenase-treated bladders. Changes in metabolism and electrolyte content should be easier to detect in a pure cell preparation than in the intact bladder composed of a heterogeneous cell population as used in previous negative studies.

As in the intact bladder, the rate of oxidation of ^{14}C-labeled glucose or pyruvate is increased by aldosterone and by vasopressin, agents that stimulate sodium transport, and is decreased by ouabain and amiloride, agents that inhibit sodium transport. In order to determine whether the change in sodium transport elicited by each agent is mediated by a change at the mucosal entry or serosal exit step, cell water, Na, K, and Cl content were measured. The following statistically significant ($P < 0.01$) percentage changes were observed: 10^{-4} M ouabain, Na + 46, K − 19, 10^{-5} M amiloride, Na − 32; 10^{-7} M aldosterone, H_2O + 6, Na + 96, K − 5, Cl + 31; vasopressin, H_2O + 16, Na + 63, K − 11, Cl + 22. These results are compatible with the interpretation that ouabain acts by inhibiting the serosal pump, amiloride by inhibiting the mucosal entry step, and the two hormones by stimulating the mucosal entry step. Each hormone also causes the phosphocreatine/creatine ratio in the cells to fall to less than half that n controls. The results are interpreted as indicating that aldosterone and vasopressin each stimulate sodium transport primarily by increasing a mucosal entry step. The stimulation of coupled energy metabolism is secondary.

142. Deoxycholic Acid Metabolism in Man. RUSSELL F. HANSON,* Minneapolis, Minn. (introduced by Ivan Frantz, Jr.**).

Deoxycholic acid, a potent inhibitor of several intracellular functions in vitro, has been implicated in the production of the diarrhea in the syndrome of cholerhetic enteropathy. Several animal models used to study bile acid metabolism rehydroxylate deoxycholic acid to bile acids which no longer exhibit this inhibition. Nevertheless little is known about the metabolic fate of deoxycholic acid after intestinal absorption in man. To investigate the metabolism of deoxycholic acid in man, deoxycholic acid-^{14}C was given intravenously to patients with complete T tube bile fistulas. The excretory products were isolated from the bile. Approximately 80% of the administered radioactivity was excreted in the first 24 hr. After hydrolysis and chromatographic separation of the bile acids, greater than 94% of the radioactivity was found in the deoxycholic acid zone. The other 6% was scattered in many small peaks, none of which migrated with cholic acid. The radioactivity in the deoxycholic acid zone migrated with authentic deoxycholic acid on paper chromatography and when mixed with unlabeled deoxycholic acid gave a constant specific activity after repeated recrystallizations. The conjugates formed from deoxycholic acid-^{14}C were measured by thin-layer chromatography of unhydrolyzed bile. Approximately 85% of the deoxycholic acid was conjugated with glycine and 13% with taurine. The other major secondary bile acid in man, lithocholic acid, has been shown to undergo 3α-sulfation. However, in these studies no radioactivity was found in the area where the 3α-sulfate ester of conjugated deoxycholate should be located on thin-layer chromatography. This study demonstrates that in man (*a*) deoxycholic acid is not rehydroxylated to cholic acid, (*b*) all deoxycholic acid is conjugated with either glycine or taurine, and (*c*) the 3α-sulfate esters of conjugated deoxycholic acid are not formed.

143. Protection by Dithiothreitol (DTT) Against Porphyrin Photosensitization. L. HARBER, B. GOLDSTEIN,* J. HSU,* AND H. HSU,* New York.

Although protection against ionizing radiation by compounds containing sulfhydryl (SH) groups such as cysteine has been reported, these agents have been unsuccessful to date in protecting mammals against nonionizing radiation (>200 nm). This study describes successful photoprotection by DTT against porphyrin photosensitization (400 nm) in RBC's obtained from patients with erythropoietic protoporphyria (EPP) and mice photosensitized by hematoporphyrin(HP). A mortality rate approaching LD_{50}/24 hr was established in 100 white mice that had received 0.10 mg HP per gm body weight intraperitoneally and were then irradiated with $5 \cdot 10^6$ ergs/cm^2 from a fluorescent light source emitting 320–450 nm radiation. Another 100 mice were treated in an identical manner except that they received, in addition, 0.08 mg DTT per g body weight. This group showed a 75% reduction in mortality ($P < 0.03$). Animals treated with DTT and/or HP in the above concentrations without 400 nm radiation had no mortality. RBC's obtained from patients with EPP and exposed to 1.10^7 ergs/mm^2 of 400 nm radiation from a Solar Simulator showed 100% hemolysis after 60 min. These cells, when irradiated under identical conditions except for the addition of 13 mM DTT manifested 12% hemolysis. Additional studies using similar photohemolysis techniques showed that RBC's from normal individuals incubated with HP or PP were also protected by DTT. Measurements of SH groups in EPP RBC's showed a progressive decrease during photohemolysis. These studies demonstrate that DTT offers photoprotection in mammalian systems against porphyrin photosensitization. It is suggested that the mechanism of the protective action is similar to that of ionizing radiation; i.e., SH protection and/or free radical traps. (Research supported by Grant ES00288-07 from NIH.)

144. Ventricular Arrhythmias Induced in Monkeys by the Inhalation of Aerosol Propellants. WILLARD S. HARRIS,* GEORGE J. TAYLOR,* AND MORTON D. BOGDONOFF, Chicago, Ill.

After inhaling fluoroalkane gases used as aerosol propellants, some people have died suddenly and unexpectedly. Seeking an explanation, we had 14 monkeys (3 awake and 11 anesthetized) inhale a 30% dichlorodifluoromethane (Freon 12)–9% dichlorotetrafluoroethane (Freon 114)–61% oxygen mixture. All developed ventricular premature beats, bigeminy, or tachycardia, which began at an average of 39 (SE ±4.2, range 20–72) sec. At this time Freon 12 and 114 levels in arterial blood, determined by gas chromatography, averaged 6.1 and 2.1 mg/100 ml, but arterial hypoxemia or hypercapnia was absent and mean arterial pressure had fallen only 12 ±4 mm Hg. Although Freon inhalation was immediately stopped, the ventricular arrhythmias persisted up to 3 min. In contrast, without fluoroalkanes, 3 min of asphyxia or of anoxia (arterial P_{O_2} 30 ±3.2 mm Hg) induced by the administration of 100% nitrogen failed to cause arrhythmias, except in one monkey, whose arterial P_{O_2} had fallen to 16 mm Hg. Since 0.07 mg/kg propranolol injected intravenously abolished them, the ventricular tachyarrhythmias caused in well oxygenated monkeys

by fluoroalkane gas may be mediated through beta adrenergic receptors. These results suggest that some human deaths after propellant inhalation may be caused by ventricular tachycardia or fibrillation.

145. Effect of Ethanol on Human Platelet Function. MICHAEL J. HAUT* AND DALE H. COWAN,* Cleveland, Ohio (introduced by John W. Harris**).

Thrombocytopenia characterized by reduced platelet life span and reduced effective thrombopoiesis occurs in nonfolate-deficient individuals ingesting large quantities of ethanol. To determine whether ethanol also affects platelet function, platelet factor III (PF3) activity, aggregation, and nucleotide release were measured by (*a*) adding varying concentrations of ethanol (50–500 mg/100 ml) to normal platelets in vitro, (*b*) comparing platelets obtained before and during an intravenous ethanol infusion, and (*c*) comparing normal platelets with those obtained from a subject who developed thrombocytopenia while ingesting 1 quart of 86 proof whiskey daily. In vitro addition of ethanol to platelet-rich plasma produced a decrease in the rate and maximal extent of aggregation in response to ADP, epinephrine, thrombin, and collagen, eliminated the second wave of ADP-induced aggregation, and prolonged the lag phase of collagen-induced aggregation. The degree of suppression was dependent upon the relative concentrations of ethanol and the aggregating agent. The rate and extent of aggregation of platelets obtained during an intravenous infusion of ethanol (blood alcohol level 190 mg/100 ml) was decreased 20–50% with each agent as compared to preinfusion platelets. Aggregation of platelets obtained from a thrombocytopenic ethanol-ingesting subject (blood alcohol level 290 mg/100 ml) was reduced by 50–75% compared with normal. The second wave of ADP-induced aggregation was again absent. PF3 activity as produced by kaolin or ADP was unaffected by in vitro addition of ethanol, but was absent from platelets obtained from the thrombocytopenic alcoholic subject. No decrease in nucleotide release occurred with in vitro addition of ethanol or with ethanol ingestion. The data indicate that ethanol suppresses platelet aggregation and PF3 activity and suggests that ethanol may alter the reactivity of the surface membrane. (Research supported by Grants AM 05420-07 and RR 05410-09 from NIH.)

