JCI The Journal of Clinical Investigation

Abstracts

J Clin Invest. 1970;49(6):58a-106a. https://doi.org/10.1172/JCI106344.

184. Two Kinds of Genetic Control of Reagin Production in the Mouse. BERNARD B. LEVINE AND NELSON A. VAZ,* New York, N. Y.

Experiments are being performed to develop a mouse model for reagin production in human atopic allergic diseases. The present studies show two distinct kinds of strain differences in reaginic antibody responses in mice. Mice of several inbred strains were immunized with hapten-protein conjugates in alumina gel. Sera drawn at intervals were assayed by PCA in CFW mice. Prior work indicates that PCA after a 2 hr sensitization period detects mainly 7S IgG_1 antibodies, and 48 hr PCA detects mainly reagins. Immunization with repeated 0.1 μg doses 4 wk apart of BPO or DNP conjugates of bovine γ-globulin or hen ovomucoid induced clear-cut responses in four strains (A/He, C3H, CBA, and AKR) and no detectable responses (at 1:5 dilution) in four strains (C57B1, C57L, SWR, and SJL). Responders made both IgG_1 and reagin (titers up to 1:640) of both hapten and protein specificities. Reagin production was prominent, was long-lived (>20 wk), and showed marked booster responses. Immune responsiveness to low doses of antigen correlated with H-2 genotype (of 20 strains, H-2a and k were responders; b, q, and d did not make detectable responses). Breeding experiments are in progress. This kind of control is of immune response in general, and reagin production is prominent in low-dose immunization. The second kind of control appears to be of reagin production specifically. In response to immunization with a single large (100 μg) dose of antigen (in alumina), most strains make both IgG_1 and reagin; SJL, AKR, and St/b made IgG_1 but virtually no reagin. More detailed studies with RF and SJL mice showed that both produced IgG_1, RF but not SJL produced reagin (titer, 1:80), and SJL × FR F_1 produced both IgG_1 and reagin like the RF parents. These results suggest that strong reaginic responses to certain antigens in low dosage may require the simultaneous presence of several different genes. (Supported by contract DADA 17-67-C-7119, United States Army Medical Research and Development Command.)

185. Combination Therapy of Reticulum Cell Sarcoma. MARTIN LEVITT,* RONALD C. DE CONTI,* JOHN C. MARSH,* MALCOLM S. MITCHELL,* AND JOSEPH R. BERTINO, New Haven, Conn.

A treatment program based on a kinetic model for patients with disseminated reticulum cell sarcoma has been tested. This model assumes that in the early, logarithmically growing stage the tumor is characterized by a short generation time (about 24 hr), with few cells in resting (G0) phase, and is sensitive to high-dose intensive antimetabolite therapy. In the advanced form, with over a kilogram of tumor tissue present, the disease is considered refractory to therapy with antimetabolites, since (1) a small percentage of cells are synthesizing DNA and (2) most cells synthesizing DNA are less inhibited by antimetabolites than are logarithmically growing cells. Since our patients were characterized by late-stage disease, initial therapy consisting of an alkylating agent and Vincristine was given, not only to achieve rapid tumor regression, but particularly to convert the remaining tumor population to logarithmic growth. Intravenous cytoxan (1.5 g/m^2) was administered on day 0, followed by Vincristine (1.4 mg/m^2) on days 1, 8, and 15. Methotrexate (80 mg/m^2) in three divided doses over 24 hr, followed by leucovorin rescue (15 mg/m^2) every 8 hr × 3, together with an i.v. push of cytosine arabinoside (300 mg/m^2) 16 hr after initiation of methotrexate therapy, was administered at weekly intervals for eight doses. This cycle was repeated two additional times. 10 patients have been entered on this protocol. Of the six patients who have received at least one course of therapy, five have achieved a complete remission, and the other a partial response. Two of the five patients who achieved a remission have relapsed after the second or third course. The other three patients have been in complete remission for 20, 34, and 44 weeks. These results indicate that the model proposed for the treatment of this disease may be valid.

186. Erythropoiesis-Stimulating Factors. JASPER P. LEWIS,* DOROTHY A. ALFORD,* WILLIE A. NEAL,* RUSSELL R. MOORES,* EDWARD GARDNER, JR.,* EMILY T. WELCH,* CLAUDE-STARR WRIGHT,** AND LINDA L. SMITH,* Augusta, Ga.

Fraction II + III, a urine concentrate of erythropoietin obtained by DEAE-cellulose chromatography, contained regulators of erythropoiesis that could be fractionated by selective membrane permeability. In a previous report we demonstrated a progressive increase in the daily erythropoietic specific activity in dialysates obtained by first dialyzing fraction II + III with the system 0.1% with respect to phenol, as recommended by Lowy and Keighley, and later removing the phenol. This report presents some aspects of erythropoiesis-stimulating factors (ESF) as derived from studies of our most highly purified fractions. The most highly purified ESF obtained from the dialysates appeared to contain no appreciable sialic acid or fucose, a trace of hexosamine, and about 11% protein. The complex appeared to be part steroid(s) having spectral characteristics similar to those of 21-deoxycortisone and 11-dehydrocorticosterone. The protein appeared to be a fragment of a glycoprotein(s). $Alpha_1$ acid glycoprotein seemed to be a protective carrier, but the erythropoietic activity was not completely dependent on that protein, as indicated by immunoelectrophoresis and the bioassay. By exhaustive dialysis a nondialyzable fraction could be obtained that had the characteristics of an erythropoietin-producing enzyme (EPE). The optimum pH for activity was about 7.4. Our data postulate that erythropoietin possibly remains in an inactive state when bound to a glycoprotein such as α_1–acid glycoprotein or the corticosteroid-binding globulin. A role of an EPE would be to act on the glycoprotein-steroid complex to produce a glycopeptide-steroid complex, thus making the active hormone available. (Supported in part by grants HE-10591-07 [HEM], FR-0061, FR-5365, and HE-12958 [formerly AM-10509] from the NIH.)

187. Inhibition of Leukocytic Proteases in Purulent Sputum by $Alpha_1$ Antitrypsin. JACK LIEBERMAN,* M. MOHAMED,* AND CHARLES MITTMAN,* Duarte, Calif. (introduced by Ernest Beutler).

It is postulated that inherited $alpha_1$ antitrypsin (A_1AT) deficiency predisposes to pulmonary emphysema by allowing

leukocytic and macrophagic proteases to digest lung alveoli during an inflammatory reaction. However, conflicting reports have appeared regarding the ability of A_1AT to inhibit the fibrinolytic action of leukocytes. Characterization of the protease activity in purulent sputum has revealed the presence of heat-labile and -stable proteases that are active on native and denatured hemoglobin, respectively. For this study, protease activity was extracted from 10% sputum homogenates by 1 M NaCl and assayed on 2% hemoglobin at pH 7.5. Both the labile and the stable proteases were found to be strongly inhibited by human serum, with sharp differences between normal, heterozygous, and homozygous A_1AT-deficient specimens. The labile protease was maximally inhibited by 1:8–16 dilutions of normal sera and 1:2–4 dilutions of serum from heterozygotes, and incompletely inhibited by undiluted serum from homozygotes. A 1:8 dilution was found best for screening and classifying multiple sera. Similar results were obtained with the stable protease, but maximal inhibition resulted from a 1:64 dilution of normal serum. Leukocytic proteases were also tested with 10% suspensions of washed human lung sediment as substrate. Lung tissue was found to be an excellent substrate for the labile protease; activity was strongly inhibited by normal human serum, less by heterozygous-deficient sera, and much less by homozygous-deficient sera. A 1:8 dilution of serum was found best for distinguishing these groups. Higher concentrations of homozygous-deficient serum could inhibit this system, apparently through the $alpha_2$ macroglobulin inhibitor; similar results were obtained with normal serum that had been shaken with chloroform to remove the $alpha_1$ inhibitor. $Alpha_1$ antitrypsin has been shown to inhibit leukocytic protease from purulent sputum with either hemoglobin or a suspension of human lung as substrate. These findings support the postulate that an inherited deficiency of A_1AT may predispose to pulmonary emphysema by allowing digestion of lung tissue by leukocytes. (Research supported by a grant from the NIH.)

188. Distribution of Antigenic Determinants in Collagen Molecules of Rat and Man. HERBERT LINDSLEY,* MART MANNIK, AND PAUL BORNSTEIN, Seattle, Wash.

Immunochemical studies of collagen have relied almost exclusively upon immunization with whole collagen. In order to localize the antigenic determinants on collagen molecules, antibodies to rat and human collagen chains were produced by immunizing rabbits with chromatographically isolated α_1- and α_2-chains, as well as with the purified intact protein. Purified, radioiodinated rat and human collagen α_1- and α_2-chains were prepared for a double-antibody radioimmunoassay. Unlabeled cyanogen bromide (CNBr)-produced fragments of rat alpha chains were used as inhibitors in this immunoassay. Rabbits immunized with native rat skin collagen produced antibodies primarily to the helical portion of the rat α_1-chain, in particular to CNBr peptides α_1-CB6 and α_1-CB8. In addition, the same antisera contained antibodies to determinants distributed throughout the helical region of the rat α_2-chain. Rabbits immunized with rat skin α_2-chains made antibodies to the same determinants. No inhibition was detected with the nonhelical, amino-terminal peptide α_2-CB1. However, antibodies to α_2-CB1 could be detected in antisera to rat skin collagen and α_2-chains by testing directly with the radioiodinated peptide. Rabbits immunized with rat skin α_1-chains produced no detectable antibodies. Rabbits immunized with human skin collagen produced antibodies both to the α_1- and the α_2-chains of the protein. Furthermore, in contrast to the rat chains, both isolated α_1- and α_2-chains were immunogenic. The use of immunochemical studies as probes of collagen structure will assist in defining interspecies differences in the amino acid composition and sequence of collagen. Preparation of antibodies to specific regions of the collagen molecule may also be of value in detecting alterations of this protein in human disease. (Research supported by NIH grants AM-1000, AM-11248, and T1-AM-5602.)

189. Effects of Cortisone on DNA Content and DNA Polymerase Activity of Weanling Rat Liver. JOHN N. LOEB* AND I. CRAIG HENDERSON,* New York, N. Y. (introduced by Nicholas P. Christy**).

The administration of cortisone to young rats is known to result in an abrupt arrest of somatic growth and in a rapid fall in liver DNA concentration. Despite this fall in concentration, we have found that the total DNA content of liver does not change, and that the effect of the hormone is to produce a prompt cessation of the normal increase in liver DNA content associated with growth. Although for the first few days of cortisone administration the livers of the rats are larger than those of control animals, the enlargement reflects the well known increases in RNA, protein, and glycogen content and is hence due to hypertrophy of liver cells rather than to the normal hyperplasia of growth. Cortisone treatment, even when maintained for several weeks, is not associated with any appreciable degradation of liver DNA, since total liver DNA content remains constant and radioactive thymidine incorporation promptly falls to less than 10% of control levels. The striking inhibition of DNA synthesis by glucocorticoids in this and a number of other mammalian systems made it of interest to examine the effect of cortisone administration on liver DNA polymerase activity. The administration of cortisone to weanling rats was found to result in a rapid fall in the DNA polymerase activity of liver extracts to approximately 50% of control levels within 24 hr and to less than 20% of control levels after 72 hr. The fall in activity is reversible when the hormone is withdrawn, and can be observed when as little as 0.3 mg cortisone per 100 g body weight is administered daily. (Supported by NIH grant GM-15022.)

190. Characterization of the Third Component of Complement on Erythrocytes from Patients with Autoimmune Hemolytic Anemia. MALCOLM MACKENZIE* AND ROGER SPITZER,* Cincinnati, Ohio (introduced by Virginia H. Donaldson).

The third component of complement (C3) is present on the erythrocytes of many patients with autoimmune hemolytic anemia (AHA). Cells from 21 such patients were tested for agglutination with goat antisera to human C3. These antisera recognize three distinct antigenic determinants, called A, B, and D, present on native C3. C3, when

studied in in vitro systems utilizing purified complement components, deposits on cell membranes as C3b (antigenic composition A, D). However, studies utilizing an immune precipitate system in the presence of fresh serum have demonstrated the deposition of a minimally altered form of C3 designated C3X (antigenic composition A, B, D). Erythrocytes from 20 patients gave positive direct Coombs tests with antisera to A, B, and D determinants, implying that the deposition product is not C3b and may be C3X. C3 antigens and IgG were eluted from erythrocyte stroma by acid treatment and transferred to normal erythrocytes. Absorption of these eluates by either anti-C3 or anti-IgG antisera removed both C3 and IgG. Thus the transferred C3 is bound to IgG. Erythrocytes from 11 patients were studied for immune adherence (IA) and hydrolysis of glycyl-L-tyrosine; only one demonstrated IA, whereas all hydrolyzed this peptide. The hydrolysis was inhibited by antisera to native C3. The following criteria distinguished the C3 bound to erythrocytes in vivo in AHA from the C3 bound in vitro: (1) The deposition product may be C3X rather than C3b. (2) Significant amounts of C3 are bound to antibody. (3) The C3 does not participate in IA, but does have peptidase activity. These differences appear to be significant physiologically in that the erythrocytes in AHA usually do not undergo intravascular hemolysis, but are removed by the reticuloendothelial system. (Supported in part by American Cancer Society grant T-500.)

191. *De Novo* Biosynthesis of Glutathione in Extracts from Human Erythrocytes. PHILIP W. MAJERUS, VIRGINIA MINNICH,* AND DANIEL MOHLER,* St. Louis, Mo., and Charlottesville, Va.

Numerous reports have suggested that human erythrocytes can synthesize glutathione (γ-glutamylcysteinylglycine, GSH). We have demonstrated and partially purified from hemolysates of human erythrocytes the two enzymes required for *de novo* GSH synthesis: γ-glutamylcysteine (GC) synthetase and GSH synthetase. GC synthetase, purified 1000-fold, requires cysteine, glutamic acid, Mg^{++}, and ATP. GSH synthetase separated from GC synthetase by DEAE-Sephadex chromatography catalyzes the formation of GSH from GC and glycine in the presence of ATP and Mg^{++}. GC synthetase activity in fresh hemolysates from 12 normal subjects was 67.4 ± 8 U (1 U = 1 mμmole GC or GSH formed per g Hb per min); over-all GSH formation in 29 normal subjects was 21 ± 3.7 U. In studying a patient with reduced levels of GSH (7 mg/100 ml, control = 35 mg/100 ml), we found that extracts from his erythrocytes did not incorporate ^{14}C-glycine into GSH in the presence of ATP, Mg^{++}, cysteine, and glutamic acid. These extracts prepared from blood stored 2 days in EDTA during shipment contained increased levels of GC synthetase (52.5 U) as compared with three controls collected at the same time, 28.1 ± 2.9 U. The inability of the patient's extract to synthesize GSH could be corrected by addition of purified GSH synthetase (free of GC synthetase), indicating that the patient lacked the enzyme GSH synthetase. The patient's parents and four children were heterozygous for GSH synthetase deficiency although they had normal GSH levels. Over-all GSH synthesis in heterozygotes was 7.3 ± 1.3 U as compared with 12.4 ± 1.2 U in three controls collected at the same time. Heterozygotes were also identified by their increased levels of GC synthetase, 37.8 U, a value intermediate between controls and the patient. (Supported by grants from the NIH and the American Cancer Society.)

192. Phagocytosis of Small Particles (Bacteriophage T_6) by Human Polymorphonuclear Leukocytes. STEPHEN E. MALAWISTA,* GRETCHEN V. GREENE,* AND KLAUS G. BENSCH,* New Haven, Conn., and Palo Alto, Calif. (introduced by Fred S. Kantor).

Polymorphonuclear leukocytes (PMN) are known to ingest both virus particles and antigen-antibody complexes. Using the bacterial virus coliphage T_6, we have quantitated phagocytic, metabolic, and enzymatic effects on PMN of small particles with and without antibody, and correlated them with structural and ultrastructural changes. Leukocytes, prepared from heparinized blood by dextran sedimentation, were suspended in 12% autologous serum-phosphate buffer, and incubated for 1 hr at 37°C with dilute (1:50) immune or nonimmune rabbit serum, and with or without tritiated T_6 at ratios of 200 to 1300:1, T_6:PMN. Phagocytosis was measured by the disappearance of recoverable T_6 from the supernatant, or, where neutralizing antibody (Ab) was added, by the disappearance of tritium counts, and was confirmed by radioautography and by electron microscope observation of leukocytes. In eight experiments, mean values for supernatant losses of T_6 were $0 \pm 12\%$ (SEM) of the inoculum without Ab, and $63 \pm 5\%$ with Ab, the latter value indicating a mean of about 400 T_6 ingested per PMN. When no Ab was used with T_6, the usual accompaniments of phagocytosis were present to some extent, despite poor ingestion, and included increased consumption of oxygen and of 1-^{14}C-glucose, and degranulation and vacuolization of PMN (seen in the light and electron microscopes and confirmed by measurements of granule-associated acid phosphatase activity). *Escherichia coli* endotoxin probably contributed to these effects. These same changes occurred to a significantly greater extent ($P < 0.001$ in all cases) in leukocytes given Ab as well as T_6, and were comparable in intensity to those seen with ingestion of bacteria such as *Staphylococcus aureus*. This quantitative model of small-particle phagocytosis may be helpful in understanding certain disorders in which PMN, virus-like particles, and antigen-antibody complexes are associated with an acute inflammatory response. (Research supported by grants from the NIH, the Arthritis Foundation, and the John A. Hartford Foundation.)

193. Marijuana Smoking: A Study of Its Effects on Alveolar Lining Material and Pulmonary Macrophages Recovered by Bronchopulmonary Lavage. PHILIP E. G. MANN,* THEODORE N. FINLEY, AND AARON J. LADMAN,* San Francisco, Calif., and Albuquerque, N. M.