146. An Alternative to the Pore Enlargement Hypothesis for Vasopressin Action. RICHARD M. HAYS, NICHOLAS FRANKI,* AND ROY SOBERMAN,* New York.

Vasopressin is believed to enlarge aqueous channels in the luminal cell membrane, permitting bulk water flow. Supporting evidence includes the finding that vasopressin increases net water flow across the toad bladder 40-fold, but the diffusion rate of tritiated water (K_{trans}THO) only 2-fold. This discrepancy could occur if pore radius (r) enlarged, with flow increasing by r^4 and diffusion by r^2. Studies of activation energy (E_A) for K_{trans}THO also supported the hypothesis: E_A fell from 9.8 to 4.1 kcal/mole after vasopressin, the latter value approximating that for water diffusion in liquid water. The present studies are based on the observation that K_{trans}THO after vasopressin is so rapid that unstirred layers of water, and the thick layer supporting the bladder epithelial cells become rate limiting for diffusion. Thus, K_{trans}THO across the cell membrane is greatly underestimated. In chambers stirred with impellers, K_{trans}THO (extrapolated to infinite stirring speed) increases 15- to 20-fold across the epithelial cell after vasopressin. An even higher value is obtained for K_{trans} across the luminal cell membrane when the retarding effects of cell cytoplasm, intercellular space, and basolateral membrane are estimated. E_A is also influenced by unstirred and supporting layers: in well stirred chambers, E_A across the epithelial layer is unchanged after vasopressin (11.7 ± 1.4 and 10.6 ± 1.1 kcal/mole before and after hormone), indicating that channel size is unaltered. These studies suggest that vasopressin increases the *number* rather than the *size* of small aqueous channels in the membrane, and also accounts for the selectivity the cell exerts towards small solutes even during high water flows. (Supported by NIH grant.)

147. On the Lipolytic Defect in Familial Type I Hyperlipoproteinemia. PETER HERBERT,* JOHN LAROSA,* RONALD KRAUSS,* SAMUEL LUX,* ROBERT I. LEVY, AND DONALD S. FREDERICKSON,** Bethesda, Md.

Familial type I is characterized by markedly decreased lipolysis of lipoprotein-bound triglyceride. Paradoxically, lipolysis of artificial glyceride emulsions by postheparin plasma (PHP) in type I is sometimes quantitively normal. A specific polypeptide activator (apoLP-glu) for lipoprotein lipase has recently been isolated from normal high density (HDL) and very low density lipoproteins (VLDL). The relationship of apoLP-glu to lipolysis in PHP and to the defect in type I have now been further studied. PHP contains at least two different triglyceride lipase activities (TGL). One (TGL_1), probably identical with lipoprotein lipase in adipose tissue and other organs, is activated by apoLP-glu and inhibited by high salt concentrations, protamine, and pyrophosphate. The other (TGL_2) is released from liver by heparin, is inhibited by apoLP-glu, and is resistant to salt inactivation, protamine, and pyrophosphate. The hepatic enzyme is very active against artificial triglyceride emulsions but is less than 10% as active as TGL_1 against chylomicron and VLDL triglyceride. A normal proportion of apoLP-glu has been found in the VLDL and HDL apoproteins of two patients with familial type I from different kindreds. Furthermore, apoLP-glu from both type I subjects activated lipoprotein lipase as effectively as equimolar amounts of apoLP-glu from a control. PHP from four type I patients hydrolyzed artificial glyceride emulsions; but the activity had the characteristics of TGL_2, being unaffected by salt, protamine, or pyrophosphate. Activity in PHP from type I patients was also not enhanced by added apoLP-glu. It is concluded that the defect in familial type I is due to a deficiency of TGL_1 activity and that this is not due to absence of the apoprotein activator of this enzyme.

148. Mechanism of Dilutional Anemia and Hyperviscosity in Multiple Myeloma (MM). CHARLES E. HESS,* DANIEL N. MOHLER,* AND CARLOS R. AYERS,* Charlottesville, Va. (introduced by Kenneth R. Crispell**).

Decreased RBC production is present in most cases of MM and has been considered to be the main cause of the anemia. Only recently has plasma volume (PV) expansion been im-

plicated in the etiology of the anemia. The mechanism of this PV expansion is poorly understood. We have studied 14 cases of MM to determine not only the extent and frequency of dilutional anemia, but also that of the hyperviscosity state and the mechanisms involved. Red cell mass (RCM) and PV were determined by the ^{51}Cr-labeled RBC and ^{125}I-labeled albumin methods respectively. The colloid osmotic pressure (COP) exerted by albumin and immunoglobulins was calculated. 12 cases had a monoclonal ("M") spike with an expanded PV and elevated serum viscosity, the extent of which was predictable from the COP, the aggregation-disaggregation pattern on ultracentrifugation, and the distribution between the intravascular and extravascular spaces of the "M" protein. In two cases there was no "M" spike and no anemia of PV expansion even though the bone marrow revealed extensive plasmacytosis. Plasmapheresis resulted in a decrease in "M" protein concentration, PV, relative serum viscosity, and COP with increase in the venous hematocrit. PV expansion was found to be a major factor responsible for anemia in MM. With increasing accumulation of "M" protein, the PV expands in an effort to maintain a normal COP. This dilution of the "M" protein tends to decrease its viscosity effects. If aggregation occurs or if the protein possesses inherently high viscosity properties relative to its COP effect, the hyperviscosity state often results. (Research supported by grants from NIH.)

149. Tumor Resistance Conferred by Intracellular Protozoa. JOHN B. HIBBS, JR.,* LEWIS H. LAMBERT, JR.,* AND JACK S. REMINGTON, Palo Alto, Calif.

Administration of live (e.g. Calmette-Guérin bacillus [BCG]) or dead (e.g. *B. pertussis*) organisms or polyanions (e.g. Poly C:I) which stimulate multiple immunologic factors and confer resistance against intracellular infection has been demonstrated to prevent tumor development or cause tumor regression. Since *Toxoplasma gondii* (TG), a common latent intracellular infectious agent of man, has been shown to confer resistance against intracellular bacteria (e.g. *Listeria*) and virus (e.g. *Mengo*) in mice (this resistance is mediated by cells and persists for more than 1 yr), studies were performed to determine if TG and a related protozoan, *Besnoitia jellisoni* (BJ), confer resistance against transplantable and autochthonous tumors. Significant resistance to development of mammary tumors was noted in C_3H/He mice (e.g. tumor incidence at 6 months: controls = 62%, BJ- and TG-infected mice = 26%) and to leukemia in AKR mice (e.g. mortality at 6 months due to leukemia: controls = 69%, TG-infected mice = 19%, BJ-infected mice = 0%). Similar resistance was noted after challenge with Friend leukemia virus (e.g. mortality at 6 months due to leukemia: controls = 90%, TG-infected mice = 20%, BJ-infected mice = 40%). Significant prolongation of time to death and increased survival were observed in protozoal-infected mice inoculated with sarcoma 180 in the ascites form. Initial studies suggest that macrophages may play a major role in this tumor resistance. Since toxoplasma remains active for years in man, it would be of interest to determine what epidemiologic effect this infection may have on tumor development in humans. (Research supported by grant from ACS, California Division.)

150. Quantitative Assessment of Blood and Tissue Pyridoxal-Phosphokinase Concentration in Patients with Vitamin B_6-Dependent States. JOHN D. HINES,* Cleveland, Ohio (introduced by George Gabuzda**).

Pyridoxal-phosphokinase (PL-kinase) is an enzyme system necessary for phosphorylation of the metabolically inert vitamin B_6 analogs, pyridoxine and pyridoxal, to their coenzyme forms. Quantitative determination of PL-kinase was performed in erythrocytes, liver, and cultured fibroblasts obtained from patients with vitamin B_6-dependent conditions including refractory sideroblastic anemia (RSA) (six patients) and cystathionuria (one patient). Vitamin B_6 dependency was demonstrated in all patients by successive hematologic (RSA) or biochemical (cystathionuria) response patterns after administration of pharmacological amounts of pyridoxine (20–200 mg/day). Erythrocyte, hepatic, and fibroblast PL-kinase activity were estimated from the pyridoxal phosphate (PLP) concentrations generated by incubation at 37°C of erythrocyte hemolysate or tissue extracts incubated with 0.6 mM ATP, 0.6 mM pyridoxal, 0.6 mM pyridoxine, and 0.06 mM magnesium with 0.05 M glycerophosphate buffer pH 7.4 (erythrocytes) or 5.8 (tissue) for 0, 30, and 60 min. Erythrocyte PL-kinase values were subnormal in five patients with RSA and in the cystathionuria patient ranging from 9 to 36% of normal control values at a time when the seven patients received no pyridoxine for 8 wk. Oral pyridoxine therapy (20–200 mg/day) resulted in an increase in erythrocyte PL-kinase activity to normal levels in only one patient (with RSA). Hepatic PL-kinase levels were determined in five patients (all RSA) and the values were subnormal in three of the five patients ranging from 11 to 41% of normal control values. Oral pyridoxine therapy (100 mg/day) did not significantly alter the hepatic PL-kinase concentrations in these three. Fibroblast PL-kinase concentrations were subnormal in the patient with cystathionuria (30% of normal controls). These studies illustrate that certain patients with vitamin B_6 dependency have associated abnormalities in the PLP anabolic pathway which may be of etiologic significance in the genesis of the B_6-dependent state. (Research supported by grant from NIMH 15735-02.)

151. Electrical Potential Profile of Rabbit Ileum: Effects of Theophylline and Cholera Exotoxin. NORBERT HIRSCHHORN* AND HOWARD S. FRAZIER, Boston, Mass.

The epithelial layer of the isolated and unstimulated rabbit ileum absorbs Na and Cl from the mucosal (M) medium. Theophylline (T), or cyclic AMP, and cholera exotoxin (CE) reduce net absorption of Na and change net Cl movement to secretion. The electrical consequences of these changes in ion movements were measured with intracellular micropipettes in order to differentiate between effects on villus (VC) and intervillus cells (IVC) and to test the hypothesis that the effect of CE is mediated by the cyclic AMP system. In spontaneously active control preparations the intracellular potential referable to M of VC was −18.7 mv and of IVC −16.1 mv ($P < 0.025$). T increased transmural potential; in IVC, it significantly reduced intracellular negativity but did not affect cellular resistance; and in VC, it increased cellular resistance without significantly altering intracellular potential.

Purified CE or inactivated CE (ICE) were added to the perfusate of ileal loops in vivo in six rabbits 4 hr before removal of the tissue for electrical measurements. Reduction in intracellular negativity of borderline significance was noted in IVC only in animals receiving CE as compared to ICE. There was no change in cellular resistance. Subsequent addition of T caused the usual increase in transmural potential in preparations treated with ICE, but no significant change in those treated with CE. These results support the hypothesis that the action of CE is mediated by the cyclic AMP system, and that CE has its major effect on intervillus cells. (Supported by Grants AM 39655, AM 05555, and HE 31106 from the NIH.)

152. Isolation of Inclusion Bodies from Rabbit Lung Parenchyma. LEE HOFFMAN,* Bronx, N. Y. (introduced by M. Henry Williams, Jr.**).

There is some evidence that the inclusion body of the type II alveolar epithelial cell is a type of lysosome; and prior studies have implicated it as the source of pulmonary surfactant, a phospholipid-containing material which, after discharge into the alveolus, is responsible for maintainig low surface tension at the alveolar air-liquid interface. The present study was undertaken to provide more information about the nature of this inclusion body and further evidence that it is the source of pulmonary surfactant. We studied the mitochondrial-plus-lysosomal fraction of rabbit lung parenchyma by equilibrium density centrifugation in sucrose density gradients (d 1.035–1.250). Cytochrome *c* oxidase, a mitochondrial marker, was present in a narrow band at density 1.18. Lysosomal marker enzymes—cathepsin, aryl sulphatase, α-mannosidase, acid phosphatase, β-glucuronidase—were present in a broad band at d 1.16–1.18, a density typical for lysosomes. Three of these lysosomal enzymes were also present in high concentration in a band at d 1.06–1.07. Phospholipid phosphorus determination of lipids extracted from each band showed that the light density band contained the highest relative specific activity of phospholipid. Thin-layer chromatography of the lipids from this band showed a large spot which cochromatographed with dipalmitoyl lecithin (the chief constituent of pulmonary surfactant) and a smaller spot which cochromatographed with phosphatidylethanolamine. Electron microscopy of material from the light density band showed a pure array of particles which bear a strong resemblance to the inclusion bodies seen in electron micrographs of rabbit lung tissue sections. These data indicate that the light density band is a pure isolation of type II alveolar epithelial cell inclusion bodies, that these bodies are the source of surfactant, and that they are part of of the cell's lysosomal system. (Supported by NIH Grant HE 10176.)

153. Mechanism of Abnormal T_3 Suppressibility in Hyperthyroidism: Relationship to Elevated T_3 Levels and Long Acting Thyroid Stimulator (LATS). CHARLES S. HOLLANDER,* TERONURI MITSUMA,* NORIYUKI NIHEI,* YOSHIMOCHI ISHIZUKI,* AND MARVIN C. GERSHENGORN,* New York and Nagoya, Japan (introduced by Saul J. Farber**).

Measurements of triiodothyronine (T_3), T_3 suppressibility, and long acting thyroid stimulator (LATS) were performed in 37 patients with toxic diffuse goiter before treatment and after they became euthyroid. T_3 was measured by a newly developed radioimmunoassay technique on unextracted serum and corroborated by gas chromatography. All 37 patients had abnormal T_3 suppressibility and elevated T_3 levels (644±286ng/100 ml) before therapy; LATS was found in 15 patients. The patients were treated with radioiodine and became euthyroid. All five with persistent LATS failed to suppress with T_3. In the (−)LATS group, 25 of 32 failed to suppress. Serum T_3 levels differed significantly in the two groups ($P < 0.005$); 174 ±27 ng/100 ml in the (+)LATS group and 131 ±26 ng/100 ml in the (−)LATS patients. Immunoglobulin concentrates from a patient with high LATS titers were shown to decrease mean serum thyroid-stimulating hormone (TSH) by 40% (2.05 ±0.15 mU/ml to 1.23 ±0.14 mU/ml; $P < 0.001$) in a group of 12 thyroidectomized rats. No significant change was found with (−)LATS immunoglobulin concentrates. We conclude that (*a*) high serum T_3 levels are present in untreated toxic diffuse goiter, (*b*) after therapy (+)LATS determinations are associated with high serum T_3 levels, (*c*) the presence of LATS, in this group, was uniformly associated with abnormal T_3 suppressibility, although this abnormality can also occur in the absence of measurable LATS, and (*d*) LATS may inhibit the normal thyroid pituitary feedback mechanisms by acting either directly or via T_3. (Research supported by Grants FR-96 and 2 RO1 AM 14314-02 from NIH.)

154. Pulmonary Oxygen Toxicity. GARY HUBER,* STEPHANIE BURLEY,* LEE PORTER,* ROBERT MASON,* AND MARC LAFORCE,* Boston, Mass. (introduced by Edward H. Kass**).

Therapeutic use of oxygen at toxic concentrations is often essential in managing many respiratory diseases. The following experiments on administration of 100% oxygen (*a*) define a progressive, dose-dependent toxicity to pulmonary structure and function after continuous exposure to oxygen, (*b*) demonstrate complete prevention of these toxic manifestations if an equivalent toxic dose is given by intermittent rather than continuous exposure, and (*c*) introduce a concept of "tolerance" in which toxic manifestations of continuous exposure are markedly reduced in animals first adapted, on an intermittent basis, to an oxygen environment. Pulmonary morphology, fluid accumulation, phospholipids, and proteins were quantified in each group studied. Pulmonary edema and hyaline membranes occurred late with continuous exposure, were absent with intermittent exposure and were markedly reduced in the tolerant group. Alveolar macrophage function was evaluated after exposure to 100% oxygen by quantitating *in situ* bacterial killing in the lungs of mice 6 hr after exposure to an aerosol of radiolabeled (^{32}P) *Staphylococcus aureus*. Controls inactivated 7.1% of the bacterial inoculum. Continuous exposure resulted in a progressive impairment of pulmonary antibacterial defenses (inactivation of 21.5% and 30.8% after 24 and 48 hr, with bacterial replication exceeding inactivation at 72 and 96 hr of exposure). Bacterial inactivation was normal with intermittent exposure at all accumulative dosages, and reduced in the tolerant group (14.9%, 24.5%, 75.3%, and 96.4% at 24, 48, 72, and 96 hr, respectively). Electron microscopy revealed progressive vacuolization in alveolar macrophages after continuous exposure to oxygen and no alterations in the intermittent or tolerant group. Thus, pretreatment with

oxygen reduces the parenchymal manifestations and ameliorates the bactericidal defect induced by prolonged exposure to 100% oxygen.

155. Relationship Between Intrarenal Control of Sodium Reabsorption and Renin Secretory Activity (RSA). M. H. HUMPHREYS,* I. A. REID,* R. C. UFFERMAN,* AND L. E. EARLEY, San Francisco, Calif.