Larger numbers of macrophages and reduced volumes of acellular alveolar lining material (ALM) were observed in the sediment obtained by centrifugation of bronchopulmonary lavage effluent from eight tobacco smokers (20 to 60 cigarettes daily) than in that from eight nonsmoking volunteers. The macrophages obtained from smokers were filled with cytoplasmic inclusions to an extent that enabled an observer unfamiliar with the smoking history of the subjects to iden-

tify them. In the present investigation, eight marijuana smokers, all but one of whom had normal pulmonary functions and chest X-rays, were studied. One smoked one marijuana cigarette a day, six smoked one to eight marijuana cigarettes a day, and one smoked 20 marijuana cigarettes a day. From a lavage volume of 300 ml, 192 ml on the average were recovered. The volume of sediment averaged 0.07 ml of ALM and 0.23 ml of cells, predominantly macrophages. The ALM values in this sediment were greater than those observed in tobacco smokers ($P < 0.05$) but less than those observed in nonsmokers ($P < 0.02$). A study of the light microscope and ultrastructural appearance of the cellular portion of the sediment showed marijuana smokers' macrophages to be indistinguishable from nonsmokers' macrophages with respect to cell size, number of multinuclear cells, and number of cytoplasmic inclusions. It appears that marijuana cigarette smoking in the amounts studied, in contradistinction to tobacco cigarette smoking, caused no distinguishing morphological alterations. (Research supported in part by NIH grants HE-12-12571-01 and 1-RO1-GM-14435-02, and by the Council for Tobacco Research.)

194. Saphenous and Colic Venomotor Responses to Prostaglandin $F_{2\alpha}$. ALLYN L. MARK,* PHILLIP G. SCHMID,* JOHN W. ECKSTEIN,** AND MICHAEL G. WENDLING,* Iowa City, Iowa.

Experiments were performed to determine the action of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on veins in the limb and mesentery, to compare its effects on veins with those of norepinephrine, and to assess the role of the sympathetic nervous system in the venous responses to $PGF_{2\alpha}$. The lateral saphenous or left colic vein was perfused in vivo at constant flow with arterial blood. Pressures at the tip of the perfusion cannula and at the tip of a catheter 15 cm downstream were recorded. Increases in the pressure gradient between these two points indicated venoconstriction. $PGF_{2\alpha}$ (12.5, 25.0, and 100 μg) injected into the perfusion tubing produced increases in the gradient of 12 ± 6 (mean $\pm$ SEM), 25 ± 10, and 38 ± 13 mm Hg, respectively, in the saphenous vein, and 7 ± 2, 9 ± 1, and 23 ± 6 mm Hg in the colic vein. Increases with norepinephrine (0.78, 1.56, and 3.12 μg) were 20 ± 7, 40 ± 14, and 59 ± 16 mm Hg in the saphenous vein, and 8 ± 6, 9 ± 5, and 14 ± 5 mm Hg in the colic vein. Bioassay indicated that the dose of $PGF_{2\alpha}$ required to cause a given response was 19.2 times the dose of norepinephrine in the saphenous vein and 13.6 times the dose of norepinephrine in the colic vein. Phentolamine blocked responses to norepinephrine, but did not reduce responses to $PGF_{2\alpha}$. Reserpine and hexamethonium did not alter responses to $PGF_{2\alpha}$. In these experiments $PGF_{2\alpha}$ constricted both saphenous and colic veins, but in relatively large doses as compared with norepinephrine. The venomotor responses to $PGF_{2\alpha}$ were not dependent on integrity of the sympathetic nervous system.

195. Glucagon: Levels and Metabolic Effects in Man under Prolonged Fasting. ERROL B. MARLISS,* THOMAS T. AOKI,* AND GEORGE F. CAHILL, JR., Boston, Mass.

Glucagon's contribution to the metabolic adaptation to prolonged fasting has been examined. Plasma immunoreactive glucagon (IRG) was measured during 6 wk fasts by an assay system which minimized nonpancreatic immunoreactivity. Protein mobilization for gluconeogenesis diminished from an initially augmented rate (urine nitrogen 9.8 ± 0.8 g/day) to a plateau by 3 wk (4.9 ± 0.4 g/day), reflecting decreasing body glucose requirement with adaptation of brain from oxidation of glucose to that of ketones. As reported by Unger and colleagues, fall in glucose (BG) and immunoreactive insulin (IRI) from postabsorptive levels to a plateau over 5 days was accompanied by a rise in IRG from 73 ± 5 to a maximum of 144 ± 16 pg/ml on day 3 ($P < 0.01$, paired t). However, IRG declined thereafter but remained above prefast levels. The peak on day 3 occurred at a time of augmented fractional splanchnic extraction (71%) of alanine (the key gluconeogenic amino acid, AA) as compared with the postabsorptive (43%) and prolonged-fasted (53%) states, as reported by Felig and associates. Constant i.v. infusion of glucagon at 20 mg/48 hr after 4–5 wk fasting increased hepatic gluconeogenesis and urea nitrogen excretion, and markedly lowered most plasma AA. BG and IRI increased; free fatty acid and glycerol levels were unchanged, though ketonuria increased. By contrast, infusion of 0.2 mg/48 hr caused a decrease only in AA, indicating extreme sensitivity to this effect of glucagon. The possibility that a peripheral effect of glucagon might contribute to the hypoaminoacidemia has not been excluded. Conclusion: The potent stimulation of hepatic gluconeogenesis by exogenous glucagon suggests that the elevated endogenous levels may act in concert with hypoinsulinemia in regulating the proportion of substrate produced in the periphery and extracted by the liver.

196. Comparison of the Cardiac Contractile State in Patients with Idiopathic Myocardial Disease and in Ventricular Hypertrophy Secondary to Prolonged Systolic Pressure Overloading. DEAN T. MASON, JAMES F. SPANN, JR.,* ROBERT ZELIS,* AND EZRA A. AMSTERDAM,* Davis, Calif.

Although similar derangements of hemodynamics and myocardial mechanics are characteristic of ventricular hypertrophy occurring either idiopathically or consequent to increased pressure loading, the possibility was considered in this study that quantitative differences in inotropic state and compensatory mechanisms might exist between these two groups. Accordingly, variables of cardiac performance and high-fidelity isovolumic left ventricular pressure (IP) and its rate of rise (dP/dt) were recorded in 12 adult patients with primary or secondary cardiac hypertrophy without valvular regurgitation who had congestive symptoms with more than ordinary activity. Left ventricular contractility was quantified by the determination of instantaneous contractile element velocity (V_{CE}) during isovolumic systole as $(dP/dt)/(32 \cdot IP)$; and construction of pressure-velocity relation thereby provided maximum V_{CE} (V_{max}), the independent measure of inotropic state. Although average resting cardiac index (CI) was normal in both types of hypertrophy, V_{max} was significantly ($P < 0.05$) below normal value of 1.50 ± 0.09 ml/sec in both groups and was significantly less (0.84) in the six patients with primary muscle disease (PMD) as

compared with 1.19 in the six patients with aortic stenosis (AS). Left ventricular end-diastolic volume (LVEDV) index was elevated in PMD (145 ml/m^2) but was normal in AS (77). Moreover, ratios of V_{max} to LVEDV and V_{max} to CI were more diminished in PMD (0.0048 and 0.36) than in AS (0.0104 and 0.45). Thus, it is suggested that in idiopathic myocardiopathy there is greater extent of abnormality of the fundamental physiologic defect in PMD (depression of contractility), and that increased utilization of the Frank-Starling principle and development of ventricular hypertrophy maintain normal basal CI. In contrast, in ventricular hypertrophy resulting from basic physiologic abnormality of chronic systolic hemodynamic burden, contractility is less compromised and CI is normal at rest with reduced encroachment upon reserve capacity of preload mechanism. In either primary or secondary ventricular hypertrophy, decompensation occurs when impaired contractility, the fundamental determinant underlying congestive heart failure, becomes so severe that maximum use of compensatory mechanisms no longer sustains normal basal cardiac output.

197. Synthesis of Dipalmityl Lecithin by Lung and Alveolar Macrophages. ROBERT MASON,* GARY HUBER,* AND MARTHA VAUGHAN, Bethesda, Md., and Boston, Mass.

Dipalmityl lecithin (DPL) is the primary physiologic constituent of pulmonary surfactant, but there is little information about its origin or mode of synthesis. Alveolar macrophages (95% pure by electronmicroscope differential count; <0.5% cells with lamellar bodies), peritoneal exudates (>90% heterophils), and slices of lung and of liver from rabbits were studied. Pure saturated lecithins were separated from other lecithins after formation of mercuric adducts. Saturated lecithin (>96% palmitate) accounted for about 20% of total lecithin from saline-washed alveolar macrophages and 30% of that from lung slices. Incorporation of radioactive precursors into lecithin was investigated with cells or tissue slices incubated 1 or 3 hr in Krebs-Ringer phosphate medium with 10 mM glucose and 2 mg/ml bovine serum albumin. Label from ^{14}C-ethanolamine and ^{14}C-methyl methionine was found in significant amounts only in the unsaturated lecithins from liver. All tissues incorporated ^{14}C-labeled choline and palmitate into saturated lecithin, but per microgram of lipid phosphorus, alveolar macrophages were the most active. Saturated lecithin isolated from cells incubated with ^{14}C-palmitate was degraded with phospholipase A. In samples from alveolar macrophages, the ^{14}C was distributed equally between alpha and beta positions, whereas in the heterophils most of the ^{14}C-palmitate was in the beta position. Both macrophages and heterophils incorporated ^{14}C-choline–labeled lysolecithin into unsaturated lecithins, but only heterophils incorporated significant amounts into saturated lecithin. We conclude that alveolar macrophages synthesize DPL mainly *de novo,* whereas heterophils form DPL by acylation of lysolecithin as well as by *de novo* synthesis. Thus the alveolar macrophage is capable of synthesis of DPL and may be an important source of DPL in pulmonary surfactant. (Research supported by a grant from the NIH.)

198. Spontanteous In Vitro Nitro Blue Tetrazolium Reduction: A Discriminatory Test for Bacterial Infection in Adults. GEORGE MATULA* AND PHILIP Y. PATERSON, Chicago, Ill.

We have confirmed and extended in adults with bacterial infection the spontaneous reduction of nitro blue tetrazolium (NBT) by polymorphonuclear leukocytes (PMN) as observed in children by Park and associates. Colorless NBT, added to freshly collected peripheral blood, is reduced to conspicuous blue-black cytoplasmic deposits of formazan crystals. In 24 adults with definitive cultural proof of active bacterial infection, "formazan-positive" PMN ranged from 5 to 58%, with a mean of 21%. Two of the three values below 10% were associated with early, localized infection lacking constitutional symptoms, i.e. cystitis. In contrast, 38 control subjects (clinically well individuals or patients where bacterial infection was excluded) had a range of 0 to 8%, with a mean of 2.3%. On the basis of these observations, a positive NBT test was arbitrarily defined as 10% or more "formazan-positive" PMN. It is of interest that two cases of *Plasmodium vivax* malaria yielded positive results. A variety of noninfectious conditions, as well as hepatitis and other viral diseases, neither precluded a positive test if bacterial infection was present, nor gave false-positive results if bacterial infection was absent. After adequate antimicrobial therapy in 16 NBT-positive patients, the NBT response became negative within 3 to 26 days. We believe the NBT test is useful for recognition of active bacterial infection and in monitoring the effects of therapy. In probing the basis for NBT-positive responses, not only bacterial cultures but also their cell-free filtrates induced formazan-positive PMN in vitro. The filtrate factor(s) is stable (−70° to 100°C) and does not appear to be endotoxin. The PMN of a given subject exhibited responses to culture filtrates which varied with different bacterial species. A given filtrate also induced varied NBT responses in PMN of different subjects. Thus, both the infecting bacterial species and host-specific factors are implicated in positive NBT responses. (This investigation was supported by USPHS training grant T1-AM-5069.)

199. Inhibition of Human Erythrocyte Pyrophosphatase Activity by Calcium, Cupric, and Ferrous Ions. D. J. MCCARTY, P. PEPE,* S. SOLOMON,* AND J. COBB,* Chicago, Ill.

The deposition of calcium pyrophosphate crystals in articular cartilage (pseudogout syndrome) is frequently associated with hyperparathyroidism (8%). Numerous cases with coincident hemochromatosis and a few cases with associated Wilson's disease have been reported. A sensitive assay was developed to measure the rate of hydrolysis of ^{32}P-O-^{32}P to ^{32}P (which was then precipitated selectively) and applied to the study of the soluble pyrophosphatase (PP_iase) in RBC hemolysates. Purity of ^{32}P-O-^{32}P was ascertained by thin-layer chromatography. Final concentrations were PP_i 1.5 mM ($100 \times K_m$), Mg^{++} 1.75 mM, Tris Cl 33.3 mM, pH 7.7. PP_iase activity in 13 randomly selected patients and normal controls averaged 0.026 μmole/min per mg protein (range 0.021–0.028). Fe^{++}, F^{+3}, Cu^{++}, and Ca^{++} did not

act as cofactors when substituted for MG^{++}, and all but Fe^{+3} markedly suppressed PP_iase activity (> 95%) even in the presence of excess Mg^{++}. The ion products of Ca^{++} and the elevated synovial fluid PP_i in pseudogout reported by Fleisch and associates are but one-tenth the values that we have found necessary for in vitro precipitation of calcium pyrophosphate, so that even in hyperparathyroidism factors other than a simple increase in ion product must be sought. If inhibitory concentrations of the divalent cations obtain in the affected tissues in the above-mentioned associated diseases, and if PP_iases vital to PP_i homeostasis are inhibited by them, then these observations provide a working hypothesis to explain the association. (Research supported by USPHS grant 1-RO1-AM-13069.)

200. The Use of Etiocholanolone to Increase Collection of Granulocytes with the IBM Blood Cell Separator. KENNETH B. MCCREDIE* AND EMIL J FREIREICH, Houston, Texas.

32 leukaphereses were performed on six normal donors. 19 served as controls, and in 13, the donor was given 0.5 mg/m^2 etiocholanolone. 12 hr before leukapheresis a mean of 9.20 liters of whole blood per leukapheresis was processed in the control series, and 9.04 liters in those given prior etiocholanolone. A mean of 1.4×10^{10} leukocytes (30% granulocytes) was collected from the controls and 1.6×10^{10} (40% granulocytes) from the etiocholanolone group. The mean number of leukocytes collected per liter of whole blood processed in the controls was 1.45×10^9 (range $0.61–2.5 \times 10^9$) and in those with prior etiocholanolone 1.95×10^9 (range $1.13–3.3 \times 10^9$), an increase of 34.6%. The mean number of granulocytes collected was increased from 0.43×10^9/liter (range $0.008–1.2 \times 10^9$) of whole blood processed to 0.78×10^9/liter (range $0.02–1.67 \times 10^9$), an increase in the etiocholanolone-treated group of 81.5%. The mean number of monocytes was increased from 0.21×10^9/liter (range $0–1.37 \times 10^9$) to 0.46×10^9/liter (range $0.01–1.1 \times 10^9$), an increase of 119%. There was no change in the number of lymphocytes collected. Side effects of the etiocholanolone were limited to local pain and fever (max 101°F) which persisted for less than 24 hr. The use of etiocholanolone has enabled the number of granulocytes collected from normal donors to be almost doubled. A total of almost 10^{10} granulocytes can now be collected in a 10 liter leukapheresis. This number of cells closely approaches the number required to be therapeutically effective in well matched recipients. (Research supported by grants CA-08859 and CA-05831 from the NIH.)

201. An Intrarenal Hormonal System Regulating the Renal Actions of Angiotensin II and Norepinephrine. J. C. MCGIFF,* K. CROWSHAW,* N. A. TERRAGNO,* AND A. J. LONIGRO,* St. Louis, Mo. (introduced by René Wégria**).

Prostaglandins (PG) E_2, $F_{2\alpha}$, and A_2 are present in canine renal medulla. The release of renal PG by vasoconstrictor hormones was correlated with simultaneous changes in arterial blood pressure, renal blood flow (RBF) (electromagnetic flowmeter), and urine flow (photoelectric drop counter) in chloralose-anesthetized dogs. Renal venous blood was assayed continuously for prostaglandin-like substances (PLS) by the superfused blood-bathed organ technique. In all nine experiments, angiotensin II released PLS in amounts unrelated to the degree of vasoconstriction. The effects of angiotensin II were nonspecific, since norepinephrine also released PLS. The appearance of PLS in renal venous blood coincided with loss of their vasoconstrictor and antidiuretic actions. On two occasions, when PLS were not released, RBF and urine flow did not recover. In three experiments, samples of renal venous blood were removed and subjected to acidic lipid extraction and thin-layer chromatography, which characterized PLS as a PGE. In six experiments, the threshold concentration of angiotensin II which released PLS varied from 0.08 to 4.4 ng/ml; in five, the concentration fell within the range reported in renal vascular hypertension (< 1.0 ng/ml). The log of the threshold dose of angiotensin II which released PLS was plotted against control values of plasma renin activity (PRA) of thoracic caval blood, the latter an index of the state of sodium balance. A linear relation obtained ($P < 0.001$ for regression coefficient). Thus, when sodium balance was negative (high PRA) the threshold dose of angiotensin II was high. These results suggest that renal PG (1) are oriented to salt and water metabolism, and (2) modulate renal vasoconstrictor hormones. Our recent demonstration that PGA_2 and PGE_2 inhibit the renal actions of angiotensin II and norepinephrine endorses the latter suggestion.

202. Increased Sensitivity to Warfarin in Thyrotoxicosis. THOMAS J. MCINTOSH,* S. FRED BRUNK,* INGRID KÖLLN,* JAMES R. FOUTS,* AND WILLIAM R. WILSON, Iowa City, Iowa.

Although drug interaction is important, drug-disease interaction also should be considered by the physician. Altered responsiveness to anticoagulants has been reported in various hypermetabolic states. We studied the prothrombin responses and the plasma decay after warfarin in five thyrotoxic patients before treatment with ^{131}I and 3 months later when euthyroid. Therapy decreased the average serum thyroxine (16 to 10 μg/100 ml) and 24 hr ^{131}I uptake (46 to 11%) significantly ($P < 0.05$). After treatment, body weight increased (150 to 170 lb.) and serum cholesterol rose (144 to 214 mg/100 ml; $P < 0.05$). Each fasted patient received warfarin sodium orally, 40 mg per square meter of body surface area. Nine blood samples were drawn for the one-stage prothrombin time and for warfarin plasma levels before and 3–84 hr after warfarin. The time required for the prothrombin time to double was the indicator of the response to warfarin. The mean doubling time before ^{131}I was 24 ± 1.5 hr; after treatment it was 31 ± 2 hr ($P < 0.05$). Warfarin plasma levels decreased more rapidly during the thyrotoxic period ($t_{\frac{1}{2}} = 44 \pm 5$ hr) than during the euthyroid period ($t_{\frac{1}{2}} = 126 \pm 29$ hr; $P < 0.05$). Mean plasma levels at 12 hr were 5.1 ± 0.4 μg/ml when thyrotoxic and 6.1 ± 0.6 μg/ml when euthyroid ($P > 0.05$). At 24 hr the concentrations were 4.2 ± 0.3 and 5.5 ± 0.5 μg/ml ($P < 0.05$). These findings indicate that patients with thyrotoxicosis were more sensitive to warfarin than three months later when they were euthyroid. This enhanced responsiveness was associated

with a shortened plasma half-life of the drug. (Research supported by the Veterans Administration [TR-105], the National Heart Institute [HE-5577], the National Institute of General Medical Sciences [GM-12675], the USPHS [MO1-FR-59], and the Iowa Heart Association.)