Arterial blood of anesthetized dogs pretreated with mineralocorticoid was circulated via a pump through isolated perfused canine kidneys (PK) and the renal venous blood returned to the dog via a reservoir initially filled with canine plasma equilibrated with the dog's blood. This arrangement permitted independent control of the dog's blood volume and the composition of the blood while perfusion pressure to PK was kept constant. Plasma renin activity (PRA) (ng angiotensin I/ml per 3 hr) was measured by radioimmunoassay, and RSA by PK calculated from arterial and renal venous PRA and directly measured blood flow. Volume expansion from the reservoir containing equilibrated whole blood increased arterial pressure (AP) and sodium excretion ($U_{Na}V$) in the dog and decreased arterial PRA from 46 ±6 (SE) to 40 ±4 ($P < 0.05$), while mean $U_{Na}V$ and RSA by PK did not change. Ringer's solution added to the reservoir diluted the circulating blood without volume expansion, and decreased AP and usually $U_{Na}V$ in the dog, but $U_{Na}V$ by PK increased from 25 ±6 to 69 ±9 μEq/min ($P < 0.001$) as RSA decreased by 63% ($P < 0.025$). Hemorrhage of the volume-expanded dog into the reservoir decreased AP and $U_{Na}V$ in the dog with inconsistent effects on renal vascular resistance, $U_{Na}V$, and RSA in PK. These results indicate that (*a*) there is no evidence that changes in a circulating factor regulate $U_{Na}V$ during volume expansion or hemorrhage; and (*b*) hemodilution is a potent suppressor of RSA, and increases $U_{Na}V$ in PK, independent of changes occurring in the animal supplying the blood or in arterial PRA. Thus, it is possible that changes in RSA may relate directly to changes in sodium reabsorption caused by intrarenal mechanisms in PK. (Supported by NIH Grants AM 06704 and AM 12753, NASA Grant NGR 02505007, and a grant from the Bay Area Heart Research Committee.)

156. Effect of Peritubular Oncotic Pressure on Reabsorption of Sodium and Water in Isolated Perfused Proximal Tubule. MASASHI IMAI* AND JUHA P. KOKKO,* Dallas, Tex. (introduced by D. W. Seldin**).

Micropuncture studies have indicated that variation in peritubular oncotic pressure influences net transport of fluid out of the proximal tubules. The present in vitro studies on isolated perfused rabbit proximal convoluted tubules (PCT) were designed to examine whether oncotic pressure must act across the peritubular capillary membrane to influence reabsorption, or whether it can exert a direct effect across the tubular basement membrane. 45 isolated PCT were perfused with ultrafiltrate (UF) made isomolal to bathing fluids, the latter having identical composition as the perfusing UF, but adjusted to three oncotic pressures: hypooncotic (hypo), protein 0.0 g/100 ml; normal control serum (C), protein 6.8 g/100 ml; and hyperoncotic (hyper), protein 12.4 g/100 ml. Net volume flux (Jv, nl/mm per min), net Na flux (Φ_n^{Na}, nEq/mm per min), unidirectional Na flux from bath to lumen (Φ_{bl}^{Na}, nEq/mm per min), and passive permeability coefficient of Na ($P_{Na} \times 10^{-5}$ cm/sec) were determined isotopically using methods previously published. When hypooncotic bath was used in random order, Jv (C, 1.02 ±0.10; hypo, 0.66 ±0.09) and Φ_n^{Na} (C, 0.158 ±0.023; hypo, 0.094 ±0.017) decreased significantly ($P < 0.001$) from control without detectable changes in Φ_{bl}^{Na} (C, 0.724 ±0.101; hypo, 0.69 ±0.119) and P_{Na} (C, 12.8 ±1.8; hypo, 14.0 ±1.6). When the hyperoncotic bath was used, Jv (C, 1.05 ±0.13; hyper, 1.25 ±0.13) and Φ_n^{Na} (C, 0.158 ±0.027; hyper, 0.198 ±0.025) increased in 9 of 12; however, the changes were not statistically significant ($0.1 < P < 0.2$). With hyperoncotic serum, Φ_{bl}^{Na} (C, 0.747 ±0.100; hyper, 0.812 ±0.112) remained unchanged. These data demonstrate that a decrease in the oncotic pressure gradient exerts a direct effect upon proximal tubular reabsorption, reducing bath Jv and Φ_n^{Na}. It is concluded that an effect of oncotic pressure on reabsorption can be exerted directly across the basement membrane, without necessary interposition of the capillary bed. (Research supported by grants from Dallas Heart Association and NIH.)

157. Mechanism of Thyroid Calorigenesis: Role of Activation of NaK-ATPase. FARAMARZ ISMAIL-BEIGI* AND ISIDORE S. EDELMAN,** San Francisco, Calif.

We recently presented evidence that thyroid calorigenesis is predominantly mediated by stimulation of energy consumption by the Na^+ pump. We now determined the effects of administration of triiodothyronine (T_3) on NaK-ATPase activity in rat liver and on intracellular electrolyte concentrations in liver and other tissues. In thyroidectomized rats, three injections of T_3 on alternate days (50 μg/100 g body weight) produced a 91% increase in the activity of NaK-ATPase but had no significant effect on the activities of Mg-ATPase or 5′-nucleotidase of the liver plasma membrane fraction. Time-course measurements of the changes in total oxygen consumption (QO_2) and ouabain-sensitive respiration [$QO_2(t)$] of liver slices and of NaK-ATPase activity in liver homogenates were made after either single or repeated T_3 injections. In normal and thyroidectomized rats injected with successive doses of T_3, the increase in QO_2 was concomitant with the increases in both $QO_2(t)$ and NaK-ATPase activity. After a single injection of T_3, the increase in QO_2, $QO_2(t)$, and NaK-ATPase activity peaked at 48–60 hr and then declined to the base line with similar half-times of ~ 48 hr. Moreover, administration of T_3 in normal and thyroidectomized rats lowered intracellular Na^+ and raised intracellular K^+ concentrations in liver and skeletal and cardiac muscle. These results imply that thyroid stimulation of NaK-ATPase enhances Na^+ pump activity and accounts for the measured increase in $QO_2(t)$ and thereby mediates the increase in QO_2. (Research supported by Bay Area Heart Research Committee and NIH Grants HE-06285 and HE-05725.)

158. Angiotensin I As an Intrarenal Hormone. HAROLD D. ITSKOVITZ,* CHARLES ODYA,* JEAN BROCK,* AND JOHN STEMPER,* Milwaukee, Wis. (introduced by William W. Engstrom**).

The renin-angiotensin system was studied in more than 25 isolated blood perfused dog kidneys. During the initial hour

of isolated perfusion, perfusate plasma levels of renin substrate were within a range usually measured in the plasma of intact animals. Radioimmunologic measurements of angiotensin I were higher in the renal vein than renal artery during this time. This was considered evidence for the formation of angiotensin I within the renal vasculature. In contrast, renal venous angiotensin II levels were no greater than renal arterial levels. As perfusion progressed through an additional 3–4 hr, the perfusate concentration of angiotensin I increased, angiotensin II did not change, and renin substrate was depleted. With substrate depletion, renal venous angiotensin I no longer surpassed renal arterial angiotensin I, indicating possible deficient intrarenal formation of angiotensin I secondary to diminished precursor. The latter state was accompanied by intrarenal hemodynamic changes characterized by a reduced ratio of cortical plasma flow (C_{PAH}) to total renal blood flow (RBF) and diminished filtration fraction (C_{Cr}/RBF) secondary to an increased extracortical blood flow probably through medullary pathways. These physiologic changes could be reversed in part by the addition of synthetic tetradecapeptide renin substrate to the perfusate. It is suggested that angiotensin I, but not angiotensin II, is formed within the renal vasculature to function as a humoral regulator of the intrarenal distribution of RBF and the glomerular filtration rate. (Research supported by grant from NIH.)

159. Experimental Production of Hereditary Spherocytosis (HS): Role of Defective Membrane Microfilaments in the Disorder. HARRY JACOB, THOMAS AMSDEN,* AND JAMES WHITE, Minneapolis, Minn.