203. The Effect of Fibrin-Stabilizing Factor on the Subunit Structure of Human Fibrin. PATRICK A. MCKEE,* PATRICK MATTOCK,* AND ROBERT L. HILL,* Durham, N. C. (introduced by Malcolm P. Tyor**).

It is thought that fibrin-stabilizing factor (FSF) promotes peptide bond formation between subunits of different fibrin monomers to form cross-linked, urea-insoluble fibrin. The present study identifies the fibrin monomer subunits involved in FSF-catalyzed cross-linking and establishes their extent, order, and rates of cross-linking. Purified human fibrinogen (97% clottable), non-cross-linked fibrin (soluble), and cross-linked fibrin (insoluble) were reduced by 0.1 M mercaptoethanol and then analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Fibrinogen and non-cross-linked fibrin (soluble) contained three subunits: γ-chain, 50,000 molecular weight (mol wt); β-chain, 60,000 mol wt; and α-chain, 73,000 mol wt. Each subunit was identified by isolation of its S-sulfo derivative from CM chromatography in 8 M urea containing a 0.005–0.1 M sodium acetate gradient. Cross-linked fibrin (insoluble) had a distinctly different distribution of subunit weights; the γ-chain had disappeared completely within the first 2 min of clotting to form a dimer of mol wt 100,000; at this time the fibrin was partially insoluble in 10 M urea. By 2 hr the α-chain had disappeared to form $(-\alpha-\alpha-)_n$ polymers which had a mol wt > 400,000; this fibrin was completely insoluble in 10 M urea. Calcium accelerated the formation of both γ-γ and $-\alpha-\alpha-$ cross-links. Amino acid compositions of the γ-γ dimer and the $(-\alpha-\alpha-)_n$ polymer confirmed their identities. It is concluded that (1) FSF rapidly catalyzes the dimerization, γ-γ, of γ-chains, but more slowly the polymerization, $(-\alpha-\alpha-)_n$, of α-chains; (2) the β-chains of fibrin monomers do not participate in cross-linking; (3) the γ-γ dimer and $(-\alpha-\alpha-)_n$ polymer can account for the three-dimensional, fibrillar structure of cross-linked, insoluble fibrin. (Supported by the USPHS, NIH grant NB-06233, and NSF grant GB-12676.)

204. Secretion of 18-Hydroxydeoxycorticosterone in Human Hypertensive Disease. JAMES C. MELBY, THOMAS E. WILSON,* AND SIDNEY L. DALE,* Boston, Mass.

18-Hydroxydeoxycorticosterone (18-OH-DOC), a mineralocorticoid secreted by rat adrenal cortex, was isolated from human adrenal vein blood and identification established. Because 18-OH-DOC has been implicated in the development of rat adrenal regeneration hypertension, its secretion was assessed in patients with hypertensive diseases including essential hypertension (13), accelerated or renovascular hypertension (4), primary aldosteronism (10), and Cushing's syndrome (4). 18-OH-DOC was measured in adrenal vein blood before and after administration of angiotensin and ACTH. Blood was obtained by percutaneous catheterization, plasma was extracted, and 18-OH-DOC was isolated, oxidized to γ-lactone, and quantitated. The secretion rate of 18-OH-DOC (18-OH-DOC SR) was determined by injecting intravenously 1,2-^{3}H-18-OH-DOC and isolating urinary 1,2-^{3}H-18-OH-TH DOC. Quantitation of urinary 18-OH-TH DOC (principal metabolite of 18-OH-DOC) was also carried out. Basal adrenal venous plasma 18-OH-DOC levels were < 0.5 μg/100 ml in 20 of 27 patients. Angiotensin infusion failed to evoke a rise in 18-OH-DOC concentration, whereas ACTH increased 18-OH-DOC to an average of 12 μg/100 ml. Of seven patients with basal measurable 18-OH-DOC, three had levels > 6 μg/100 ml. These had suppressed plasma renin activity (PRA) and low aldosterone secretion (ASR), and became normotensive with spironolactone. In healthy subjects 18-OH-DOC SR averaged 100 μg/24 hr. Urinary 18-OH-TH DOC excretion averaged 20 μg/24 hr. ACTH increased 18-OH-DOC SR 10-fold. Three of four essential hypertensives with low PRA and ASR and four patients with Cushing's syndrome had elevated urinary 18-OH-TH DOC excretion (48–3030 μg/24 hr). The remaining hypertensive patients had urinary 18-OH-TH DOC excretion of 5–31 μg/24 hr. 18-OH-DOC is a mineralocorticoid elaborated by the human adrenal cortex. It is not a precursor of aldosterone, nor is its formation stimulated by angiotensin. Corticotropin appears to regulate its discharge. It is secreted excessively in Cushing's syndrome and in certain patients with hypertension associated with suppressed PRA and ASR and hypotensive response to spironolactone. (Supported in part by the following grants: USPHS AM-12027-03, 5-PO2-AM-08675-06, TO1-AM-05446-05.)

205. Density Gradient Electrophoresis in Hyperbetalipoproteinemia. JOHN S. MELISH* AND CHRISTINE WATERHOUSE,** Rochester, N. Y.

Pratt and Dangerfield, using acrylamide gel density gradient electrophoresis (DGE), described resolution of lipoprotein classes in normal and "hyperlipemic" plasma not possible by other techniques. With modification of their technique, which permits greater separation of lipoprotein bands, we have used DGE to study fasting plasmas from normal subjects and from patients with Frederickson's type II hyperbetalipoproteinemia (hypercholesterolemia, normal triglycerides by silicic acid chromatography; hyperbetalipoproteinemia by paper electrophoresis). Prestained plasma was electrophoresed in continuous density gradient columns (2–8% acrylamide) 10 cm in length. Reproducible electrophoretic patterns, resulting from the increasing sieving property of the gel, showed a heavily stained low density lipoprotein (LDL) band in normal plasma which could easily be resolved into two and occasionally three components. These bands were preceded and followed by one or two faintly staining lipoprotein bands. Type II hyperbetalipoproteinemia usually showed increased staining intensity in the heavy LDL bands and up to five or six more slowly moving bands, clearly separable from the yet more slowly moving very low density lipoproteins (VLDL) as defined by ultracentrifugation. As compared with normal, type II plasma showed marked reduction in faster-moving high density lipoprotein staining. Some subjects in families of type II probands showed one or more of the slower-moving components despite having

normal plasma cholesterol and triglycerides. Increased staining of the slower-moving bands persisted in type II patients despite dietary therapy and return of lipid chemistries to more normal values. There is no superimposition or trailing of VLDL over LDL that may confuse the qualitative interpretation of paper electrophoresis patterns, and this technique appears to offer improved clarity diagnostically as well as in evaluating treatment results in specific lipoprotein disorders. (Research supported by grants from the NIH.)

206. Rapid Induction of Increased Alpha Aminoisobutyric Acid Uptake by Phytohemagglutinin-Stimulated Lymphocytes. JOHN MENDELSOHN,* SR. ANN SKINNER,* AND STUART KORNFELD,* St. Louis, Mo. (introduced by Carl V. Moore**).

Studies of early phytohemagglutinin (PHA)-induced changes were performed on a highly purified preparation of human peripheral blood lymphocytes containing fewer than one phagocytic cell, platelet, and erythrocyte per 100 lymphocytes. The nonutilized amino acid, α-aminoisobutyric acid (AIB), enters lymphocytes with saturation kinetics (apparent $K_m = 2 \times 10^{-3}$ M). Within 30 min after addition of PHA to lymphocyte cultures, there is an increase in the rate of AIB uptake, which continues to rise for 6–9 hr and then remains stable for 3 days. Kinetic studies show that after PHA stimulation the K_m for AIB remains unchanged, whereas the V_{max} increases by 4- to 6-fold. Thus the increased rate of uptake is due either to an increased number of transport sites or to an increased turnover per site, rather than to an altered affinity of the transport site for AIB. 75% of ^{131}I-PHA bound to lymphocytes can be rapidly removed by addition of a glycopeptide extracted from erythrocyte membranes which competitively binds PHA. Removal of membrane-bound PHA effects a partial reversal of the PHA-induced increase in AIB uptake. Another reported early PHA effect, increased ^{32}P incorporation into cell membrane phospholipids, is also reversed by removal of PHA. In summary, we have demonstrated a direct effect on lymphocyte membrane function produced by binding of PHA to the lymphocyte surface. This effect is dependent on the continued presence of PHA on the cell membrane and can be reversed by removal of PHA, suggesting that PHA can function without entering the cell. (Supported by grants from the NIH and the American Cancer Society.)

207. Lobular Glomerulonephritis and Mesangioproliferative Glomerulonephritis. NINA MENDOZA,* NICHOLAS MANDALENAKIS,* CONRAD L. PIRANI,* AND VICTOR E. POLLAK, Chicago, Ill.

Lobular glomerulonephritis (LGN) has been thought to be a stage in the evolution of membranous GN. Renal biopsies from 900 patients with primary glomerular disease were reviewed. LGN was identified in eight cases; in 13 a similar morphologic entity, mesangioproliferative GN (MPGN), was recognized. Analysis of 39 specimens revealed, in both LGN and MPGN, an accentuated glomerular lobular pattern, and mesangial and some endothelial hypercellularity. In LGN, centrilobular hyaline nodules were found; increased mesangial cells and matrix preceded nodule formation, but mesangial cellularity may persist. In MPGN, mesangial matrix was proportional to mesangial hypercellularity and extended peripherally, simulating basement membrane duplication; mesangial and subendothelial deposits were striking. Save that nephrotic syndrome was more frequent in MPGN (8 of 12 vs. 3 of 8), the two morphologic entities were similar clinically. Recurrent sore throats and tonsillitis were frequent (17 of 22), but evidence of streptococcal infection was equivocal and the relation of infection to onset of renal disease uncertain. Azotemia was found initially in 18 patients, but renal functional deterioration was slow. Five patients with LGN, five with MPGN have died. Thus, LGN and MPGN are distinct morphologic entities. They do not evolve from membranous GN; rather, they may evolve rarely from diffuse endocapillary proliferative GN, possibly streptococcal in type. They might occur with repeated subliminal immunologic stimulation by infectious agents. (Research supported by grant AM-10314 from the NIH.)

208. The Liver during Fasting in Pregnancy: An Example of Anabolic Catabolism. BOYD E. METZGER,* FRANCIS S. AGNOLI,* AND NORBERT FREINKEL, Chicago, Ill.

Maternal survival and continued fetal growth during starvation in late pregnancy are rendered possible by acceleration of all the conventional responses to fasting. Whether the acceleration is wasteful of maternal resources or attended by unique conservation mechanisms has not been examined heretofore. Accordingly, we have monitored disposition of alanine carbons and nitrogen during perfusion of isolated livers from 24 hr fasted and 19 day pregnant (P) and age-matched virgin (V) rats. Recirculating perfusion media consisted of KRB containing washed bovine RBC (hematocrit 20), fat-free albumin (3%), and U-^{14}C-alanine (20 mM). Per micromole DNA phosphorus, livers from pregnant rats extracted more alanine ($P < 0.05$) and formed more glucose ($P < 0.005$), lactate ($P < 0.005$), and glyceride-glycerol ($P < 0.001$) than did those of nonpregnant rats. Glucose and glyceride-glycerol also constituted a larger fraction of assimilated alanine carbons ($P < 0.05$). Thus, hepatocytes of fasted pregnant rats make more glucose than do those of fasted nonpregnant rats by "trapping" more gluconeogenic precursor and shunting greater proportions away from oxidation and toward triose phosphates. The more efficient gluconeogenic utilization of amino acid carbon is attended by potentially greater frugality in disposition of amino acid nitrogen. Release of urea and ammonia (ΣN) accounted for more than 90% of glucose production in P as well as V. However, after 60 min perfusion, 70% of ΣN in V consisted of the nonutilizable end product, urea, whereas 65% in P was present in metabolically useful form as ammonia. Such ammonia could contribute to fetal pyrimidine synthesis, since aspartate transcarbamylase is increased and urea cycle enzymes are diminished in the fetus. It could also support fetal growth via transaminative recapture after transplacental passage. (Research supported by grants AM-10699 and AM-06071 from the NIH.)

209. Hepatic Porphyria: Inappropriate Induction of Delta Aminolevulinic Acid Synthetase by Enhanced Hemoprotein Turnover. U. A. MEYER* AND H. S. MARVER, San Francisco, Calif.

Overproduction of porphyrin precursors in both human and chemically induced experimental porphyria results from inappropriate induction of δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in porphyrin and heme biosynthesis. However, the cause of this excessive enzyme induction has remained unexplained. The following findings indicate that in the animal model this abnormality results from increased hepatic turnover of the microsomal hemoprotein, cytochrome P-450 (P-450), the terminal oxidase in drug and steroid metabolism. (1) Heme participates in repression of ALAS as a principal mechanism in regulation of this enzyme as directly demonstrated in hepatocyte cultures. (2) Compounds such as phenobarbital that increase microsomal oxidases induce ALAS to provide heme for new synthesis of P-450; no other hemoprotein is involved. Phenobarbital sequentially produces these proportional increases in rat liver: (a) ALAS, 3–5 ×; (b) microsomal heme synthesis, 4–5 ×; (c) P-450, 3 ×. Substances of this type do not produce experimental porphyria because derepression is controlled and, therefore, the increase of ALAS is modest and of short duration (6–12 hr). (3) By contrast, porphyria-inducing drugs such as allylisopropylacetamide affect ALAS and P-450 disproportionally, rapidly lowering P-450 and microsomal heme (0.3–0.7 ×) but markedly inducing ALAS (8–15 × for 24–36 hr) and microsomal heme synthesis (6–10 ×). This picture of increased microsomal heme synthesis without a corresponding net increase in product suggests enhanced hemoprotein turnover. This is directly demonstrated by pulse labeling, with ^{3}H-δ-aminolevulinic acid, the heme moiety of submicrosomal particles containing P-450, as the sole hemoprotein. The $t_{\frac{1}{2}}$ of the predominant phase of decay of ^{3}H-heme crystallized from these particles was about 16 hr in controls or after treatment with phenobarbital, and 1–3 hr after allylisopropylacetamide. Thus, in experimental porphyria enhancement of P-450 turnover diverts heme from repression of ALAS, resulting in inappropriate induction of the enzyme. Because of the biochemical parallelism between experimental and human porphyria, these findings seem relevant to the hepatic porphyrias of man. (Supported by grants from the NIH.)

210. Glomerular Polyanion: Nature and Alteration in Renal Disease. ALFRED F. MICHAEL,* EDWARD BLAU,* GUNNAR WESTBERG,* AND ROBERT L. VERNIER, Minneapolis, Minn.

Light and electron microscope examination of colloidal iron (CI)-stained (pH 1.9) specimens of human and rat kidney reveal a sialic acid–containing polyanion (GP) restricted to the surface of the glomerular epithelial cells and foot processes adjacent to the glomerular basement membrane (GBM). Alcian blue (AB) stains confirm the distribution. Isolated rat and human GBM (121 *g* sediment of sonicated glomeruli) do not contain detectable polyanion, and react very weakly with AB and not at all with CI, even though the concentration of sialic acid is similar to that in intact glomeruli. The polyanion can be separated at high *g* forces from sonicated glomeruli and GBM, or by incubation with proteolytic enzymes and sialidase but not collagenase or hyaluronidase. In 55 of 58 rats given aminonucleoside of puromycin, a decrease and alteration in CI and AB staining of glomeruli were seen coincident with the development of proteinuria, when compared with 48 controls. Electron microscope examination reveals loss of GP of the epithelial cells adjacent to the GBM. Similar alterations were seen in 45 rats given rabbit anti–rat GBM serum 4 and 10 days later in kidney stained with CI. These changes were associated with a significant decrease in glomerular sialic acid content (six nephrotoxic glomerular preparations, 6.30 ± 0.62 μg/mg; nine controls, 8.36 ± 1.06 μg/mg). These studies suggest that there is an alteration in the negative charge of the glomerular filter in both metabolic and immune injury. Whether these changes are responsible for increased permeability of the glomerular filter or are secondary to proteinuria or inflammation is unknown. Preliminary observations of kidneys from human glomerulonephritis and nephrotic syndrome will be discussed.

211. Alcohol-Induced Fatty Liver: Importance of Endogenous Intestinal Lipoproteins. STEVEN P. MISTILIS* AND ROBERT K. OCKNER,* San Francisco, Calif. (introduced by I. S. Edelman**).

In the continuing controversy surrounding the pathogenesis of ethanol-induced fatty liver, little attention has been paid to the intestine as a source of endogenous triglycerides (TG). However, even in the absence of dietary fat, the small intestine contributes TG-rich very low density lipoproteins to plasma via intestinal lymphatics. Furthermore, it absorbs most of the ingested ethanol and contains ethanol-metabolizing enzymes. Our data suggest that a major portion of the hepatic lipid accumulation following ethanol is derived from endogenous TG of intestinal origin. Fasting male rats, with or without intestinal lymph fistula, received continuous intraduodenal infusions of ethanol (5–7.5 g/kg) or of isocaloric glucose over 8 hr, followed by saline. Lipids were measured in lymph and liver. Although similar to controls at 0–8 hr and after 24 hr, lymph TG output in ethanol-treated rats was 25% greater at 8–16 hr and 50% greater at 16–24 hr ($P < 0.025$). Lymph cholesterol and phospholipid also increased during these periods. In nonfistula rats, ethanol caused marked increases in total hepatic TG by 24 hr (159 mg, vs. 33 mg in glucose controls; $P < 0.001$). This ethanol-induced fatty liver was prevented by lymph fistula; in fistula rats, total hepatic TG after ethanol was only 43 mg, differing markedly ($P < 0.0025$) from that in nonfistula rats. It is noteworthy that after ethanol, total hepatic TG in nonfistula rats (159 mg) exceeded that in fistula rats (43 mg) by approximately their 24 hr lymph TG output (120 mg). Furthermore, after ethanol, triglyceride fatty acid compositions of lymph and fatty liver were similar. These results strongly suggest that endogenous intestinal lipoproteins are a major source of the hepatic TG accumulation following ethanol administration, and that ethanol enhances intestinal lipoprotein formation. As

fatty liver in man occurs despite low fat intake, these observations suggest an important role for endogenous intestinal lipoproteins in the pathogenesis of ethanol-induced fatty liver. Increased intestinal lipoprotein production could also contribute to ethanol-induced hyperlipidemia. (Research supported by grants from the NIH.)