Microfilamentous proteins, morphologically prominent in RBC membranes, occur throughout phylogeny under circumstances suggesting they provide cell plasticity and shape. Thus, virtually identical actin-like proteins underlie slime-mold creeping, muscle contraction, and the platelet's discoid shape. Our demonstration of mutant membrane protein in hereditary spherocytes suggests that defective microfilament formation might underlie the peculiar shape, rigidity, hyperpermeability, fragmentability, and survival of these RBC. If so, altering microfilamentous protein in normal RBC might reproduce the hereditary spherocyte. Four widely disparate substances (vinblastine, strychnine, colchicine, and 5 mM Ca^{++}) shared one property: they specifically precipitated purified microfilamentous proteins extracted from RBC membranes, skeletal muscle, brain, mitotic spindles, and cilia. All transformed normal RBC into cells identical in all parameters to hereditary spherocytes. For example, 0.2 mM vinblastine rapidly caused disc-sphere transformation while osmotic fragility increased 4 SD. Transmission and scanning electron microscopy using thorium dioxide to mark membranes demonstrated gradually shrinking surface material without net membrane loss; spheroidicity without volume change resulted. Simultaneously, RBC plasticity (filterability) decreased 10-fold to that observed in HS. Sodium fluxes increased as did glycolytic rates. Overnight incubation increased osmotic fragility further; as in HS, this now reflected membrane fragment loss (25% symmetrical lipid depletion). Glucose supplementation virtually prevented increased 48 hr autohemolysis. Vinblastine-treated, ^{51}Cr-labeled erythrocytes underwent specific splenic entrapment in humans and rats; liver radioactivity remained constant even in splenectomized individuals; rat spleen radioactivities exceeded other organs by 50 times. These results support the view that membrane microfilamentous protein not only underlies cellular shape and motility (plasticity) in unicellular organisms, but is crucial to shape, deformability, cation permeability, and survival of red cells as well. Mutations in such protein would predictably produce the abnormalities of the hereditary spherocyte. (Supported by a VA Hematology Training Grant.)

160. The Mechanism of Puberty in the Rat. H. S. JACOBS,* R. S. SWERDLOFF,* AND W. D. ODELL, Torrance, Calif.

The current hypothesis of the mechanism of puberty involves the concept of a reduction of the sensitivity of the hypothalamic pituitary unit to feedback inhibition by gonadal steroids during the period of sexual maturation. We have tested this hypothesis directly in rats. Immature (21 days) and mature (75 days) rats were castrated and 5 days later treated with graded doses of testosterone (1–1000 μg/100 g body weight per day) for 5 days. Serum LH and FSH (radioimmunoassay) and prostate weight were measured. In control groups 10 days after castration, serum LH had risen 5 and FSH 2.5 times the intact levels. With testosterone treatment, prostate weight increased and serum LH and FSH fell in both groups. There was no effect of sexual maturation on the dose of testosterone required for feedback suppression of LH or FSH. Since this data indicates that the hypothesis stated above is incorrect, we studied the response of the gonad to gonadotropin treatment. Either LH-NIH-P7 or FSH-NIH-S4 was injected for 5 days into mature and immature rats which had been hypophysectomized 5 days earlier. Sexual maturation did not affect the response to FSH, as assessed by the increase of testicular weight. In contrast, although doses of LH above 11 μg/100 g body weight per day resulted in a dose-related increase of prostate weight in mature animals, immature rats did not respond to doses up to 2000 μg LH/100 g body weight per day. We conclude that, in the rat, there is no effect of puberty on the sensitivity of the feedback control of gonadotropins, and that other factors, such as the changing responsiveness of the gonad, are of major importance in the process of sexual maturation. (Supported by Research Grant AM-5550-04.)

161. Radioimmunoassay for Prostaglandins. BERNARD M. JAFFE,* JAY W. SMITH,* AND CHARLES W. PARKER, St. Louis, Mo.

Prostaglandins are 20 carbon carboxylic acids with a substituted cyclopentane ring and two aliphatic side chains. Prostaglandin (PG)E_1, A_1, and A_2 were conjugated to human serum albumin using a water-soluble carbodiimide. Conjugates contained 2–3 moles of PGE or PGA per mole of carrier protein as determined by the 278 nm absorbancy in alkali. Conjugates were administered to rabbits in complete Freund's adjuvant at 2- to 4-month intervals and the animals were bled 7–10 days after the third and subsequent boosts. Antibody activity was demonstrated by specifically inhibitable binding of PGE_1-^3H. Using ammonium sulfate precipitation to separate antibody-bound from unbound ligand, >90% of the PGE_1-^3H label can be bound; with antibody obtained after 16 months of immunization 0.1 ml of 1:10,000 dilution of several sera specifically binds at least 50% of the label. Specificity is directed both

against the cyclopentane ring and the side chains. Significant cross-reactivity is demonstrated by arachidonic acid (an unsaturated fatty acid PG precursor), but saturated fatty acids, steroids, and fat-soluble vitamins cross-react to only a minimal degree. The maximal sensitivity of the radioimmunoassay for PGE_1 and PGA_1 (using anti-PGE_1 and anti-PGA_1 respectively) is less than 0.1 pmole (<30 pg) and subpicomolar amounts of PGE_2 and PGA_2 can also be measured. A variable degree of prostaglandin-like activity has been demonstrated in human serum, strongly suggesting that the immunoassay is sufficiently sensitive and specific to detect physiologic concentrations of prostaglandins. The radioimmunoassay may provide the first quantitative method for evaluating the biological significance of the prostaglandins. (Supported by NIH Grants GM 371 and AI00219.)

162. Micropuncture of the Renal Papilla of Rats with Hereditary Diabetes Insipidus. REX L. JAMISON,* JOHN BUERKERT,* AND FRANK B. LACY,* St. Louis, Mo. (introduced by S. Wessler**).

Micropuncture samples were collected from the left renal papilla in 21 rats with hereditary diabetes insipidus to study fractional solute and water reabsorption in Henle's loop and the collecting duct during water diuresis and after vasopressin-induced antidiuresis. In the right kidney, the urine-to-plasma osmolality ratio increased from 0.52 ±0.02 SE in water diuresis to 4.26 ±0.25 in antidiüresis, the excretion of filtered water decreased from 6.3% ±0.5 to 0.7% ±0.1, GFR decreased from 338 ±23 to 283 ±22 μl/min ($P < 0.001$), and blood pressure remained unchanged. In the papilla, the tubular fluid-to-plasma osmolality ratio in Henle's loop was 1.80 ±0.06 in water diuresis (indicating a hypertonic papilla), and increased to 3.03 ±0.23 in antidiuresis ($P < 0.001$). The fractions of filtered solute and sodium unreabsorbed by the juxtamedullary nephron up to the hairpin turn were statistically unchanged (mean difference between the two conditions, 7.6% ±5.2 and − 4.4% ±4.9, respectively), but the fraction of filtered water unreabsorbed decreased from 29.9% ±1.37 to 20.1% ±1.55 ($P < 0.001$). Solute unaccounted for as sodium or potassium chloride in loop fluid increased from 62 ±16 to 322 ±40 mOsm ($P < 0.001$). The fraction of filtered water entering the terminal millimeter of collecting duct in water diuresis (6.41% ±0.77) was strikingly reduced in antidiuresis (1.76% ±0.15) ($P < 0.001$). Fractional water reabsorption by this terminal segment was actually less in antidiuresis (0.58% ±0.08) than in water diuresis (1.58% ±0.32) ($P < 0.005$). These findings suggest that the sodium load reaching the ascending limb is relatively unvarying (at least in fractional terms) and independent of urine osmolality and that the extent to which volume reduction occurs in proximal collecting tubules is of prime importance in the effectiveness of the urinary concentrating mechanism. (Supported by grants from NIH and Kidney Foundation, Metropolitan St. Louis.)

163. Specific Inhibition of Collagen Synthesis in Rat Granulomas by Incorporation of the Proline Analog *cis*-4-Hydroxyproline into Intracellular Collagen. SERGIO A. JIMENEZ* AND DARWIN J. PROCKOP, Philadelphia, Pa.

Rosenbloom and Prockop previously demonstrated that the proline analog *cis*-4-L-hydroxyproline (c-Hypro) is incorporated into intracellular collagen in vitro and that the collagen containing c-Hypro is not extruded at a normal rate. Carrageenin granulomas were produced in 150- and 300-g rats maintained on a proline-free diet by a subcutaneous injection of 5 ml of 1% carrageenin on day 1. From days 5 to 13 the rats were given two daily injections of *cis*-Hypro and the granulomas were removed on day 14. In 150-g rats the weight of the granulomas was 4.21 g ±0.25 (SE) in the controls, 2.69 g ±0.39 in the rats receiving 100 mg/kg per day of *cis*-Hypro, and 1.80 g ±0.26 in the rats receiving 200 mg/kg per day of *cis*-Hypro. The total collagen content of the granulomas was 64.5 mg ±1.91, 32.27 mg ±4.30, and 26.15 mg ±3.03, respectively. Similar effects on weight and collagen content of the granulomas were observed in the 300-g rats receiving *cis*-Hypro. Amino acid analyses showed that on the average each α-chain of total intracellular and extracellular collagen in the granuloma contained 14 residues of the analog. The final weight of the treated rats was 10–15% less than controls but further experiments showed that limiting the growth of rats by 10% did not have any significant effect on the weight or collagen content of the granulomas. The results indicate that collagen synthesis in carrageenin granulomas in rats can be specifically inhibited by the administration of a proline analog which is incorporated into the molecule of collagen. (Supported in part by USPHS Grants FR-107 and AM-14, 526.)

164. Evolution of Papain Emphysema in Rats. W. G. JOHANSON, JR.,* R. C. REYNOLDS,* AND A. K. PIERCE,* Dallas, Tex. (introduced by J. P. Sanford).

Pulmonary deposition of papain in experimental animals produces a stable lesion which resembles human emphysema. Study of the sequential development of this lesion may provide insight into mechanisms of lung destruction by proteolytic enzymes. Lungs of young male rats, exposed to an aerosol of 10% papain for 4 hr, were examined histologically from 1 hr to 90 days after exposure. After anesthesia and tracheostomy, the chest was opened widely, and the lungs inflated *in situ* with cold glutaraldehyde under 20 cm H_2O pressure. Mid-coronal thick (40 μ) and thin (5 μ) sections were stained with hematoxylin and eosin, Gömöri's trichrome, aldehyde fuchsin, or Wilder's reticulin stains. Intra-alveolar hemorrhages appeared within 1 hr. By 2 hr, these were associated with interstitial edema and disappearance of the finest reticulin fibers. Elastin fibers demonstrated a distinctive beaded appearance by 6 hr and definite alveolar duct ectasia was present. By 12 hr maximum hemorrhage had occurred; numerous alveolar walls appeared hyalinized and contained no demonstrable cells or fibers. These empty septa were less frequently observed over the next 48 hr as progressive enlargement of central air spaces occurred. Over the next several days, transient accumulation of hemosiderin-laden macrophages was seen. Changes in elastin staining persisted but became less prominent. Minimal fibrosis developed; alveolar duct ectasia progressed minimally beyond that observed at 48 hr. Endobronchial lesions were not seen. Electron microscopically, principal features of the acute lesion were margination of leukocytes in alveolar capillaries, interstitial edema, blebbing of endothelial cells, and focal cytoplasmic swelling of type 1 epithelial cells with occasional desquamation. These findings indicate that

papain attacks all components of alveolar septa, leading to dissolution of septa and dilatation of centrilobular air spaces. The resultant lesion closely mimicks human emphysema.

165. Immunofluorescent Hepatic Localization of Complement Proteins: Evidence for a Biosynthetic Defect in Hereditary Angioneurotic Edema (HANE). A. MYRON JOHNSON,* CHESTER A. ALPER, FRED S. ROSEN, AND JOHN M. CRAIG,* Chapel Hill, N. C., and Boston, Mass.

Specific cytoplasmic immunofluorescence in hepatic parenchymal cells has been shown to correlate qualitatively and quantitatively with hepatic synthesis of plasma proteins. In the present study, liver sections were evaluated by the indirect immunofluorescent technique using primary antisera monospecific for C3, C4, C5, $C\bar{1}$ inhibitor (EI), and, as a control, transferrin. The percentages of hepatic parenchymal cells with specific cytoplasmic fluorescence were as follows: C3, 1.5 and 3.3%, C4; 2.8, 4.4, and 1.9%; C5, 5.5, 2.8, and 6.8%; EI, 6.8 and 7.5%; and transferrin, 9.0–19.7%. No immunofluorescence was found in sections of lymph node, stomach, or duodenum. In two patients with HANE (and low serum concentrations of EI), complement component and transferrin hepatic localization was normal but no EI was detectable in the liver sections. These findings suggest that the third, fourth, and fifth components of complement and EI are synthesized, at least in part, in hepatic parenchymal cells. They further suggest that in patients with HANE and low serum concentrations of $C\bar{1}$ inhibitor protein there may be deficient hepatic synthesis of the protein, and that the disease results from this biosynthetic error. (Supported by USPHS grants and a grant from the Tara Soper fund.)

166. Growth and Morphology of Transformed Fibroblasts are Regulated by Cyclic AMP and Prostaglandins. GEORGE S. JOHNSON,* CHARLES V. PEERY,* JACQUES OTTEN,* WILLIE D. MORGAN,* AND IRA PASTAN, Bethesda, Md.

The biochemical changes leading to the defective regulation of the growth and morphology of cancer cells are unknown. Since cyclic AMP (cAMP) regulates many reactions in normal cells, we have begun to investigate cAMP metabolism in cancer cells and have found that some cancer cells of fibroblastic origin acquire many characteristics of normal cells when treated with cAMP or its derivatives (Johnson et al., *Proc. Nat. Acad. Sci.*, February, 1971). The response to cAMP varies, but in general, the cell body lengthens, major cell processes are greatly extended, and the cells appear flatter and less refractile. The growth rate is decreased, and the cells frequently align in parallel arrays. Time lapse photography has been used to verify these findings. We have now investigated many cell lines and find that the following cells respond: L-929, RSV-induced rat tumor (XC) and hamster tumor, three human osteosarcomas and one melanoma, and several mouse fibrosarcomas. No response is observed with untransformed mouse embryo fibroblasts (MEF), BHK cells, a reticulum cell sarcoma, and various normal or transformed epithelial cells (HeLa, VERO, mouse mammary tumor, and human choriocarcinoma). Prostaglandins E_1, E_2, and $F_{2\alpha}$, which activate adenyl cyclase in L-929 cell extracts, dramatically alter cell morphology and decrease the growth rate, like cAMP. PGA_2 fails to activate or alter cell morphology. Adenyl cyclase activity in L-929 cell extracts is 8 pmoles/mg per min and is increased by fluoride (162 pmoles/mg per min) and by 25 μg/ml PGE_1 (157 pmoles/mg per min), PGE_2 (92 pmoles/mg per min), or $PGF_{2\alpha}$ (30 pmoles/mg per min). These results suggest that prostaglandins and cAMP regulate the morphology and growth rate of transformed cells and that the actions of prostaglandins are mediated by cAMP.

167. The Mechanism of Intestinal Uptake and Transcellular Transport of IgG in the Neonatal Rat. E. A. JONES* AND T. A. WALDMANN, Bethesda, Md.

The transport of immunoglobulins across the gastrointestinal tract of neonatal rats provides an excellent model for the study of transcellular protein transport. The mechanism of intestinal uptake and transcellular transport of plasma proteins has been studied in 14-day-old rats using intraduodenally administered radioiodinated proteins. Appreciable quantities of mouse IgG and all four subclasses of human IgG were taken up by the intestinal wall (19–52% of administered dose at 4 hr) and transported to the animal (10–36% of administered dose at 4 hr). In contrast there was little or no uptake or transport of human IgM, IgA, IgD, IgE, albumin, transferrin, and ceruloplasmin. Both the uptake and transport of labeled IgG were significantly inhibited by unlabeled IgG. An appreciable proportion of the label of IgG in intestinal wall homogenates, but not in plasma or intestinal washings, migrated in a sucrose ultracentrifugation gradient much more rapidly than did the administered 7S molecules. This pattern was not observed with other proteins studied. This apparent binding of labeled IgG was also markedly inhibited by unlabeled IgG. In subcellular fractionation studies of intestinal homogenates the complexed labeled IgG was shown to be associated with cell membrane rather than cell sap fractions. It is concluded that in the neonatal rat: (*a*) both intestinal uptake and transport of IgG are specific saturable processes, (*b*) intestinal transport is associated with complexing of IgG molecules probably with membranes, and (*c*) the part of the IgG structure involved in this process is probably similar to that involved in the concentration-catabolism effect but is not identical with that mediating other nonantibody functions of IgG. These data are consistent with the existence of specific receptors for IgG on enterocyte microvillus membranes. Such receptors would be necessary for the specific uptake and transport of these molecules.

168. The Measurement of Hepatic Synthesis of Bilirubin and Its Delivery to Plasma in Man. E. ANTHONY JONES,* JOSEPH R. BLOOMER,* PAUL D. BERK,* AND NATHANIEL I. BERLIN,** Bethesda, Md.

Methods have been developed for measuring in vivo the rate of synthesis of bilirubin from hepatic heme turnover and for calculating the amount of hepatic-synthesized bilirubin which appears as plasma unconjugated bilirubin. After administration of δ-aminolevulinic acid-4-^{14}C (ALA-^{14}C) and unconjugated bilirubin-^{3}H, the plasma curves of unconjugated bilirubin-^{14}C and -^{3}H were defined in seven patients, three of

whom had acute intermittent porphyria (AIP). The proportion of ^{14}C incorporated into plasma unconjugated bilirubin (13.1–23.5% in nonporphyrics and 5.4–13.6% in porphyrics) was determined by deconvolution of the two plasma curves. Total bilirubin production (BRP) was calculated from the plasma bilirubin-^{3}H disappearance rate. In five patients total ^{14}C incorporation into bilirubin (21.9 and 25.3% in nonporphyrics and 8.5–25.3% in porphyrics) was determined by multiplying BRP by the mean stercobilin-^{14}C specific activity in a 7–10 day pool of feces. These results indicate that 52.0–90.4% of hepatic-synthesized bilirubin entered the plasma as unconjugated bilirubin. An inverse relationship between urinary porphobilinogen (PBG) excretion and the incorporation of ^{14}C into bilirubin was apparent. The hepatic pool of PBG is a common precursor of both urinary PBG and hepatic-synthesized bilirubin and can be selectively labeled by ALA-^{14}C. By applying the labeled precursor-product relationship to this system, the proportion of BRP derived from hepatic hemes can be calculated from the ^{14}C specific activities of fecal stercobilin and urinary PBG. Estimates were made in the three patients with AIP, as their high urinary excretion of PBG facilitated PBG-^{14}C specific activity determination. The rate of hepatic bilirubin synthesis was 0.42, 0.47, or 0.98 mg/kg per day, or 13.3, 15.8, and 20.7% of BRP. This is the first determination of hepatic bilirubin production in man. Since BRP in patients with AIP is not increased, these values are probably similar to those in normal man.