212. Protein-Lipid Interactions and the Maintenance of Red Cell Membrane Integrity. CHARLES F. MOLDOW,* VERNE HOSPELHORN,* DOROTHEA ZUCKER-FRANKLIN,* AND ROBERT SILBER, New York, N. Y.

The erythrocyte membrane contains approximately equal amounts of lipid and protein. This study deals with the interaction between these molecules, as related to the preservation of membrane structure. Erythrocyte ghosts were "solubilized" by succinylation in the presence of urea. This treatment results in the addition of a succinate half amide moiety onto the free amino groups of lysine and increases the negative charge of the protein. Dissociation of protein from lipids after succinylation was demonstrated in the following manner: Over 85% of the protein stayed in the supernatant fluid after centrifugation for 1 hr at 100,000 *g*, whereas 80% of the cholesterol, approximately two-thirds of the phospholipid, and the remainder of the protein sedimented to the bottom of the tube. Analyses of the ghosts, solubilizing ghosts, and supernatant fluid revealed no differences in their amino acid content. Optical rotatory dispersion, circular dichroism, and infrared spectroscopy studies on the succinylated membrane showed a decrease in the α-helical configuration of the protein and a concomitant increase in its β- and random configuration. The solubilized membrane was further characterized by Sephadex gel filtration and acrylamide gel electrophoresis. In the presence of sodium dodecyl sulfate (SDS) 12 or more bands were observed on electrophoresis, and at least four groups of proteins were separated on Sepharose 6B or Sephadex G-200. In the absence of SDS, aggregation of the proteins was demonstrated by Sephadex gel filtration and acrylamide gel electrophoresis. These experiments show that when the negative charge of membrane proteins is increased by the introduction of succinyl groups, the three-dimensional structure of the protein is changed and the interaction with lipids is reduced. These observations are consistent with a role of protein-lipid interactions in the maintenance of the erythrocyte membrane structure.

213. Studies with Ion Exchange Calcium Electrodes: Does the Cirrhotic Produce Albumin with Normal Calcium-Binding Properties? EDWARD W. MOORE, Boston, Mass.

Previous studies from our laboratory indicate that in both normals and cirrhotics, serum total calcium [Ca] indirectly measures serum albumin; change in [Ca] was almost totally accounted for by corresponding change in calcium proteinate. In cirrhotics there was no detectable binding to serum globulins (vs. 20% in normals), suggesting deficiency of a normal globulin. The over-all dissociation constant (K'_d) for CaAlb $\rightleftarrows Ca^{++} + Alb^-$ was markedly elevated in several patients. The present studies indicate that K'_d variation in 41 cirrhotics was not random, but varied as a rectangular hyperbola with $\bar{\nu}$, i.e. the molar ratio [CaAlb]/[Alb]. As $\bar{\nu} \rightarrow 0$, $K'_d \rightarrow \infty$ and k'_a (association constant) $\rightarrow 0$. Thus, calcium "binding-affinity" ($dk'_a/d\bar{\nu}$) of albumin varied with the number of calciums on the albumin molecule and was opposite in direction to that for electrostatic interaction between binding sites for the multiple equilibria k'_1, k'_2 . . . k'_n. K'_d was not related to serum pH or globulin/albumin ratios (i.e.? protein-protein interaction), but tended to rise with increase in the ratio [diffusible calcium complexes]/[Alb], suggesting possible protein-ligand interaction. It was not due to cirrhosis per se, since K'_d values in 21 normals fell exactly on the same hyperbola. To evaluate normal albumin over the entire cirrhotic $\bar{\nu}$ range, serial NaCl dilution and $CaCl_2$ addition studies (13) of pooled normal sera were made (constant pH, temperature, ionic strength). As in cirrhosis, as $\bar{\nu} \rightarrow 0$, $k'_a \rightarrow 0$. In contrast with cirrhotics, at higher $\bar{\nu}$ levels, k'_a remained essentially constant (mean $pK'_d = 2.14$). We conclude that calcium binding by cirrhotic albumin is normal at normal $\bar{\nu}$ levels and below, and somewhat increased at higher $\bar{\nu}$ values. The paradoxical decrease in k'_a as $\bar{\nu} \rightarrow 0$ is a previously undescribed property of normal albumin. This suggests that binding of the first calcium may be related to the configurational state of albumin. Binding then appears to stabilize normally (i.e. $dk'_a/d\bar{\nu}$ nears 0) with attachment of this first calcium to the albumin molecule. (Supported by NIH grants.)

214. Protein Synthesis in Human Lung Alveolar and Peritoneal Macrophages. ROGER M. MORRELL,* Kalamazoo, Mich. (introduced by Robert W. Heinle**).

Although the precise role of macrophages (M) in the immune response is not established, it is clear that many facets of their metabolism are altered when they ingest antigens. More knowledge is at hand on RNA synthesis than on protein synthesis. However, M produce specific proteins (interferon) in response to certain stimuli, as reported by Smith and Wagner. Rabbit and mouse peritoneal cells (mostly M) in preliminary studies incorporate amino acid into protein as stated by Myrvik. Morrell has reported enhanced incorporation of amino acid and uridine into protein and RNA in polysomes of rabbit lung M from animals immunized with tuberculoprotein (PPD) and challenged with PPD in vitro. In these experiments the first account is given of polysome, protein, and RNA synthesis in human lung alveolar and peritoneal M. Lung cells were obtained from bronchial washings, peritoneal cells from dialysates. Cells from both areas have not yet been obtained from the same patient. Lung M polysomal profiles are more differentiated than those of peritoneal M. Lung M incorporate more amino acid into protein in the resting (unchallenged) state, but RNA synthesis is the same for the two cell types, suggesting a useful system for the study of certain regulatory mechanisms in protein synthesis. The ease with which human lung and peritoneal M are obtained, and their brisk metabolic activity, suggest an important role for them in detailed studies of the afferent limb of the immune response in man.

215. Evidence of a Pathogenetic Role of Hyperparathyroidism in the Renal Tubular Dysfunction of Patients with Fanconi's Syndrome. R. CURTIS MORRIS, JR., ELISABETH MCSHERRY,* LOUIS M. SHERWOOD,* AND ANTHONY SEBASTIAN,* San Francisco, Calif.

The renal acidification defect induced experimentally by parathyroid hormone (PTH) resembles that of patients with renal tubular acidosis and Fanconi's syndrome (RTA-FS). In RTA-FS, renal bicarbonate reabsorption ($THCO_3^-$) at normal plasma bicarbonate concentrations is reduced by > 15%, a finding indicating reduced bicarbonate reabsorption in the proximal tubule. To investigate the possible pathogenetic role of hyperparathyroidism in RTA-FS, we measured serum PTH (PTH_s) and $THCO_3^-$ at normal plasma bicarbonate concentrations before and during intravenous administration of calcium gluconate, in seven studies of five affected patients, including two unrelated children with cystinosis and three unrelated adults. Whenever measured (four studies), PTH_s was initially supernormal (0.45–1.05 mμg/ml). As serum calcium was increased approximately 2 mg/100 ml, PTH_s decreased to normal values (< 0.2), urinary pH decreased, and $THCO_3^-$ increased, in six studies by 25–40%. The increase in $THCO_3^-$ disappeared minutes after initiation of intravenously administered PTH (Lilly) (two patients), despite progressively increasing serum calcium concentrations. In four studies, GFR decreased slightly during calcium administration, in one by 25%, but improved renal tubular function could not be attributed solely thereto: $THCO_3^-$ increased even when GFR remained constant; absolute reabsorption rates of HCO_3^- and phosphate, α-amino nitrogen, and urate increased even when filtered loads decreased; chloride excretion increased even when its filtered load decreased. These results (a) strongly suggest that hyperparathyroidism plays a pathogenetic role in the tubular dysfunction of FS, (b) may explain why vitamin D has improved renal tubular function in patients with FS, and (c) suggest the possibility that parathyroidectomy might interrupt a critical amplifying "loop" in the renal tubular dysfunction and structural deterioriation characteristic of FS. (Research supported by grants from the NIH and the American Cancer Society.)

216. Diversity of Biliary Secretory Mechanisms. THOMAS Q. MORRIS,* New York, N. Y. (introduced by Stanley E. Bradley**).

Active transport of bile salts and other organic anions is recognized as the principal driving force for the secretion of water and electrolytes as bile at the level of the hepatic cell. Secretin has been shown to produce its bicarbonate-rich choleresis at a site lower in the hepatobiliary system. The properties of these agents may be differentiated during their respective choleresis by measurement of the biliary clearance of a freely diffusible inert solute such as erythritol. Studies in fasted, cholecystectomized dogs equipped with Thomas duodenal cannulae were undertaken to assess comparatively the influence of a variety of choleretic agents including sulfobromophthalein, glucagon, hydrocortisone, and acetazolamide on the composition of bile and clearance of ^{14}C-erythritol during constant infusion of sodium taurocholate (10–12 μEq/min) and cholinergic blockade (Piptal). Choleresis associated with all these agents were always accompanied by an increase in ^{14}C-erythritol clearance. A characteristic change in composition was observed only after acetazolamide, which produced a chloride-rich increment in bile flow. The remainder of the materials tested stimulated secretion of a fluid resembling an ultrafiltrate of plasma. The ability of aminophylline, a phosphodiesterase inhibitor, to induce a similar response suggested that some of the choleretic effects might be mediated by cyclic AMP. Sequential administration of acetazolamide and any of the other choleretics tested always produced a cumulative response. These studies indicate that choleretics may act at the hepatocellular level independently of taurocholate excretion, by at least two additional mechanisms which are additive and not dependent upon their osmotic activity. (Supported by NIH grant AI-08890.)

217. Myocardial Metabolic Adaptations to Coronary Shock and the Response to L-Norepinephrine, Isoproterenol, and Phase Shift Balloon Pumping. HILTRUD MUELLER,* STEPHEN M. AYRES,* STANLEY GIANNELLI, JR.,* E. FOSTER CONKLIN,* AND WILLIAM J. GRACE,* New York, N. Y. (introduced by Daniel S. Lukas**).

Hemodynamics and myocardial metabolism were evaluated in 13 patients in cardiogenic shock following acute myocardial infarction. The response to L-norepinephrine was studied in seven, to isoproterenol in four, and to phase shift balloon pumping in three. Cardiac index (CI) was markedly reduced, averaging 1.49 liters/min per m^2. Mean arterial pressure ranged from 50 to 60 mm Hg; systemic vascular resistance varied widely, averaging 1489 dynes·sec·cm^{-5}. Coronary blood flow (CBF) was decreased in all but three patients (range 60–95, mean 73 ml/100 g per min). Diastolic coronary vascular resistance was uniformly decreased, suggesting that the low measured CBF reflected mainly perfusion of the noninfarcted myocardium. Myocardial oxygen consumption (MVO_2) was normal or increased, ranging from 6.1 to 11.4 ml/100 g per min. Myocardial oxygen extraction was above 70% and coronary sinus oxygen tension was below 22 mm Hg in most of the patients. 11 of 13 patients demonstrated myocardial lactate production; two had extractions below 10%. Excess lactate was present in nine patients. During L-norepinephrine infusion CI increased insignificantly. Increased arterial pressure was associated in all patients with increases in CBF, averaging 32% ($P < 0.01$). Increases in MVO_2 mainly paralleled increases in CBF. Myocardial lactate production shifted to extraction in three patients, and extraction improved in three. During isoproterenol infusion CI increased uniformly, averaging 63%. Mean arterial pressure remained unchanged, but diastolic arterial pressure fell. CBF increased in three patients, secondary to decreases in coronary vascular resistance. Myocardial lactate metabolism deteriorated uniformly: lactate production increased or extraction shifted to production. Phase shift balloon pumping following resistant shock improved CI and arterial pressure in two patients. CBF rose in all three, and myocardial lactate production shifted to extraction. In acute coronary shock, the primary therapeutic concern should be directed toward the myocardium, and

not toward peripheral circulation. L-Norepinephrine appears to be superior to isoproterenol; phase shift balloon pumping should be considered early when pharmacologic therapy is unsuccessful. (Supported by USPHS National Heart Institute research grants HE-12323-01 and HE-08074-7.)

218. Abnormal Alpha Cell Function in Diabetes. WALTER A. MULLER,* GERALD R. FALOONA,* AND ROGER H. UNGER, Dallas, Texas.

Fasting glucagon levels of diabetics are normal, despite hyperglycemia, suggesting unresponsiveness of diabetic α-cells to hyperglycemic suppression. Studies were designed to determine whether diabetes is characterized by constant relative or absolute hyperglucagonemia, and, if so, to elucidate its mechanism. Radioimmunoassayable glucagon responses to meals were compared in nondiabetics and diabetics. In 14 nondiabetics, > 40 g of protein elicited a prompt glucagon rise, averaging 102 ± 11 (SEM) pg/ml; hyperglycemia, induced by glucose infusion, prevented the protein-induced glucagon rise. In 12 adult and 12 juvenile-type diabetics, glucagon rose 106 ± 29 pg/ml and 95 ± 16 pg/ml, despite hyperglycemia > 225 mg/100 ml. In nondiabetics, carbohydrate ingestion (> 60 g) elicited prompt declines in glucagon, averaging -45 ± 6 pg/ml, with glycemia < 140 mg/100 ml; glucagon failed to decline in the diabetic groups, despite hyperglycemia > 310 mg/100 ml, and frequently rose. Because diabetic α-cell unresponsiveness to hyperglycemia might reflect reduced glucose penetration secondary to insulin lack, glucagon was studied in experimental hypoinsulinemia in dogs. Mannoheptulose infusion abolished insulin secretion acutely. As glucose rose, glucagon rose 3-fold; after the infusion, insulin reappeared, whereupon glucose and glucagon returned to normal. In alloxan-induced hypoinsulinemia (FBS > 380 mg/100 ml), glucagon averaged 4650 pg/ml, 5–10 times normal; insulin infusion promptly lowered glucose and glucagon. In two adult-type diabetics, however, insulin (0.12 U/kg), infused during carbohydrate ingestion, lowered but failed to normalize glucagon. In conclusion: Normally glucagon is suppressed by carbohydrate-induced hyperglycemia and stimulated by protein, except during hyperglycemia. In diabetes, glucagon is not suppressed by hyperglycemia after carbohydrate and rises normally after protein, despite hyperglycemia. Since experimental insulin deficiency induces hyperglucagonemia, hyperglucagonemia of human diabetes may be secondary to insulin lack, although less readily corrected by acute insulin infusion, suggesting restraining factors. Whatever the mechanism, diabetics are constantly either relatively or absolutely hyperglucagonemic, which must exaggerate the metabolic consequences of hypoinsulinemia and increase therapeutic requirements. (Supported by a NIH grant.)

219. The Effect of Contrast Medium Injection on Left Ventricular Function in Dogs. CHARLES B. MULLINS,* STEPHEN J. LESHIN,* DONALD S. MIERZWIAK,* AND JERE H. MITCHELL, Dallas, Texas (introduced by Carleton B. Chapman**).

An analysis was made of left ventricular function in six studies on four open-chest dog preparations before, during, and after the injection of 1 cc/kg contrast (76% meglumine diatrizoate) or normal saline at 200 psi pressure. Six lead beads were placed near the endocardium of the left ventricle, and biplane cinefluorographic exposures were taken while measurements were made of the left ventricular pressure (LVP) and its derivative (dp/dt), aortic pressure, and ECG. Left ventricular end-systolic (ESV) and end-diastolic (EDV) volumes were calculated for an assumed ellipsoidal shell of cardiac muscle which includes the left ventricular cavity. EDV, ESV, stroke volume (SV), left ventricular end-diastolic pressure (EDP), dp/dt, and ejection fraction (EF) were analyzed continuously for 3 beats before and for 13–17 beats after the injection of either contrast or saline into the left ventricle. Repeat measurements were made at 1 and 15 min after injection. Within one to seven beats after either saline or contrast injection, EDP and EDV increased, with a corresponding rise in dp/dt, SV, and EF, indicating a Frank-Starling effect. 9 to 16 beats after saline injection, EDP and EDV decreased, with an associated fall in dp/dt, SV, and EF; whereas after contrast, EDP and EDV increased further with a fall in dp/dt, SV, and EF, indicating a negative inotropic effect. This negative effect was not evident at 1 min, and by 15 min all values had returned to control after either injection. Thus, the injection of either saline or contrast causes an immediate alteration in function mediated by the Frank-Starling effect. This is followed by a very transient negative inotropic effect after contrast injection that is not seen after saline.

220. Induced Human Cholera. STANLEY I. MUSIC,* RICHARD P. WENZEL,* JOSEPH P. LIBONATI,* MERRILL J. SNYDER,* RICHARD B. HORNICK, AND THEODORE E. WOODWARD,** Baltimore, Md.

Asiatic cholera continues to ravage endemic areas of the world and recently has spread beyond these boundaries to pose international control problems. Knowledge of the immunology and pathophysiology of cholera has been hampered by the lack of a suitable animal model. Studies by others in dogs showed that large numbers of unwashed organisms, large volumes of liquid culture media, and either $NaHCO_3$ or direct duodenal challenge by intubation were required to induce cholera in this animal. This high innate resistance makes the assessment of vaccines difficult. Human epidemiological studies suggest only short-term protection by current vaccines, but they raise questions about vaccine type, what strains should be included, and whether antitoxic antibodies are associated with immunity. We have endeavored to produce a human model to answer these important questions. 35 well informed volunteer inmates from the Maryland House of Correction were challenged with a virulent, toxin-producing *Vibrio cholerae,* classical biotype Inaba serotype, strain 569B. Volunteers were given 10^4 to 11^{11} washed organisms, with and without $NaHCO_3$. Human cholera infection was induced with a wide spectrum of presentation, from patients with vibrio-positive stools without diarrhea to those with severe cholera diarrhea requiring intravenous fluid replacement for several days. 10^{11} organisms was required to induce diarrheal disease. If the challenge was preceded by $NaHCO_3$ (2 grams), diarrhea could be produced by 10^6 organisms. Induced disease mimicked nat-

urally acquired human cholera. Anticholera antibody production was recorded. An attempt to correlate susceptibility to cholera with gastric acid production was made. (This work was supported by a grant from the National Institute of Allergy and Infectious Diseases.)