169. Adrenal Failure in Man and Animals Induced by the Treatment of Renal Enterobacterial Infection with Vaccine. ROBERT K. JORGENS* AND KATHLEEN E. ROBERTS,** New York.

Evidence that renal enterobacterial infection is the causal factor in several degenerative diseases of man has been shown. The initial studies have been monotonously confirmed on more than 500 patients. Among the diseases directly related to the renal infection are emphysema, arthritis, diabetes mellitus, nephro- and cholelithiasis, lupus erythematosa, rheumatic heart disease, and a variety of dermatological lesions. The strongest evidence presented, relating the renal defect and degenerative disease, has been the complete reversal of the disease by the use of antibiotics and a specific vaccine. During the course of treatment, it became increasingly apparent that adrenal insufficiency was the outstanding complication incurred with therapy. This was manifested in the usual clinical signs and symptoms and confirmed by biochemical analysis. Many of the patients displayed this complex before treatment was instituted. However, the majority developed adrenal exhaustion during treatment. The studies indicate that either the renal infection per se and/or the vaccine triggers adrenal exhaustion in an already damaged adrenal. This suggests that the adrenal axis is a primary focus in management. Confirmatory evidence has been obtained by parallel studies on cattle, sheep, and dogs. Adrenal failure was an outstanding feature in the management of the diseases plagueing these animals. Although these degenerative diseases are assigned a different label, the diagonsis, causal relationship, and management followed an identical pattern in both man and the animals subserving him.

170. Immunologic Activation of Hageman Factor and Its Relationship to Fibrinolysis, Bradykinin Generation, and Complement. A. P. KAPLAN,* I. GIGLI,* AND K. F. AUSTEN, Boston, Mass.

Activation of partially purified unactivated Hageman factor was accomplished by incubation with an immune complex consisting of a monoclonal human IgM antibody to human IgG. The IgM antibody reacts with a single antigenic determinant present in IgG and therefore forms only soluble complexes, binding a maximum of 5 moles IgG/mole IgM. Activation of Hageman factor was recognized by the development of two separate functions: correction of the coagulation defect of Hageman factor–deficient plasma and initiation of the generation of bradykinin. In separate experiments activated Hageman factor was incubated with streptokinase-activated plasminogen resulting in the formation of a fragment possessing the ability to correct Hageman factor deficiency and convert prekallikrein to kallikrein; kallikrein in turn cleaved kininogen to generate bradykinin. Thls prekallikrein activator had an isoelectric point 4.9, migrated as a prealbumin on disc-gel electrophoresis at pH 9.3, and had an estimated mol wt 35,000. Partially purified kallikrein markedly enhanced the activity of $C\bar{1}$ as assessed by an effective molecular titration in a hemolytic system. Further fractionation of kallikrein on Sephadex G-150 revealed peaks of $C\bar{1}$-enhancing activity at the position of kallikrein and at 80% bed volume, a subunit apparently derived from kallikrein. Evidence thus exists that an immune complex can directly activate the complement and the clotting systems and indirectly the kinin-forming system: activation of Hageman factor and digestion of activated Hageman factor with plasmin yields a Hageman factor fragment which converts prekallikrein to kallikrein; kallikrein can then digest kininogen to yield bradykinin or interact with $C\bar{1}$ to markedly enhance the effectiveness of $C\bar{1}$ as an activator of the complement sequence.

171. Complement-Dependent Opsonization of Human Red Blood Cells by Secretory IgA Isohemagglutinins. MANUEL E. KAPLAN, AGUSTIN P. DALMASSO,* AND MILDRED WOODSON,* Minneapolis, Minn.

Secretory IgA (S-IgA) present in colostrum and other secretions are thought to protect mucosal surfaces from invasion by foreign antigens. Previous studies suggest that S-IgA is not opsonic and that the mechanism whereby the antibody prevents ingress of antigenic substances remains unclear. To investigate further the biological properties of S-IgA, postpartum serum and colostrum were obtained from a type O, Rh+ mother of a B+ erythroblastotic infant. Maternal serum, in the presence of either heat-inactivated (56°C, 30 min) or fresh, complement-containing normal serum, caused phagocytosis of B RBC by type O leukocytes (monos $\gg$ polys). IgM and IgG serum fractions were separated by G-200 gel filtration. The IgM fraction was nonopsonic; the IgG fraction exhibited weak opsonic activity which was enhanced by addition of fresh serum. Unfractionated colostrum, containing principally S-IgA and much smaller concentrations of IgM and IgG, induced phagocytosis of B BRC only in the presence of fresh serum. An IgM-rich colostral fraction obtained by DE-52 chromatography was nonopsonic. Colostral IgA was isolated

by DE-52 chromatography, G-200 gel filtration, and preparative ultracentrifugation. The purified S-IgA, in which no IgM or IgG could be detected, opsonized B RBC only when fresh serum was added. When heat-inactivated or M/1 KSCN-treated serum was substituted for fresh serum, total inhibition of S-IgA opsonic activity occurred. These studies demonstrate that colostral IgA from certain hyperimmunized mothers opsonizes incompatible RBC when heat- and KSCN-labile serum factors, presumably complement components, are present. The identification of these factors and the mechanism whereby they interact with S-IgA to induce phagocytosis of incompatible RBC remain to be elucidated. (Research supported by the VA and NIH Grant AM-13717.)

172. The Importance of Comorbidity in the Outcome of Diabetes Mellitus. MORESON H. KAPLAN* AND ALVAN R. FEINSTEIN, West Haven, Conn.

The outcome of adults with newly discovered diabetes has previously not been quantitatively related to the associated comorbid diseases. From existing medical records and other solicited data, complete 5-yr follow-up was obtained for 90 patients who were treated in 1959–1960 at the West Haven VA Hospital and whose diabetes was diagnosed during admission or within the preceding 6 months. Comorbid conditions present at the zero time (ZT) admission, when or shortly after diabetes was first diagnosed, were classified exclusively on the basis of data available at ZT. Specific criteria were used to designate the type of ZT comorbidity as vascular or nonvascular and its functional severity as none, slight, moderate, or severe. The 5-yr fatality rate after zero time was 43% (39/90) for all patients, but was 61% (33/54) in those whose ZT comorbidity was substantial, i.e. moderate or severe, and 17% (6/36) in those whose comorbidity was minimal, i.e., slight or none. Of the 54 patients with substantial ZT comorbidity, 26 (48%) later died of it, and 7 (13%) died of other causes. Although the over-all death rate in younger patients (age 55 and below) was lower (27%; 12/45) than in older ones (60%; 27/45), severity of comorbidity was more cogent than age alone in affecting survival. Among 5-yr survivors, the occurrence rate of new vascular events (or "diabetic complications") was: 50% (4/8) in patients with substantial vascular ZT comorbidity; 20% (1/5) in those with severe nonvascular ZT comorbidity; and 8% (3/38) in all others. These data indicate that the outcome of maturity-onset diabetes mellitus is directly related to the type and functional severity of the comorbid diseases present when the diabetes is discovered. An appropriate analysis of comorbidity is crucial for evaluating the results of different modes of therapy.

173. Mechanism of Type I Hypercoproporphyrinuria in Liver Disease. NEIL KAPLOWITZ,* NORMAN JAVITT, AND ATTALLAH KAPPAS, New York.