221. Mechanism of Formation of the Hemoglobin-Haptoglobin Complex. RONALD L. NAGEL* AND QUENTIN H. GIBSON,* New York and Ithaca, N. Y. (introduced by Helen M. Ranney**).

The binding of haptoglobin (Hp) and hemoglobin (Hb) is believed to be stoichiometric and effectively irreversible. Utilizing the quenching of the 250 mμ fluorescence emission of Hp by Hb, previous kinetic experiments of Nagel and Gibson suggested that the reaction between Hb and Hp involves hemoglobin subunits. As more quantitative data on the dissociation of Hb have become available, we have utilized the fact that deoxyhemoglobin does not bind Hb to undertake a detailed analysis of the Hb-Hp complex formation. The dissociation of liganded hemoglobin into subunits can be made, under appropriate conditions, comparatively slower than the rate of binding of CO by deoxyhemoglobin. Carbon monoxide Hb, which is initially almost entirely in the tetrameric form, was prepared in the stopped-flow apparatus by mixing deoxyhemoglobin with CO in high concentrations. When this reaction was performed in the presence of Hp, a marked lag in Hb binding by Hp suggested that tetrameric Hb did not react with Hp. The results were satisfactorily fitted, utilizing hybrid computation, by a model in which only the Hb dimer binds Hp. Dissociation constants of 1.3×10^{-6} M for the hemoglobin tetramer, 2 sec^{-1} for the dissociation rate constant, and 5.4×10^{5} M^{-1} sec^{-1} for the haptoglobin dimer reaction were the optimum values obtained for the experimental data. We therefore conclude that the hemoglobin-haptoglobin reaction involves the previous dimerization of the liganded hemoglobin and a consequent combination rate of about 5.5×10^{5} M^{-1} sec^{-1} between the liganded dimer and haptoglobin. These data provide independent support for a dissociation constant of about 1.5×10^{-6} M for the dimerization of the liganded tetramer. (Research supported by grants from the NIH and the United States Army Research Office.)

222. A Mechanism Regulating Intracellular Cation Content during Contraction Alkalosis. MARK A. NEEDLE* AND ARTHUR G. GOLDMAN,* New York, N. Y. (introduced by Louis Leiter**).

During production of gastric alkalosis in dogs, 73 mEq of intracellular (ICF) K^+ and 23 mEq of unmeasured cations ("H^+") exchanged for 96 mEq of extracellular Na^+. Correction of K^+ deficit by rapid infusion of $KHCO_3$ was accompanied by calculated K^+-"H^+" exchange, 107 mEq of infused K^+ for 107 mEq of ICF "H^+." Correction of K^+ deficit with KCl was accompanied by K^+-Na^+ exchange, 101 mEq of infused K^+ for 90 mEq of ICF Na^+. The shifts of Na^+ ($P < 0.002$) and "H^+" ($P < 0.007$) were significantly different as between the $KHCO_3$ and KCl groups. Over 12–16 hr after the infusion, 52 mEq of K^+ left and 75 mEq of "H^+" reentered ICF in the $KHCO_3$ dogs, while only 21 mEq of K^+ left and 20 mEq of "H^+" entered ICF in the KCl group. The shifts of K^+ ($P < 0.002$) and "H^+" ($P < 0.03$ were significantly different as between the two groups; the ICF K^+ deficit of contraction alkalosis could not be replenished without providing Cl^-. Cellular K^+ replenishment requires normalization of serum Cl^- concentration and extrusion of Na^+ from cells. In vitro, activity of Na^+-K^+ ATPase, which may provide energy from ATP for cation transport across cell membranes, is sensitive to changes in pH. If the pumps for Na^+ extrusion and K^+ inclusion are linked metabolically, ICF Na^+ and K^+ content in contraction alkalosis may vary with serum Cl^-/HCO_3^- ratio. We propose that the titer of Cl^- bathing the cells influences the transmembrane potential and ATP degradation by Na^+-K^+ ATPase, thus governing ICF cation content. (This study was supported in part by grant 5-RO1-AM-09086 from the National Institute of Arthritis and Metabolic Diseases, NIH.)

223. Male Pseudohermaphroditism Due to 17-Hydroxylase Deficiency. MARIA I. NEW* AND RALPH E. PETERSON,** New York, N. Y.

This is the first report of a male with 17-hydroxylase deficiency resulting in male pseudohermaphroditism, ambiguous external genitalia, absence of male secondary sexual characteristics, and gynecomastia at puberty. Diagnosis was based on extensive studies of steroid metabolism, including: low urinary excretion of 17-ketosteroids and 17-hydroxycorticoids which did not increase after ACTH; no response of very low plasma testosterone and dehydroepiandrosterone to ACTH or chorionic gonadotrophin; low urinary aldosterone and plasma renin which increased after dexamethasone. Secretion rates of 17-hydroxylated steroids, cortisol (F), and 11-deoxycortisol (S) were very low, whereas deoxycorticosterone (DOC) and corticosterone (B) secretion rates were increased 7-fold. Results expressed as mg/m^2 per day were as follows: F, 1.3; S, 0.023; DOC, 0.35, B, 16 (mean normal values were: F, 7.5; S, 0.26; DOC, 0.055; B, 2.2). Plasma gonadotrophins were markedly increased (FSH 106, LH 364 mIU/ml). Testicular biopsies revealed interstitial cell hyperplasia and early spermatogenesis. The karyotype was 46/XY. The pedigree showed no other affected members. At laparotomy, ovaries, uterus, and fallopian tubes were absent, vas deferens was incomplete, prostate was present. External genitalia consisted of small phallus, bifid scrotum, third-degree hypospadias, and small vagina. At puberty there was no growth of body hair or phallic enlargement. Biopsy of marked gynecomastia showed both ducts and acini. Testosterone administration produced virilization. Sexual ambiguity demonstrates strong dependence of external genitalia on androgens for male differentiation. Suppression of müllerian structures occurred despite female levels of testosterone, indicating that this step in male differentiation is not testosterone dependent. Pubertal breast development in this male supports the concept of femaleness during ontogeny unless counteracted by male hormone. Diagnosis of other adrenocortical enzymatic deficiencies is excluded by the steroidal studies. The clinical response to testosterone excludes testicular feminization. Deficiency of 17-hydroxylation must be added to the causes of male pseudo-

hermaphroditism. (Support: NIH, HD-72, HE-12239, FR-47; contract I-481 with the Health Research Council of the City of New York; and American Heart Association 69-686).

224. Defective Cellular Immunity in Uremia: Depression of Reactivity of Lymphocytes to Phytohemagglutinin by Uremic Sera. W. M. NEWBERRY* AND J. P. SANFORD, Dallas, Texas.

The prolonged survival of allografts in uremia suggests a defect in cellular immunity, yet studies confirming and defining the defect have yielded variable results. These studies were designed to confirm a cellular defect and, if this is demonstrable, to define whether it is associated with a factor(s) in uremic serum. Lymphocyte function was assessed by in vitro uptake of thymidine (^{3}H-T) by normal lymphocytes stimulated with various concentrations of phytohemagglutinin (PHA). Cells were processed from healthy donors and uremic serum was compared simultaneously with normal serum from a third donor. Sera from each of six uremic patients exhibited 9–94% depression of normal lymphocyte response. Sera from two patients demonstrated profound suppression before dialysis. Immediate postdialysis samples allowed 1.5–4 times greater ^{3}H-T incorporation, which was still subnormal. In one patient with acute renal failure, depression by serum obtained while uremic was lost after recovery 3 wk later. Temporal relations were further defined by the following: 24 hr preincubation of normal cells in normal and uremic sera, followed by PHA stimulation in normal serum, revealed "normal" ^{3}H-T uptake. Initiation of PHA stimulation in normal serum, followed by change after 1, 6, and 24 hr to uremic serum which contained no PHA, resulted in depression of the cellular response, with 24 hr < 6 hr < 1 hr. These studies indicate a defect in cellular immunity due to the presence of a dialyzable factor(s) in uremic serum which inhibits the ability of peripheral blood lymphocytes to respond to a mitogenic stimulus. Inhibition is not due to cell lysis, and requires the continued presence of the serum factor. Cells in the active state of transformation are less susceptible to inhibition than cells which are stimulated from a resting state. (Research supported by NIH grants RO1-HD-00851 and TO1-AI-00030.)

225. Control Mechanisms for Puberty. W. D. ODELL, C. A. KIDDY,* AND M. A. HESCOX,* Torrance, Calif., and Beltsville, Md.

Studies in our laboratory and others have led to the following information on gonadal control prior to puberty in children: (1) Luteinizing hormone (LH) is secreted in all children. (2) LH ratios of normal girls:agonadal girls:normal women:castrate women are 1:2:5:20. (3) At puberty, agonadal girls increase to adult castrate levels. We have extended our studies of puberty into other species and report the following studies of LH in cattle (cattle are sexually mature at 12–13 months): (1) In females, mean LH at ½–3 months of age was 1.88 ± 0.36 (SE) mμg/ml; 3½–6 months, 2.36 ± 0.73; 6½–9 months, 3.02 ± 0.80; and $> 13\frac{1}{2}$ months, 1.52 ± 0.11. (2) In males, levels at ½–6 months were 2.86 ± 0.65; at $> 13\frac{1}{2}$ months, 2.60 ± 0.82. (3) Castration of females at 1–5 months increased LH from 2.11 ± 0.35 to 12.34 ± 4.74. (4) Castration of males at <6 months increased LH from 2.86 ± 0.62 to 15.82 ± 5.62. (5) Castration of adult males increased LH to 8.36 ± 1.25. (6) Serial weekly measurements of LH for over a year in animals castrated at <6 months failed to reveal any secondary rise at 12–13 months as would be expected in humans. We conclude that (1) pubertal control mechanisms are different in cattle and in humans, (2) in cattle, prepubertal LH levels are not lower than adult, and (3) castration results in similar changes in prepubertal and adult cattle.

226. Micropuncture Study of Proximal Tubule Albumin Concentration in the Rat. DONALD E. OKEN AND WALTER FLAMENBAUM,* Boston, Mass.

The concentration of albumin in proximal tubule fluid (PTF) of rat kidney was assessed with an ultramicro modification of disc gel electrophoresis. This technique permits the measurement of picogram amounts of albumin and concentrations below 0.1 mg/100 ml. Because of possible contamination with serum, technical aspects of tubule fluid collection are of paramount importance. Collections must be made in the shortest time feasible and performed with extremely sharp pipettes carefully inserted with minimal trauma into the middle of the tubule lumen. In an initial series of 19 collections in six rats, the PTF albumin concentration was 8.3 ± 2.4 (SE) mg/100 ml, a value comparable to those reported recently in other micropuncture studies. With fastidious regard to avoiding contamination, the mean values in 65 samples obtained from 20 female rats and 23 samples from eight male rats were 1.2 ± 0.2 (SE) and 0.7 ± 0.2 mg/100 ml respectively (range, < 0.1–6.8 mg/100 ml). The albumin concentration of 62.5% of these 88 samples was 1.0 mg/100 ml or less, the occasional high values raising the mean value appreciably. It seems likely that the higher concentrations found were the result of inapparent contamination with trace amounts of serum protein. We conclude that normal glomerular capillaries are far less permeable to albumin than was previously believed. The scant amount of protein in proximal tubule fluid is grossly insufficient to account for the degree of proteinuria which occurs in the nephrotic syndrome. (Supported by USPHS grant 10919.)

227. Isolation of a New Progesterone "Receptor" from Oviduct Target Tissue. BERT W. O'MALLEY* AND MERRY M. RUBIN,* Nashville, Tenn., and Bethesda, Md. (introduced by C. R. Park**).

Administration of progesterone (P) to estrogen-treated (E) chicks induces synthesis (in vivo or in vitro) of the specific oviductal protein, avidin. We have previously demonstrated that P exerts specific effects on nuclear RNA synthesis, but the initial interaction of hormone with target tissue was not defined and is the subject of this study. After injection of ^{3}H-P into chicks, Sephadex G-200 chromatography revealed the radioactivity to be bound primarily to a macromolecular component distinguishable from the bulk of oviductal protein. P was incubated (3°C) in vitro with oviduct cytosol (105,000 *g* supernatant). This

steroid was bound to macromolecular components of the cytosol by standard sucrose gradient methods and was distinguishable from plasma transcortin and albumin by newly developed acrylamide electrophoresis and agarose gel filtration methods. Sucrose gradient centrifugation of cytosol incubated with ^{3}H-P showed two peaks of radioactivity which became homogeneous ($S_{20,w}$ 3.7) in 0.3 M KCl. Extraction and rechromatography of the bound ^{3}H revealed > 93% as unmetabolized P. P binding (0.3 M KCl) to oviduct cytosol has the following properties: (1) tissue specificity; (2) dissociation constant of $\sim 8 \times 10^{-10}$ M; (3) heat lability; (4) destruction by proteases but not nucleases; (5) requirement for intact —SH (destroyed by PCMB); (6) major competitive displacement by other biologically active progestational steroids but not by estrogens or glucocorticoids; (7) Stokes radii of 55–63 A for binding protein. Finally, pretreatment with E (a steroid which enhances P induction of avidin) stimulates the synthesis of P-binding protein. In conclusion: (1) this study reports the first progesterone-binding macromolecule isolated from a target organ which cannot be detected in plasma; (2) this "receptor" may function to mediate the induction of avidin synthesis by P in the chick oviduct; (3) the isolation of a P receptor together with previous documentation of a cell receptor for E (and recently, androgens) now allows us to develop a general scheme for the sequence of molecular events in steroid hormone action. (Supported by NIH grant HD-04473-01.)

228. Amino Acid Regulation of Messenger RNA Synthesis Studied in the Isolated Perfused Rabbit Liver. MURRAY ORATZ,* SIDNEY S. SCHREIBER,* AND MARCUS A. ROTHSCHILD, New York, N. Y.

Isolated livers from fasted rabbits, perfused with plasma levels of amino acids, synthesize one-half as much albumin as do livers from fed rabbits. Supplementation of the perfusate with excess tryptophan or isoleucine increased albumin synthesis 175% and 89% respectively, and was associated with an increase in heavy polysomal aggregates. To determine whether this reaggregation was with preformed messenger RNA (mRNA) or also with stimulated newly synthesized mRNA, livers were perfused with ^{3}H-uridine. Polysomes associated with the endoplasmic reticulum (synthesizing albumin) and free polysomes were isolated and analyzed by sucrose gradient centrifugation. Radioactivity paralleled polysomal profiles except in the region of free ribosomes, where there was relatively less radioactivity than in the region of heavier aggregates. RNA from the free and bound polysomes was isolated and fractionated by sucrose gradient centrifugation. Radioactivity coincided with the optical density. There was also an area of high specific activity in a region of low optical density between 4S and 18S. Pretreatment of polysomes with RNase eliminated this region. The fraction sedimenting at 14S was taken to represent mRNA, and the incorporation of ^{3}H-uridine into this fraction was compared with the 28S RNA to correct for differences in the uridine pools. Feeding, tryptophan, or isoleucine supplementation increased uridine incorporation in endoplasmic bound mRNA by 113, 127, and 83%, respectively, as compared with the fasting state. On the contrary, free mRNA was decreased 53, 64, and 38% respectively. The data suggest that fasting stimulates synthesis of mRNA for intracellular proteins or enzymes necessary to maintain amino acid pools, while feeding or the addition of tryptophan and isoleucine stimulates synthesis of mRNA coded for albumin. (Supported in part by NIH grant AM-02489.)

229. Comparative Studies on Normal and Mutant Isozymes of Erythrocyte Pyruvate Kinase. DONALD E. PAGLIA,* WILLIAM N. VALENTINE,** AND KENNETH O. WILLIAMS,* Los Angeles, Calif.

An abnormal isozyme of erythrocyte pyruvate kinase, previously demonstrated in two kindreds afflicted with hereditary hemolytic anemia, has been found in erythrocytes of a third family. The mutant form (designated PK_2) was catalytically ineffective at very low concentrations of its substrate, phosphoenolpyruvate (PEP). The PK_2 Michaelis constant (K_m) for PEP was almost tenfold greater than that of the wild enzyme (designated PK_1), but the maximum activity obtainable in vitro under optimal conditions (V_{max}) was only slightly less than normal. PK_2 in crude hemolysates displayed allosteric kinetics when PEP was the variable substrate, whereas kinetics of PK_1 was hyperbolic under the same assay conditions. Minute concentrations of fructose diphosphate (FDP) converted the sigmoidal kinetic curve of PK_2 to a rectangular hyperbola, thus correcting the K_m (PEP) to normal levels. In the presence of 0.1 mM FDP, the kinetic curve of PK_1 remained hyperbolic but shifted, reducing the K_m (PEP) by 50%. V_{max} of neither isozyme was significantly affected by FDP at this concentration. Electrophoretic patterns of the two isozymes differed in starch gel with succinate buffer at pH 5.0. Cathodal migration of the fast band of either PK_1 or PK_2 was reduced almost to the velocity of the slow bands when the medium also contained 0.1 mM FDP. It is hypothesized that a genetically determined molecular disconformation of PK_2 relative to PK_1 reduces its affinity for PEP, and that the presumed disconformation is largely corrected by small amounts of the activator, FDP, thereby affecting both its K_m (PEP) and its electrophoretic migration. (These studies were supported by grants from the Veterans Administration, the USPHS [HE-1069], and the Leukemia Research Foundation of Los Angeles.)

230. Hypobetalipoproteinemia in Childhood: Evidence for Two Types by Histological, Ultrastructural, and Immunological Analysis. JOHN C. PARTIN* AND WILLIAM K. SCHUBERT,* Cincinnati, Ohio (introduced by A. Ashley Weech**).