We have examined the hepatic excretory mechanism for coproporphyrins (copro) to determine the (*a*) basis for the predominance in normal bile of the copro I over copro III isomer, and (*b*) nature of the impairment of hepatic transport for these isomers which results in the excessive type I coproporphyrinuria associated with genetic and acquired cholestatic liver disorders. Copro I and III isomers were infused intravenously into bile fistula rats, then isolated and quantitated after excretion by appropriate methods. After continuous infusion of equimolar amounts of copro isomers (10–100 μg/hr) the ratio of I/III in bile was always 2:1. An equimolar ratio of isomers in bile could be achieved only by doubling the concentration of infused copro III isomer relative to copro I. After single injections of isomers in equimolar amounts, ratios of isomers remained 1:1 in plasma; but in bile I/III ratios were again 2:1 while copro III predominated in liver. Estrogen (to produce cholestasis) or phenoldibromophthalein disulfonate administration (to compete for excretion) elicited type I coproporphyrinuria and decreased total coproporphyrin excretion into bile but did not alter the preferential biliary excretion of copro I over III in a 2:1 ratio. These findings indicate that copro I and III share a common hepatic-biliary transport mechanism having a sterically determined preference for the I isomer. This preference may be determined by the symmetrical distribution of the four carboxyl groups of copro I which would permit this isomer to interact with the hepatic transport carrier twice as frequently as the asymmetrical copro III. The type I coproporphyrinuria of liver disease reflects an impairment of a hepatic excretory mechanism common for both isomers, resulting in the expected diversion from bile to urine of more copro I than III.

174. A New Hormone, "Coagulopoietin-K." M. H. KARPATKIN* AND S. KARPATKIN, New York.

The purpose of this investigation was to determine whether there is hormonal regulation of vitamin K–dependent coagulation factors. New Zealand white rabbits were divided into two groups: donors and recipients. Donor animals received either intramuscular coumadin or saline control. Coumadin maintained factors II, VII, and X at $< 20\%$ of normal for 1 wk; factor V (not vitamin K dependent) did not change. All blood samples were drawn by clean ear artery puncture into one part 3.8% Na citrate–0.1 M EACA to nine parts blood. Donor animals were exsanguinated after intravenous injection of EACA (70 mg/kg) and heparin (2 mg/kg). Platelet-poor plasma was separated at 4°C and aliquots stored at − 30°C. Factors II, V, VII, and X were assayed by one-stage techniques using serial dilutions of plasma. Aliquots of donor plasma were injected intravenously for varying time periods into recipient rabbits who had received 1 mg vitamin K before and during the experiment. Factor levels of recipient animals were assayed from 1 ml blood samples drawn before, during, and after the injection period. Each set of assays on an individual animal was performed after completion of the injection schedule by the same worker on the same day using a fresh frozen aliquot for each. Levels obtained on 3 different days before injection did not vary significantly over a 3 wk period. Their average in each animal was designated 100%. Reproducible results were obtained in each of four sets of experiments using eight donor rabbits and 23 recipients. After injection of as little as 0.25–2 ml of plasma, factors II, VII, and X of recipients reached peak levels of 170, 154, and 255% respectively. Peak levels occurred 24–72 hr after the first injection and returned to control values 4–6 days after the last injection; factor V levels remained constant. In control experiments, factors II, VII, X, and V of recipients did not change after use of plasma from donor animals injected with

saline. It is concluded that a humoral factor, "Coagulopoietin-K," controls the level of vitamin K–dependent coagulation factors. (Supported by NIH Grant HE 1336–02.)

175. Characterization of the Hormonal Response to Luteinizing Hormone-Releasing Hormone (LH-RH) in Prepubertal and Adult Subjects. A. J. KASTIN,* A. V. SCHALLY,* D. S. SCHALCH,* S. G. KORENMAN, C. GUAL,* AND E. PEREZ PASTEN,* New Orleans, La., Rochester, N. Y., Iowa City, Iowa, and Mexico, D. F.

Administration of LH-RH causes increased levels of LH and follicle-stimulating hormone (FSH) in the blood of men and women. The effects of this hypothalamic hormone were further characterized in five normal men, two normal women, two prepubertal boys, and one prepubertal girl. Intravenous injection of 300 μg of highly purified porcine LH-RH caused a 3- to 10-fold increased release of LH and FSH in all subjects but had no consistent effect on plasma GH, TSH, or cortisol levels. Although a marked rise of 3.5- and 8-fold was found in plasma estrone in the two women during their follicular phase after LH-RH, plasma estradiol values were not changed. The men and prepubertal boys demonstrated an increase of 2- to 4-fold in plasma estradiol after injection of LH-RH. Prepubertal children showed approximately the same degree of response to LH-RH as did the adults. Three normal men received, at intervals of 1 or more wk, seven different doses of LH-RH ranging from 1.1 to 810 μg. As little as 10 μg of LH-RH caused a statistically significant increase in plasma LH, and a highly significant linear trend in the log dose-response curve was found. In summary, LH-RH is a potent and specific hypothalamic releasing hormone for LH and FSH in prepubertal children as well as normal adults; it produces a secondary rise in plasma estrone in women and estradiol in males and elicits a dose-response relationship in I H release.

176. B_{12} Deficiency Due to an Abnormal Intrinsic Factor. MAX KATZ,* SHEILA K. LEE,* AND BERNARD A. COOPER, Montreal, Canada.

Two forms of juvenile megaloblastic anemia due to B_{12} deficiency have been described, one with an isolated absence of intrinsic factor (IF) secretion in otherwise normal stomach, the other due to a selective ileal malabsorption of B_{12}. We wish to report a 13-yr-old male, product of a consanguineous marriage, who presented with a megaloblastic anemia due to B_{12} deficiency. Gastric acid secretion was normal and his gastric juice (GJ) contained 60 ng U of IF per ml (normal) when measured by immunoassay. Gastric biopsy was normal. The patient did not absorb radioactive B_{12} when it was given orally alone but absorbed normal amounts when given radioactive B_{12} with normal human GJ. Other tests of small intestinal function were normal. Patient's GJ did not correct the B_{12} malabsorption of a totally gastrectomized patient. By guinea pig mucosal homogenate assay we could demonstrate no functional IF in his GJ. Gel filtration, DEAE-cellulose chromatography, and isoelectric focusing revealed no major differences in the B_{12} binders of normal and patient GJ's. Thus, the patient secretes an IF which is normal immunologically but inactive biologically. To our knowledge this is the first such case to be described. (Supported by Medical Research Council Grant MT-802.)

177. Species Differences in Interferon Stimulation: Minimal Response in Primates and Man as Compared to Rodents. HERBERT E. KAUFMAN AND YSOLINA CENTIFANTO,* Gainesville, Fla.

Although poly I:C stimulates interferon in mice and rabbits it causes modest interferon production in man when given intravenously or in high concentrations topically to the eye. This production lasts only about 48 hr even if poly I:C administration is continued. Tilorone, a new small molecular weight interferon inducer, is similarly effective in mice but produced no interferon when given systemically or in high concentrations topically to the eye of man. In addition it was toxic. These findings suggesting that the interferon system of man responds only minimally to exogenous stimulation were further supported by studies in owl monkeys indicating species differences in prophylaxis against infection as well as interferon response. Topical poly I:C not only produced no interferon response in monkeys but failed to protect the monkeys against infection with 10 ID_{50} of herpes simplex virus whereas the same batch of poly I:C given concurrently to rabbits induced interferon and prophylaxis against infection for about 6 wk. These studies suggest that the interferon system of primates and man may be much less susceptible to interferon inducers than that of rodents in terms of stimulating interferon or inducing protection against infection, and that this great species difference must be considered in evaluating the potential of these drugs. (Research was supported by NIH.)

178. Sources of Urinary Ammonia Transported to Kidney as Glutamine. W. D. KERR,* A. G. HILLS,** AND E. L. REID,* Miami, Fla.

L-Glutamine extracted by the kidney is the principal immediate source of urinary ammonia; all tissues contributing glutamine to the circulation are accordingly ultimate sources of urinary ammonia in the postabsorptive animal. Data obtained in fasting mammals (*a*) document the importance of the peripheral tissues (hind leg) as glutamiue contributors, and (*b*) suggest that net peripheral glutamine release represents removal from the peripheral cells of ammonia formed there as an end-product of their protein metabolism. (*a*) In 12 greyhounds and 21 mongrel dogs fasted overnight, hind leg glutamine output was consistently observed and averaged respectively 0.230 and 0.096 μmole/min per kg body weight ($P < 0.001$); net output was estimated as the product of plasma femoral venous-arterial glutamine difference and leg plasma flow calculated from blood flow (magnetic meter) and hematocrit. Output of glutamine-N could not be accounted for as ammonia uptake. Findings in the spider monkey *Ateles paniscus* were similar. (*b*) Femoral arterial infusion of an ammonia salt consistently enhanced net glutamine output by the leg; the increase averaged 0.220 and 0.178 μmole/min per kg in six greyhounds and 14 mongrels respectively ($P < 0.025$). Increased glutamine output during ammonia infusion is interpreted as reflecting glutamine synthetase activity in peripheral tissues, probably chiefly skeletal muscle. Other regions also contribute to circulating glutamine; the system serves two interrelated functions at the organismal level: (*1*) provision of much of the urinary buffer ammonia for excess protons, and (*2*) defense against ammonemia. (Supported by grants from NIH.)