A girl (S.E.) 14 months and a boy (J.R.) 9 yr presented with growth retardation and steatorrhea. Acanthocytosis was absent. Neurological examination was normal. Reduced low density lipoproteins and absent very low density lipoproteins were demonstrated by agarose electrophoresis. β-lipoprotein was reduced by immunoelectrophoresis. Goat anti-human β-lipoprotein antiserum demonstrated four components (numbered from the antigen well) in normal serum. In S.E., component 4 was absent, 1 and 2 reduced; in J.R., component 1 was absent, 3 and 4 reduced. Jejunal biopsy from S.E.

showed snowy white epithelial cells; histochemically, triglyceride (TG) was stored in the epithelium but not in the villous core. Electron microscope examination demonstrated TG storage in epithelial cells but not in the lateral epithelial space (LES) or the lamina propria. After fasting, TG was reduced and electron microscope observation showed that TG was originating in dilated portions of endoplasmic reticulum. Fusion of small Golgi-like vesicles with endoplasmic reticulum containing TG was evident. In contrast, the jejunal biopsy of J.R. revealed normally translucent epithelial cells. Manipulation of the biopsy caused it to shed chyle into the suspending formalin. TG was present in the LES and villous core. Electron microscope examination showed two classes of lipid particles: small, electron-opaque, uniform-sized particles (approximately 600 angstroms) and variable-sized, electron-transparent particles more than 1000 angstroms in diameter, resembling chylomicrons. Tissue lipid composition was, for S.E. and J.R., respectively: tissue wet wt (mg/100 ml), 12.04, 12.49; total lipid (% of wet wt), 8.3, 6.7; cholesterol (% of total lipid), 3.1, 4.3; ester (% of total lipid), 2.4, 2.4; TG (% of total lipid), 70, 55.5; TG-fatty acids (%), *14:0*, 1.2, 1.2; *16:0*, 24.9, 20.7; *16:1*, 3.6, 7.0; *18:0*, 4.7, 8.9; *18:1*, 27.5, 25.3; *18:2*, 27.2, 4.8. The findings demonstrate two histological types of hypobetalipoproteinemia, each associated with deficiency of an antigenically different β-lipoprotein: one with impaired excretion of TG from the epithelial cell, and one with impaired clearance of TG from the villous core.

231. Immunoglobulin E–Mediated Respiratory Responses in Rhesus Monkeys. Roy Patterson, Catharine Talbot,* and Bernard Booth,* Chicago, Ill.

Rhesus recipients accept systemic passive sensitization with human IgE reaginic antibody and demonstrate an acute response after antigenic challenge to the respiratory tract. Rhesus IgE has been demonstrated by the cross-reactivity of heterologous anti–human IgE with rhesus IgE. Reaginic antibody of the IgE class against *Ascaris* antigen has been demonstrated in certain monkeys. In the current studies, rhesus monkeys were evaluated for their degree of immediate-type reactivity to graded cutaneous challenge with porcine *Ascaris* antigen purified by dextran gel chromatography. Three groups of two animals each were selected: highly sensitive (HS), moderately sensitive (MS), and slightly sensitive (SS). These reacted to dilutions of 10^{-6}, 10^{-3}, and 10^{-1} of a standardized solution of *Ascaris* antigen (SSA), respectively. Respiratory challenge of these groups was accomplished by delivery of constant amounts of a standard solution of *Ascaris antigen* (SSA) in separate experiments. SS animals did not react to repeated respiratory challenge. MS animals reacted initially, but the reactivity disappeared after several challenges without a decrease in skin titer. HS animals have responded consistently over a 14 month period with a reproducible respiratory response demonstrated by measurement of change in respiratory flow rates, volumes, breathing frequencies, and inspiratory-expiratory time ratios. The reproducibility of the respiratory response patterns in HS animals with controlled challenge permitted evaluation of the effect of various agents. Diphenhydramine altered but did not eliminate the response. Isoproterenol partially reversed the acute response. Propranolol did not potentiate the respiratory response to SSA nor alter the rate of recovery after onset of the acute phase. Respiratory responsiveness of the groups of animals to methacholine indicated that in HS animals, the degree of methacholine reactivity paralleled the respiratory response to SSA.

232. Adenyl Cyclase Activity of Guinea Pig Gastric Mucosa: Stimulation by Histamine and Prostaglandins. Claude V. Perrier* and Leonard Laster, Bethesda, Md.

Others have implicated cyclic adenosine-3′,5′-monophosphate in gastric acid secretion. To explore this, we determined the presence, localization, and properties of adenyl cyclase in mammalian stomach preparations. We detected activity in guinea pig, hamster, rat, rabbit, cat, monkey, and man. Detailed studies on guinea pig revealed cyclase in mucosa and muscularis of antrum and fundus. Relation of activity to pH, Mg^{++}/ATP ratio, and concentration of substrate or fluoride was studied. Activity required Mg^{++}, and was enhanced by K^{+}, inhibited by Ca^{++}, and unaffected by Na^{+}. Hormonal and pharmacological agents active either on other cyclase systems or on gastric function were tested in vitro for effects on gastric cyclase. Histamine 10^{-4} M and its analogues betazole and aminoethyltriazole (both gastric secretagogues) consistently stimulated parietal mucosa cyclase 3- to 4-fold; choline esters and gastrin had no effect. The antihistaminic chlorpheniramine reduced histamine stimulation. Cyclase from other guinea pig organs (liver, pancreas, duodenum, gall bladder, heart, and skeletal muscle) was unaffected by histamine. Prostaglandin E_1, which stimulates cyclase in several tissues, and some other prostaglandins were nearly as active as histamine on gastric cyclase. Direct assay excluded the possibility that histamine and prostaglandin E_1 act by inhibiting cyclic nucleotide phosphodiesterase. The effects of histamine, histamine analogue, and an antihistaminic on cyclase activity of a stomach preparation unresponsive to choline esters and gastrin might support the theory that histamine is, or is related to, the final stimulus for gastric acid secretion. The relevance of stimulation by prostaglandins is not clear, since some prostaglandins inhibit gastric secretion in vivo. Several features of the observed gastric cyclase responses to prostaglandins and histamine suggest that these compounds may act on different elements of the parietal mucosa.

233. Insulin Stimulation of Amino Acid Transport in Bone: Dependence on Protein Synthesis and Sodium-Potassium ATPase. James M. Phang* and Theodore J. Hahn,* Bethesda, Md. (introduced by Nathaniel I. Berlin**).

We recently reported that insulin stimulates amino acid transport and collagen synthesis in fetal membranous bone in vitro. Our finding that amino acid transport was stimulated by insulin at physiologic concentrations suggests that this hormone plays a role in regulating bone matrix formation. We now report additional studies on the mechanism of the insulin effect. In fetal rat calvaria, insulin stimulation of α-aminoisobutyric acid (AIB) transport is mediated by pro-

tein synthesis. In 30 min uptake studies, the magnitude of stimulation was proportional to the duration of insulin exposure. Analysis of transport kinetics for AIB showed that insulin increased transport V_{max} but did not change K_m or K_D. Furthermore, the presence of puromycin abolished the insulin effect. Insulin stimulation of AIB transport did not require medium glucose, nor was it affected by the presence of glucose. Insulin had no effect on uptake of 3-*O* methylglucose, but it did increase glucose oxidation to CO_2. By using inhibitors of glucose intermediary metabolism, we showed that the insulin effect on amino acid transport was independent of increased glucose utilization. AIB transport in bone is mediated by Na^+-sensitive as well as Na^+-insensitive mechanisms. When Na^+ was replaced by $Tris^+$ in the medium, active transport of AIB persisted, but with decreased V_{max}. Insulin, however, had no effect on V_{max} or K_m in the absence of Na^+. Ouabain, an inhibitor of Na^+-K^+ ATPase, also abolished manifestation of the insulin effect. We conclude that insulin stimulation of amino acid transport in bone is mediated by increased synthesis either of a membrane carrier protein dependent on Na^+-K^+ ATPase activity or of the membrane-bound Na^+-K^+ ATPase itself.

234. Cytogenetic Studies in Myelomonocytic Leukemia and Its Preleukemic Phase. ROBERT V. PIERRE,* H. CLARK HOAGLAND,* AND JAMES W. LINMAN, Rochester, Minn.

Myelomonocytic leukemia is our preferred designation for that type of leukemia which involves more than one marrow cell line either simultaneously or sequentially. In our experience this disorder is often preceded by a preleukemic phase characterized by a refractory anemia or other peripheral blood cytopenia(s) and/or quantitative and qualitative marrow abnormalities such as hypercellularity, maturation defects, and megaloblastoid morphology. Cytogenetic studies (direct bone marrow preparations) have been obtained in 30 patients with overt myelomonocytic leukemia, in 61 patients with peripheral blood and bone marrow findings suggesting preleukemia, and in 26 control subjects with nonleukemic disorders. In each analysis at least 25 consecutive metaphases of evaluable quality were karyotyped according to the Denver classification. 19 of the 30 patients with overt leukemia had significant abnormalities (7 were hypodiploid, 8 were hyperdiploid, and 4 had other changes). Chromosome studies were abnormal in 32 of the 61 patients with suspected preleukemia; there were clones of cytogenetically abnormal marrow cells in 17 of these 32 cases. In addition to the direct marrow method, chromosome analyses of 72 hr peripheral leukocyte cultures were carried out in 3 of these 17 patients and failed to reveal a clonal abnormality. To date, 5 patients with preleukemia have evolved into overt leukemia without changes in their marrow karyotype. These studies suggest that chromosome analyses may be of value in establishing the preleukemic nature of certain ill defined hematologic disorders such as "refractory anemia." Positive identification of the preleukemic phase of leukemia will permit the development of new investigations into the etiology and therapy of this myeloproliferative disorder.

235. Inducible Heme Oxygenase in the Kidney: A Model for the Homeostatic Control of Hemoglobin Catabolism. NEVILLE R. PIMSTONE,* PETER ENGEL,* PAUL T. SEITZ,* HARVEY S. MARVER, AND RUDI SCHMID,** San Francisco, Calif.

We have recently described NADPH-dependent microsomal heme oxygenase, the major enzymatic mechanism for the conversion of hemoglobin-heme to bilirubin IXα. Specific enzyme activity normally is highest in tissues involved in red cell breakdown, i.e. spleen, liver, and bone marrow, but is negligible in the kidney. After splenectomy and in experimental hemolysis, hepatic heme oxygenase is increased. Similarly, enzyme activity is enhanced in macrophages exposed to heme pigments, e.g. in hematomas and in hemoperitoneum. The following observations indicate that this functional adaptation reflects induction of heme oxygenase by the substrate, hemoglobin. Hemoglobinemia exceeding the plasma haptoglobin-binding capacity and resulting in hemoglobinuria stimulates renal heme oxygenase activity 30- to 100-fold. Maximal stimulation in rats is reached 9 to 12 hr after a single intravenous injection of 30 mg of hemoglobin per 100 g body weight; activity returns to basal levels at 48 hr. At peak levels, total renal enzyme activity exceeds that of the spleen or liver. The apparent $t_{\frac{1}{2}}$ of the renal enzyme is less than 24 hr. Prevention of the stimulatory effect of hemoglobin by cycloheximide, puromycin, or actinomycin D demonstrates that the increase in enzyme activity is dependent on new synthesis of RNA and protein. Filtered rather than plasma hemoglobin appears to regulate renal heme oxygenase activity. Thus stabilization of plasma hemoglobin in its tetrameric form with *bis*(*N*-maleimidomethyl)ether diminishes its glomerular filtration and retards its plasma clearance; concomitantly, enzyme induction in the kidney is attenuated but is enhanced in the liver. This indicates that in hemoglobinuria, induction of renal heme oxygenase serves to enhance catabolism of hemoglobin that has been filtered and reabsorbed by the renal parenchyma. These observations exemplify the adaptive capacity of the organism for maintaining homeostasis in hemolytic states. (Research supported by grants from the NIH.)

236. Activation of Blood Coagulation by Epithelial Glycoprotein. GRAHAM F. PINEO* AND ERWIN L. REGOECZI,* London, England, and Hamilton, Ontario, Canada (introduced by J. Fraser Mustard).

Intravascular coagulation (thrombosis or defibrination) is frequently associated with mucus-producing adenocarcinomas. Our studies have been carried out to test the hypothesis that mucus entering the circulation activates coagulation. Extracts of human mucus and of mucus-producing adenocarcinoma, after partial purification by column chromatography, had thromboplastic activity. This activity was stable to wide changes in pH and temperature, and was not affected by 20% trichloroacetic acid. The active material had a very high carbohydrate-protein ratio, blood group specificity, and electrophoretic mobility and staining properties of a sulfated glycoprotein. In a two-stage factor X activation test, the glycoprotein activated factor X in the presence of factor VII and Ca^{++}, and had a potency equivalent to 1:20,000 Russell's

viper venom. The glycoprotein contained only trace amounts of phospholipid, and the addition of phospholipid was not necessary for factor X activation. The glycoprotein shortened the coagulation time of plasmas deficient in factor XII, XI, IX, or VIII. There was no effect on other coagulation factors or on platelets. The intravenous infusion of the glycoprotein into rabbits resulted in a moderate fall in platelet count and plasma fibrinogen levels, and an increase in the thrombin-fibrinogen time. There was a fall in the intravascular pool of ^{125}I-fibrinogen after the infusion, which persisted for several hours, and an increase in nonclottable ^{125}I-labeled protein, indicative of the formation of fibrin degradation products. Glycoprotein inactivated by pepsin had no comparable effect. These findings suggest that epithelial glycoprotein on entering the circulation can activate coagulation and may explain the frequency of coagulation disorders in patients with adenocarcinoma.

237. A Method for Distinguishing the Breakdown Products of Skin Collagen from Those of Bone Collagen in Man. SHELDON PINNELL,* ROBERT FOX,* AND STEPHEN KRANE, Boston, Mass.

Urinary hydroxyproline has been used as an index of collagen turnover although it has not been possible to distinguish whether the collagen comes from bone or skin. Hydroxylysine, another amino acid unique to collagen, serves as a convenient, specific marker for this protein. In states of acclerated collagen turnover, total hydroxylysine urinary excretion is correspondingly increased. Certain hydroxylysine residues in collagen are linked to galactose and glucose as glucosylgalactosylhydroxylysine (GGH) and galactosylhydroxylysine (GH). GGH, GH, and free hydroxylysine were determined in human tissues and urine in an attempt to determine the extent of collagen breakdown and distinguish its source. GGH, GH, and hydroxylysine were measured on an amino acid analyzer after alkaline hydrolysis and gel filtration on Bio-Gel P-2. GGH/GH ratios were determined for matched sets of human dermis and bone collagen. The mean GGH/GH for dermis, from six patients, of 1.6 (range 1.3–2.0) differed markedly from that for bone, 0.40 (range 0.33–0.50). GGH plus GH represented one-third of the total hydroxylysine content. A patient with extensive cutaneous burns who might be expected to turn over large amounts of skin collagen showed a GGH/GH in urine of 1.3. In contrast, a patient with extensive Paget's disease and high turnover of bone and markedly increased total urinary hydroxylysine showed urinary GGH/GH of 0.38; a patient with extensive osteitis fibrosa of hyperparathyroidism showed urinary GGH/GH of 0.45. Measurement of urinary hydroxylysine and its glycosides GGH and GH may serve to distinguish the source of collagen breakdown products in urine. (Supported by NIH grants.)

238. The Rate of Extrathyroidal Conversion of Thyroxine to Triiodothyronine in Man. CONSTANCE S. PITTMAN,* JOSEPH B. CHAMBERS, JR.,* AND VIRGINIA H. READ,* Birmingham, Ala. (introduced by Thomas N. James).

Extrathyroidal conversion of thyroxine (T_4) to triiodothyronine (T_3) was demonstrated by recent studies in both athyreotic patients (by Braverman and associates) and normal man (by Pittman and associates). The rate of this conversion was measured in the present study. Two normal subjects were given daily intravenous injections of a thyroxine labeled with ^{14}C in the nonphenolic ring and side chain for 10 days (1.4 μc or 8.1 μg per day). 7 days after the last injection the subjects were given one intravenous injection of a second thyroxine labeled with ^{3}H in the alanine side chain (15 μc or 97.4 μg). Sera were obtained at 12 hr intervals during the following 3 days, and T_4, T_3, and tetraiodothyroacetic acid were fractionated by chromatography. The ^{3}H activity in the T_3 fraction obtained at 10 min after the ^{3}H-T_4 injection was used for correction of chromatographic artifacts. The difference between the ^{14}C and ^{3}H activities in the T_3 fraction, or the T_3 actually converted from the parent hormone, was found to be 0.6, or 1% of the total serum radioactivity. The ^{3}H/^{14}C ratios of the T_3 fraction approximated unity during 36 to 72 hr (1.02–1.07), suggesting that the conversion reaction reached equilibrium 36 hr after an injection of T_4. It can be shown that $dT_3/dt = \lambda_2 T_4 - \lambda_1 T_3$ if $\lambda_1 = T_3$ fractional turnover rate and $\lambda_2 = T_4$ to T_3 conversion rate. At equilibrium, λ_2 was found to be 0.0051–0.0213. Therefore, 2.6–10.7% of the total body T_3 pool was produced by extrathyroidal conversion of T_4 to T_3. (Research supported by NIH grant AM-08181.)

239. Relation between Total Body Potassium and Growth Hormone and Insulin Response in Cirrhosis. STEPHEN PODOLSKY,* HYMAN J. ZIMMERMAN,** AND BELTON A. BURROWS,** Boston, Mass.

The relation of potassium depletion to carbohydrate intolerance, as determined by the response of plasma insulin and growth hormone to stimulation, has been studied in patients with cirrhosis. The effect of supplementation of potassium chloride on ^{40}K balance was also correlated with changes in glucose, arginine, and insulin tolerance tests. 18 normokalemic cirrhotics were found to have lower endogenous ^{40}K than subjects with similar body habitus without liver or kidney disease, which may reflect increased body water. The total body potassium of some cirrhotics was not increased by large doses of KCl (up to 200 mEq K per day), which may reflect reduction of lean body mass, as K replenishment should be possible in the presence of secondary hyperaldosteronism. 13 of 18 cirrhotic patients had a frankly impaired oral glucose tolerance test (GTT). These patients had modest elevation of fasting insulin level (15.7 ± 2.2 [SEM] μU/ml as compared with a normal mean value of 8.0 ± 1.3 μU/ml). Some of the patients had exaggerated release of insulin after glucose but reduced release after intravenous arginine. Fasting growth hormone level was elevated (11.9 ± 2.6 [SEM] mμg/ml) as compared with normal levels of below 5.0 mμg/ml. There was normal suppression of growth hormone after glucose, followed by marked rebound. Growth hormone response to arginine appeared to be less than that seen in normals, despite the high fasting levels. All five patients in whom efforts to replenish K stores were successful showed enhanced growth hormone response and insulin release; two patients showed improved or normal GTT. It would appear that potassium depletion in cirrhosis is associated with reduced output of both insulin and growth hor-

mone, in some cases with impaired carbohydrate tolerance. These changes are reversible by K replenishment.

240. Streptococcal Strains and Immune Responses Associated with Different Phases of Epidemic Acute Nephritis in Trinidad. ELIZABETH V. POTTER,* JESSE ORTIZ,* A. RICHEY SHARRETT,* JUANITA BRAY,* JOHN F. FINKLEA,* THEO POON-KING,* AND DAVID P. EARLE,** Chicago, Ill., and San Fernando, Trinidad, West Indies.

Bacteriological evidence is presented for an association of acute glomerulonephritis (AGN) with several streptococcal M types isolated from skin lesions in Trinidad: types 52 and 55 during the first wave and type 49 during the second wave of the 1964–1965 epidemic; atypical "type 2" in a subepidemic rise during the following endemic year; type 57 during an epidemic period in 1967; and type 60 thereafter. Type-specific antibody (TSA) responses to these strains in groups of patients from each period of observation confirm: association of type 55 with AGN in the 1965 epidemic and its disappearance thereafter; disappearance of type 12 strains during the study; and the newness of type 57 to the Trinidad population. The TSA studies further demonstrate responses in skin infections equal to or better than those following throat infections. Patients' immune responses to streptolysin O decreased during our observations, whereas antihyaluronidase responses remained high during the first 3 yr of the study, decreasing somewhat during the last 2 yr. Emergence of at least five types of apparently nephritogenic streptococci during this 5 yr period suggests that each recurrent epidemic wave of nephritis is the result of newly introduced or newly developed strains, and that skin infections may provide particularly suitable environment for development of new streptococcal antigens. (This work was supported by USPHS contract PH-108-66-217 and grant HE-07057, the American Heart Association, the Otho S. A. Sprague Foundation, and the Trinidad-Tobago Ministry of Health.)

241. Biochemical Effects of Zinc Deficiency. ANANDA S. PRASAD, DONALD OBERLEAS,* E. R. MILLER,* AND R. W. LUECKE,* Detroit and Lansing, Mich.

Zinc is known to be essential for function of several metalloenzymes, and its role in polymeric organization of RNA and DNA molecules has been postulated; however, conflicting results have been reported concerning changes occurring in activities of zinc-dependent enzymes and content of RNA and DNA in zinc-deficient tissues. The present studies were carried out in young pigs made zinc deficient by diet. Control pigs were pair-fed and received supplemental zinc (90 ppm). Pigs were sacrificed on the 30th day. Zinc content was measured by atomic absorption spectrophotometry. RNA and DNA content was determined by the Schmidt-Thannhauser method. Activities of the following zinc-dependent enzymes were determined in tissue homogenates under optimal conditions by standard methods and expressed per milligram of DNA: alcohol dehydrogenase (ADH), lactic dehydrogenase (LDH), and aldolase (ALD) in liver; ADH, ALD, and alkaline phosphatase (AP) in kidneys; carboxypeptidase (CPD) and LDH in pancreas; and ADH, AP, LDH, and ALD in bones. Succinic dehydrogenase (SDH), an iron-dependent enzyme, and isocitric dehydrogenase (ICDH), a magnesium-dependent enzyme, were also assayed in kidneys as controls. Zinc content and RNA/DNA ratios were significantly reduced ($P < 0.001$) in liver, kidneys, pancreas, and bone, as compared with their pair-fed controls, but DNA per mg wet weight remained unaltered. Only zinc-dependent enzymes revealed significantly reduced activities ($P < 0.001$) in zinc-deficient tissues, exceptions being LDH in liver and pancreas and ALD in bones. Addition of EDTA (1×10^{-4} M) to tissue homogenates significantly reduced activities of ADH, AP, and CPD, but not those of LDH and ALD, thus revealing differences in affinity of varous enzymes for zinc. Our studies demonstrated a decrease in RNA content and reduction in activities of various zinc-dependent enzymes in zinc-deficient tissues. These studies provide a basis for an understanding of the role of zinc in cellular functions.

242. The Effects of [Alpha Ketoglutarate] and Hydrogen Ion Concentration on Ammonia Production from Glutamate by Rat Kidney Slices. HARRY G. PREUSS* AND FREDERICK R. WEISS,* Pittsburgh, Pa. (introduced by H. V. Murdaugh).

In acidosis the accelerated removal of α-ketoglutarate through increased gluconeogenesis is a suggested way of increasing ammoniagenesis from glutamate through the glutamate dehydrogenase reaction. This theory implies that [α-ketoglutarate], at least in part, controls glutamate deamination. Incubation of rat kidney slices in 10 mM glutamate and 2 mM α-ketoglutarate decreased ammonia production as compared with incubation in 2 mM glutamate alone ($45 \rightarrow 11$ μmoles NH_3 per g per 90 min, $P < 0.001$). The addition of 1.0 mM arsenite (blocking α-ketoglutarate metabolism) to the medium returned ammonia production to 41 μmoles/g per 90 min. Gluconeogenesis decreased with arsenite ($32 \rightarrow 6$ μmoles/g per 90 min, $P < 0.01$). This suggests that it is the metabolism, not the [α-ketoglutarate], that affects ammoniagenesis. Observations of $\dot{Q}_{O_2}$ in increasing glutamate (10, 20, 50 mM) or α-ketoglutarate (2, 5, 10 mM) media showed no change ($5.0 \rightarrow 5.1$ μl/mg per hr). Similarly, combining 10 mM glutamate and 2 mM α-ketoglutarate failed to increase $\dot{Q}_{O_2}$ (5.2 μl/mg per hr). An average of 40 μmoles ammonia per g per 90 min was produced during these experiments despite increasing concentrations of glutamate. This apparent maximal $\dot{Q}_{O_2}$ and ammonia production indicates that glutamate and α-ketoglutarate oxidation involve a common rate-limiting factor. 2,4-Dinitrophenol (10^{-5} M) and methylene blue (5×10^{-4} M) in 10 mM glutamate medium increased slice $\dot{Q}_{O_2}$ 23% ($P < 0.01$) and 44% ($P < 0.01$), and slice ammonia production 59% ($P < 0.01$) and 60% ($P < 0.01$). That $\dot{Q}_{O_2}$ and ammonia production both increase in the presence of dinitrophenol and methylene blue suggests that ammoniagenesis is controlled by the availability of NAD^+. Further studies show that in glutamate 10 mM medium, pH 7.0, rat kidney slice $\dot{Q}_{O_2}$ averages 5.2 μl/mg per 90 min and ammonia production averages 72 μmoles/g per 90 min as compared with an average $\dot{Q}_{O_2}$ of 4.3 μl/mg per 90 min and ammonia production of 47 μmoles/g per 90 min at pH 7.7. It has been shown that [NAD^+] is increased in acidosis.

243. Mechanism of Action of Parathyroid Hormone on Phosphate Transport in the Dog Proximal Tubule. JULES B. PUSCHETT,* ZALMAN S. AGUS,* DOROTHY J. SENESKY,* AND MARTIN GOLDBERG, Philadelphia, Pa.

Parathyroid hormone (PTH) is phosphaturic and minimally natriuretic. Saline loading inhibits proximal fractional reabsorption (FR) of sodium and is also phosphaturic. To evaluate relations between sodium and phosphate and the role of cyclic AMP in PTH action, proximal tubular recollection micropuncture experiments were performed in 22 dogs. Dogs received either (1) nothing; (2) "saline" (25 ml/kg) containing calcium (5 mEq/liter); (3) highly purified PTH (60 U/hr); or (4) dibutyryl cyclic AMP (DB-cAMP) (100 mg/hr). In controls, mean ± SD tubular fluid/plasma (TF/P)inulin (In) was 1.41 ± 0.14, (TF/P) phosphate was 0.73 ± 0.22, (TF/P)phosphate/(TF/P)In was 0.58 ± 0.15. On re-collection, no changes occurred. (TF/P)phosphate did not vary with (TF/P)In. FR of phosphate approached 0.70–0.80 when FR of sodium approached 0.50. With "saline," (TF/P)In fell from 1.42 ± 0.16 to 1.23 ± 0.17 ($P < 0.001$); (TF/P)phosphate was unchanged. Proximal FR of phosphate fell, 0.47 to 0.34 ($P < 0.005$). Final urine FR of phosphate fell, 0.92 to 0.83, and sodium 0.992 to 0.983. After PTH, (TF/P)In fell, 1.40 ± 0.22 to 1.25 ± 0.15 ($P < 0.001$); (TF/P)phosphate did not change. Proximal FR of phosphate fell, 0.57 to 0.48 ($P < 0.02$). In whole kidney, GFR was unchanged; phosphate FR fell, 0.98 to 0.72, and sodium FR was unchanged. With DB-cAMP, (TF/P)In fell, 1.29 ± 0.16 to 1.18 ± 0.12 ($P < 0.05$); (TF/P)phosphate did not change. Proximal FR of phosphate fell, 0.41 to 0.28 ($P < 0.02$). Final urine FR of phosphate fell, 0.91 to 0.66. FR of sodium decreased slightly. GFR did not rise (35 → 31). In all groups (TF/P)phosphate/(TF/P)In never exceeded 1.0. Conclusions: (1) Phosphate is mainly reabsorbed proximally without evidence of net secretion; (2) proportional inhibition of proximal sodium and phosphate FR by "saline," PTH, and cyclic DB-cAMP suggests dependence of phosphate transport on sodium transport. Therefore, PTH-induced phosphaturia is probably mediated via cyclic AMP and inhibition of proximal sodium reabsorption.

244. Shape, Size, and Function of the Left Ventricle in Heart Disease. CHARLES E. RACKLEY,* MORRIS FRIMER,* C. MCGAVOCK PORTER,* AND HAROLD T. DODGE, Birmingham, Ala.

The relation between shape, size, and function of the left ventricle was investigated in 68 patients with various forms of heart disease. Biplane angiocardiography was performed at filming rates of 6 or 12 films per second, and chamber axes, wall thickness, volume ejection fraction (EF), and circumferential (S_1) and meridional (S_2) wall stress were determined. The principal internal radii of curvature (R_1 and R_2) were calculated at the equator of the ventricle, and by addition of wall thickness the principal radii (RO_1 and RO_2) for the external surface were derived. The ratio of the radii was taken as an index of sphericity of the left ventricle. In the 68 patients, end-diastolic volume (EDV) ranged from 85 to 649 ml and EF from 0.12 to 0.88. The diastolic ratio R_2/R_1 varied from 0.16 to 0.69 and the systolic ratio from 0.11 to 0.62. The ratio of external radii RO_2/RO_1 ranged from 0.22 to 0.72 in diastole and 0.20 to 0.67 in systole. EDV was not related to systolic R_2/R_1 ($r = 0.53$). Diastolic and systolic RO_2/RO_1 were compared with EF, with $r = 0.56$ and 0.65 respectively. The systolic ratio R_2/R_1 correlated with end-systolic volume ($r = 0.73$, $P < 0.01$) and EF ($r = 0.78$, $P < 0.01$), and was significantly related to S_1/S_2 ($r = 0.81$, $P < 0.01$). These data indicate that (1) the systolic shape of the left ventricle is related to the mechanical function, and (2) the mechanically failing ventricle alters its systolic shape from ellipsoid to spheroid to distribute the wall forces more uniformly. (NIH grants HE-11310 and MO1-FR-3209.)

245. The Krypton-85 Disappearance Rate as an Index of Capillary Blood Flow in Clubbed Fingers. STEFAN RACOCEANU,* NOSRAT NAFTCHI,* ABYSSINIA SUCK,* AND MILTON MENDLOWITZ,** New York, N. Y.

Previously, digital blood flow when determined calorimetrically after indirect heating had been found to be increased in acquired clubbing and normal in hereditary clubbing. Such flow, however, is largely through arteriovenous anastomoses. The only flow that is nutritional and hence presumably relevant to the genesis of clubbing is capillary flow, an estimate of which can be made from the krypton-85 (^{85}Kr) disappearance rate. Therefore, after heating with an electric blanket or cradle baker for 1 hr, 0.03 ml of an ^{85}Kr solution was injected subcutaneously into the pulp of the fingertip in seven control subjects, five patients with acquired clubbing, and two with hereditary clubbing. The subjects were all supine, and the disappearance rate of the radioactivity of the injectate was recorded with a collimated sodium iodide crystal scintillation detector probe at heart level beginning 4 to 5 min after injection. The half-time disappearance rate of the radioactivity ($t_{\frac{1}{2}}$) varied from 2 min 15 sec to 6 min 40 sec, with a mean of 4 min 26 sec, in normal subjects. In patients with acquired clubbing, the $t_{\frac{1}{2}}$ varied from 1 min 30 sec to 1 min 52 sec, with a mean of 1 min 41 sec. In the two patients with hereditary clubbing, the $t_{\frac{1}{2}}$ was 5 min and 5 min 10 sec, respectively. Since the major factor influencing the ^{85}Kr disappearance rate has been shown to be capillary blood flow, it is concluded that under these conditions, capillary blood flow is above normal in patients with acquired clubbing, and within normal limits in patients with hereditary clubbing. (Supported by NIH grant HE-05802.)

246. Intestinal Absorption of Polyaromatic Hydrocarbons. E. DOUGLAS REES* AND PAUL MANDELSTAM,* Lexington, Ky. (introduced by J. William Hollingsworth).

The polyaromatic hydrocarbons are strongly hydrophobic and lipophilic molecules; some (e.g. benzo[*a*]pyrene) are potent carcinogens found in the human environment. Though they are readily absorbed, little is known about the mechanism of their intestinal absorption. We have studied the absorption of several of these compounds, especially benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene, by the everted intestinal sac technique. Uptake of compound by sac

tissue resembles the uptake of fatty acid and cholesterol (as reported by others) in that it is not appreciably influenced by inhibition of respiration and/or glycolysis. However, polyaromatic hydrocarbons are unique in that accumulation in intestinal sac tissue increases exponentially with increase in the incubation medium concentration. To account for this unexpected finding, we postulated that first unilayer (Langmuir) adsorption occurs on the mucosal surface, and multilayer (Polanyi) adsorption follows. The latter would explain the exponential relation and may reflect the extreme hydrophobicity and the planar structure of these compounds. The passage from sac tissue into the fluid within the sac occurs in direct proportion to sac tissue concentration, a finding consistent with passive diffusion. In vivo experiments show that an exponential relation exists between the intragastric dose given in sesame oil and accumulation in the adipose tissue and mammary glands of rats. This suggests that polyaromatic hydrocarbons enter other tissues by the same nonspecific mechanism observed in intestinal absorption. In fact, the feature of exponential absorption is not limited to living tissue, for adsorption isotherms and time curves of nonliving material incubated in the same media have the same forms as those obtained for the sacs. (Supported by Eastern Utilization Research and Development Division, United States Department of Agriculture.)

247. Conversion of L-Thyroxine to Triiodothyronine by Human Fibroblasts in Tissue Culture. SAMUEL REFETOFF* AND REUBEN MATALON,* Chicago, Ill. (Introduced by Richard Landau**).

This study was undertaken to determine whether cells in tissue culture convert L-thyroxine (T_4) to triiodothyronine (T_3), as is demonstrated to occur in athyreotic patients by Braverman and associates. Cultures of fibroblasts from skin of five normal individuals were incubated with ^{125}I-T_4 for 1 to 7 days. Presence of conversion products was examined in medium and in sonicated cells by paper chromatography. In the presence of 4.6×10^{-8} M T_4 (labeled T_4 and endogenous T_4 from fetal calf serum) in the culture medium, the cellular uptake of T_4 ranged from 0.5 to 1.8%. 1.5% of the ^{125}I-T_4 was converted to T_3 in 1 day. The rate of conversion increased with incubation time to reach 8.19% by day 5. The total conversion of T_4 to T_3 in cells was 5×10^{-15} moles/mg tissue protein per day. No conversion of T_4 to T_3 was found in controls with medium only or presonicated cells. Increasing the concentration of unlabeled T_4 in the medium (up to 1.073×10^{-6} M) increased the uptake of ^{125}I-T_4 to 5.0% and the total conversion to T_3 in cells by 200-fold. However, a drop in the percentage of conversion of the ^{125}I-T_4 to T_3 was noted. Similar effects were produced by diminishing the concentration of fetal calf serum in the medium. T_4 to T_3 conversion was linearly proportional to the concentration of dialyzable T_4 in the medium. Larger amounts of unlabeled T_3 were required to produce an increase in ^{125}I-T_4 uptake by the cells; however, total T_4 to T_3 conversion remained unchanged. We conclude that (a) human skin fibroblasts convert T_4 to T_3 in vitro; (b) intracellular T_4 concentration and the rate of its conversion to T_3 is dependent on the availability of extracellular free T_4; (c) T_3 may inhibit the conversion of T_4 to T_3; (d) the conversion of T_4 to T_3 in man, estimated from the in vivo studies, is 4.7×10^{-9} mole/kg of total body protein per day, which closely approximates the value of 5×10^{-9} mole/kg protein per day observed in fibroblast cultures. (Supported by grants from the USPHS, AM-13377, AM-05996, and FR-00305.)

248. Myocardial Function and Metabolism in Chronic Diabetes Mellitus. T. J. REGAN, M. I. KHAN,* M. U. JESRANI,* H. A. OLDEWURTEL,* AND P. O. ETTINGER,* Newark, N. J.

Myocardial disease in diabetes mellitus (D) is usually attributed to coexistent coronary atherosclerosis. To examine the influence of D on the left ventricle (LV), a mild non-insulin-requiring diabetes was produced in eight male mongrel dogs after three 20 mg/kg doses of i.v. alloxan at monthly intervals. There was a persistent decline in glucose tolerance from a G_k of 3.43 ± 0.18 to 1.61 ± 0.23. At terminus, pancreatic insulin was only 35% of that in eight controls similar in age, weight, and diet. Anesthesia was induced after 1 yr. LV end-diastolic volume and cardiac output were measured by indicator dilution. During increased afterload with angiotensin, there was a significantly lower stroke output and stroke work in D. Though controls exhibited a significant increase of LV end-diastolic volume during the rise in afterload, this was reduced in D ($P < 0.01$). LV weight was comparable. Since plasma free fatty acids had risen during the 12 months without alteration of other lipids, LV metabolism was examined during systemic infusion of 100 μc ^{14}C-oleic acid in the intact animal. Using arterial-coronary venous differences and coronary blood flow (^{85}Kr), uptake of oleic acid was normal, but $^{14}CO_2$ production was diminished. The normal fatty acid incorporation into myocardial phospholipid was shifted to mainly triglyceride in D. LV triglyceride concentration in D was higher (5.1 ± 0.72 μmoles/g) than controls (2.08 ± 0.46 μmoles/g) without alteration of other lipid classes or soluble protein, presumably related to reduced oxidation and altered distribution of fatty acid. Coronary blood flow and coronary artery lipid composition were normal. Thus in mild untreated D an abnormality of LV function and metabolism can be demonstrated, apparently independent of vascular effects.

249. Renal Hemodynamics and Oxygenation in Peruvian Natives Living Permanently at 4300 Meters (14,200 Feet). DRUMMOND RENNIE,* RODOLFO LOZANO,* CARLOS MONGE,* FRANCISCO SIME,* AND JOSE WHITTEMBURY,* Chicago, Ill., and Lima, Peru (introduced by Robert M. Kark**).

Since the renal cortex normally operates at high tissue P_{O_2} levels, renal oxygenation in fully acclimatized native Peruvians, living at an altitude where the arterial P_{O_2} would necessarily be reduced to about half the normal sea level value, was studied. Six young adult male volunteers of native Peruvian high-altitude stock were investigated. Two, who had each resided in Lima (160 m) for more than 10 years, were studied in Lima, and four, who had always resided in Cerro de Pasco (4300 m), were studied in Cerro. In each, the right femoral artery, right renal vein, and bladder were

catheterized. The four Cerro de Pasco subjects had a mean arterial hematocrit of 68%, a slight reduction in inulin clearance (104 ml/min per 1.73 m^2), but a rise in filtration fraction to 27% due to a considerable fall in PAH clearance (mean 391 ml/min per 1.73 m^2). EPAH was normal (92%) and true renal blood flow was also normal (1320 ml/min per 1.73 m^2). Despite the very low Pa_{O_2} (mean 50.8 mm Hg), Ca_{O_2} was higher than normal (mean 23.9 vol %) because of the compensatory polycythemia, and so renal oxygen delivery, renal arteriovenous oxygen content gradients, and renal oxygen uptake were all normal (315 ml/min per 1.73 m^2; 1.19 vol %; and 15.9 ml/min per 1.73 m^2, respectively). The P_{O_2} gradient across the kidney was only 4.1 mm Hg (compared with 29.5 mm Hg for the two Lima subjects, in whom results were all normal). In the high-altitude group the oxyhemoglobin dissociation curve had shifted to the right. It is concluded that EPAH is normal, so that calculation of renal blood flow from PAH clearance is justified, and there is no evidence of deficient oxygenation of the kidneys in high-altitude natives acclimatized to life at 4300 m. (This work was supported by contract DADA 17-68-C-8019, United States Army Medical Research and Development Command, Surgeon General's Office.)

250. L-Dopa Absorption and Metabolism by the Human Stomach. L. RIVERA-CALIMLIM,* J. P. MORGAN,* C. A. DUJOVNE,* J. R. BIANCHINE,* AND L. LASAGNA,** Baltimore, Md.

High doses of L-dopa (LD) are generally needed in treatment of parkinsonism, presumably because much of the LD is "wasted" through extracerebral metabolism. Since preliminary animal and in vitro data suggested that LD was metabolized in part by the stomach, we studied the gastric absorption and metabolism of LD in nine patients with parkinsonism. After administration of 200 ml solution of 25 μc (500 mg) of L-dopa and 1 gram of polyethylene glycol (PEG), the total radioactivity (RA) in gastric juice, serum, and urine aliquots obtained at timed intervals was determined. Gastric absorption, expressed as both rate of absorption and gastric clearance, was measured by determining ^{14}C-LD-PEG concentration ratio in gastric juice using the method of Schanker. LD metabolites, phenylcarboxylic acids (PCA), dopamine (D), total catecholamines (CA), and unchanged LD were determined chromatographically. Rate of absorption was 65.1 mg/hr (±6.1 SEM) and gastric clearance was 36.7 ml/hr (± 1.9 SEM). Within a gastric emptying time of 2 hr, 73.4 ml of gastric contents were cleared of LD, and 26.0% (± 6.1 SEM) of the total dose of LD was absorbed from the stomach. Since total urinary excretion of RA was 80% of the dose given, the remaining RA was presumably absorbed from the intestines. PCA, CA, and D were present in the gastric juice as early as 5 min after oral ingestion of LD. Patients with alkaline basal gastric juice or with rapid gastric emptying achieved higher peak levels more rapidly. The most rapidly achieved and highest serum levels were observed in a gastrectomized patient, and the lowest serum levels were observed in a patient who retained the drug in the stomach for 7 hr. These data suggest both limited gastric absorption of LD and metabolism of the drug by the stomach mucosa.

251. The Relation of Cold Climate, Renal Enterobacteriaceae Infection, and Arthritis. ARDEN W. ROBERTS* AND KATHLEEN E. ROBERTS,** Owego, N. Y.

Renal *Proteus* infection, found in 200 arthritic patients, has been postulated to be causally related. It is generally accepted knowledge that exacerbations of arthritic symptoms are more common in cold weather, but the reason has remained obscure. Renal blood flow, known to increase in cold weather, could fortify the infection simply by an increased delivery of urea, a known substrate for *Proteus* metabolism. The experiments outlined here were carried out to determine whether an experimentally induced acute increase in renal blood flow did indeed promote the growth or release of renal bacteria. Pulsed high-frequency waves (PHFW), known to increase renal blood flow, were applied to the renal area in arthritic patients known to carry the infection. After showering with PHFW, individual urines were collected for 24 hr. 46 of the 50 patients showered with PHFW had an increased colony count in the urine after this maneuver. These findings suggest that increased renal blood flow does indeed promote the growth and/or release of bacterial organisms from the kidney and trigger exacerbations of arthritic symptoms.

252. Arginine Vasopressin: A Minority of Vasopressin Immunoreactivity in Plasma. GARY L. ROBERTSON,* LESTER A. KLEIN,* JESSE ROTH, AND PHILLIP GORDEN,* Bethesda, Md.

Our radioimmunoassay for arginine vasopressin (AVP) using standard methods is sufficiently sensitive (0.5 pg/ml) and specific (oxytocin is less than 1/1000 as reactive) for measurements of plasma AVP. However, in plasma, arginine vasopressin represented less than 5% of AVP immunoreactivity. When plasma was filtered on Sephadex G-25, AVP was recovered as a discrete peak (peak III) just after the salt, free of other immunoreactive components (peaks I and II). Peak III was indistinguishable from pure AVP in its migration on Sephadex G-25 and G-15 and in its biological activity in rats. Over a 36-fold range of dilutions, peak III differed slightly in immunoreactivity from pure AVP, but this difference was inconsequential for quantitation of plasma AVP. Physiological amounts of AVP, added to hormone-free plasma or to normal saline, were recovered in the peak III region with yields of 70–90%. In all plasmas from five normal adults and three with nephrogenic diabetes insipidus, concentrations of peak III immunoreactivity were closely related to the plasma osmolality, being <2 pg/ml at osmolalities <285 mOs/kg and rising progressively from 2 to 26 pg/ml as osmolalities increased from 285 to 320 mOs/kg. In both patients with pituitary diabetes insipidus, peak III immunoreactivity was <2 pg/ml even when plasma osmolality exceeded 300. Concentrations of plasma immunoreactivity in peaks I and II were unaffected by the state of hydration and were high even in patients with pituitary diabetes insipidus. Peaks I and II, constituting about 80% and 15% of plasma immunoreactivity, were each separated

into four components by further fractionation on Sephadex G-100 and G-15 respectively. Their relation to arginine vasopressin is as yet unknown.

253. A Theory of Antibody Formation. JOHN P. ROBINSON* AND VICTOR A. NAJJAR,** Nashville, Tenn., and Boston, Mass.

The theory proposed by Najjar pictures the antibody as a γ-globulin molecule with a covalently inserted polypeptide segment that is complementary to a definite area on the antigen. This inserted polypeptide could be antigen derived. Its interaction with antigen would be similar to the specific interaction of monomers to form dimers or tetramers, or similar to the specific binding within segments of a polypeptide chain to mold its tertiary structure. The inserted segment could also be a piece of host cell receptor. This receptor-derived polypeptide should be reasonably specific. The reaction of phytohemagglutinins with cell receptors is one of several prototypes. The theory affords simple and reasonable interpretations of some manifestations of the immune response. Specificity and diversity of antibody relate to the inserted polypeptide. The neutralization of a biologically active antigen results from the unfolding of the antigen molecule secondary to the competition of certain peptide segments on the antigen for the same binding sites with identical peptide segments inserted on the antibody molecule. The stimulation or rejuvenation of enzyme activity by minute amounts of antibody is equally specific. Here the antibody would supply, in proper apposition to the active site, a peptide fragment originally related to that site. The toxicity of autologous soluble antibody-antigen complexes and the simple allergic state would result from the distortion of normal cytophilic γ-globulin with consequent cell injury. Rabbits with high antibody levels to two antigens were reinjected with both antigens, one radioactive with ^{14}C, ^{3}H, or ^{125}I. Antigens were bacterial protein, BSA, ribonuclease, and porcine globulin. In all experiments the antibody to the labeled antigens, up to the 5th day after reinjection, was highly radioactive as compared with antibody to the nonradioactive antigens. (USPHS grant AI-09116.)

254. The Effects of Rifampin on Chick Embryo Cell Growth and Infection and Transformation of Cells by Rous Sarcoma Virus. WILLIAM S. ROBINSON,* Palo Alto, Calif. (introduced by Thomas C. Merigan).

The antibiotic rifampin inhibits RNA synthesis in bacteria and inhibits the *Escherichia coli* RNA polymerase reaction in vitro by specific binding of the enzyme. In similar concentrations, the drug does not inhibit RNA synthesis in animal cells in tissue culture and does not inhibit the in vitro RNA polymerase reaction. Recently rifampin has been shown to reduce vaccinia virus yields in tissue culture and to inhibit focus formation by Rous sarcoma virus (RSV), but only at much higher drug concentrations than those which inhibit bacterial growth and RNA synthesis. Experiments were performed to determine the effects of rifampin on (1) replication of RSV in chick embryo fibroblast (CEF) cultures, (2) focus formation by RSV in CEF cultures, (3) growth of uninfected and RSV-transformed CEF, and (4) RNA and DNA synthesis in uninfected and RSV-transformed CEF. The results indicate that rifampin concentrations up to 80 μg/ml do not affect the production of RSV by cells in the first 48 hr after infection, but 80 μg/ml or higher concentrations reduce virus yield after 48 hr. Focus formation was reduced 4-fold by 20 μg/ml rifampin, and 100-fold by 40 μg/ml. Studies of logarithmically growing cells revealed that 20–40 μg/ml rifampin greatly inhibits the rate of cell growth, and 80 μg/ml reduces the cell number in cultures, results in severe cytopathic changes, and inhibits cellular RNA synthesis after 24 hr of drug exposure. The growth of RSV-transformed cells is inhibited by slightly lower concentrations of drug than is the growth of uninfected cells. It is concluded that inhibition of focus formation by rifampin is related to the inhibition of growth of transformed cells rather than to inhibition of virus infection and replication. The drug concentrations required to block focus formation and inhibit uninfected and transformed cell growth are 100 to 1000 times higher than those which inhibit growth of and RNA synthesis in sensitive bacteria.

255. Measurement of Diaphragmatic Blood Flow and Oxygen Consumption in the Dog. DUDLEY F. ROCHESTER,* New York, N. Y. (introduced by Réjane M. Harvey**).

Oxygen consumption and hence energy expenditure of the diaphragm could be evaluated if diaphragmatic blood flow and arteriovenous oxygen content difference were known. To estimate these parameters, a technique based on sampling diaphragmatic venous blood was developed. In 14 mongrel dogs anesthetized with pentobarbital, the common trunk formed by the veins draining the left hemidiaphragm was catheterized. After infusing krypton-85 to saturate the tissues, the maximum diaphragmatic venous tracer concentration and the integrated diaphragmatic arteriovenous krypton difference were measured. From these data, blood flow per 100 g of diaphragm was determined by the Kety-Schmidt method. Diaphragmatic oxygen consumption was calculated from blood flow and the diaphragmatic arteriovenous difference in oxygen content. Blood flow per 100 g of diaphragm averaged 18.7 ± 5.0 ml/min during quiet breathing; this represented $0.9 \pm 0.3\%$ of the cardiac output. Diaphragmatic blood flow correlated poorly with ventilation, but varied directly with cardiac output ($r = 0.501$, $P < 0.02$). Diaphragmatic oxygen consumption averaged 1.3 ± 0.5 ml/min per 100 g, and bore no relation to cardiac output, but correlated well with minute ventilation ($r = 0.613$, $P < 0.005$). The diaphragmatic arteriovenous oxygen difference was 7.0 ± 2.3 vol % at rest, and rose to over 13 vol % during CO_2-induced hyperventilation. This study demonstrates the feasibility of utilizing diaphragmatic venous blood to study the metabolism of diaphragm. In the anesthetized dog, diaphragmatic blood flow lies at a level intermediate between skeletal muscle and myocardial blood flow. Under the conditions of this study, the oxygen requirements of the diaphragm are satisfied to a greater extent by extraction of oxygen from the blood than by increasing diaphragmatic blood flow. (Research supported by grants from the New York Heart Association and the NIH.)

256. Dissociation between Two In Vitro Correlates of Cellular Hypersensitivity: Production of Migration Inhibitory Factor and Lymphocyte Transformation. Ross E. Rocklin,* Winthrop H. Churchill,* Albert Sheffer,* and John R. David,** Boston, Mass.

In vitro studies were carried out to assess lymphocyte function in diseases associated with depressed delayed hypersensitivity, in hope of further delineating the cellular abnormality responsible for anergy. Blood lymphocytes from 34 patients were assayed for production of migration inhibitory factor (MIF) and for antigen-induced incorporation of ^{3}H-thymidine with one or more of the following antigens: streptokinase-streptodornase (SKSD), PPD, and *Candida albicans*. It was found that MIF production correlated with the skin test in 30 patients. However, lymphocytes from 10 of these (two Hodgkins, four sarcoid, one chronic mucocutaneous candidiasis, one Felty syndrome, one thymoma, one idiopathic anergy) were stimulated to increased incorporation of ^{3}H-thymidine despite negative skin test and absence of MIF production. The level of thymidine incorporation by lymphocytes from these 10 patients in response to SKSD was similar to that seen in the MIF-positive group, but the response to PPD was less. Thus, in some cases the proliferative lymphocytic response to antigen can be dissociated from production of MIF. It is not known whether these two functions are performed by the same sensitive cells or by two different subpopulations of sensitive cells. If a significant part of the proliferative response results from recruitment of nonsensitive lymphocytes by a blastogenic factor described by Valentine and Lawrence, our data imply that blastogenic factor can be produced in the absence of MIF. Lymphocytes from four other patients produced MIF despite a negative skin test to the same antigen, suggesting that the patients had sufficient sensitive cells to produce MIF and that their anergy resulted from another deficiency. (Supported by NIH grants AI-7685 and AI-8026.)

257. Production and Utilization of Energy in Isolated Renal Tubules. C. C. Rohrs,* M. De Hartog,* and E. E. Gordon, New York, N. Y.

A study of energy relations was undertaken in isolated tubules from rat renal cortex prepared by the collagenase technique. Mitochondrial energy production was assessed by measuring O_2 consumption with the Clark electrode over periods of time not exceeding 15 min. An indication of the degree of coupling of mitochondrial oxidation to phosphorylation was obtained by incubating the tubules in the presence of various amounts of oligomycin, an agent that inhibits the phosphorylation of mitochondrial ADP. A plot of respiratory inhibition vs. oligomycin concentration showed that the basal respiratory rate (24 mμatoms of O per mg protein per min) was depressed to a maximum of 45% in the presence of 0.3 μg oligomycin per cell protein. At the same time the ATP content of the cells fell from approximately 4 nmoles/mg protein to negligible values within 1 min after addition of oligomycin. A similar rapid decline in ATP was observed in the presence of inhibitors of the respiratory chain, i.e., rotenone (5×10^{-7} M), antimycin (8 μg/ml), and cyanide (3×10^{-4} M). Inhibition of the cell membrane, and coupled Na^+-K^+ transport ATPase with 10^{-3} M ouabain, resulted in a 20% inhibition of respiration. Under these conditions there was no decline in the ATP content of the cell. Moreover, when ouabain was present initially, the dramatic fall in ATP content upon addition of oligomycin did not occur. These experiments indicate that (1) approximately half of the energy generated by mitochondria is in the form of ATP; (2) there is an extremely rapid turnover of ATP in these cells; and (3) ATP generated in the mitochondria of renal cortex is used principally for cell membrane transport of monovalent cations.

258. The Intestinal Maladaptation Syndrome: A New Approach to "Functional" Gastrointestinal Disease. Norton S. Rosensweig,* Robert H. Herman,* Fred B. Stifel,* and Louis Hagler,* Denver, Colo., and New York, N. Y. (introduced by Peter R. Holt).

In normal jejunum, glycolytic enzyme activities (hexokinase, glucokinase, fructokinase, fructose-1-phosphate and fructose-1,6-phosphate aldolases) adapt within hours to dietary sugars (glucose, fructose, and sucrose) and folic acid. Failure of this adaptive response may result from or lead to gastrointestinal disease. Studies of jejunal glycolytic enzyme activities in seven patients with damaged jejunal mucosa (tropical sprue, celiac disease, and nodular lymphoid hyperplasia) showed that the glycolytic enzyme activities were low and failed to adapt to the dietary sugars tested. Folic acid therapy restored the adaptive response to normal in tropical sprue but had no effect in the other conditions. Six patients with "functional" gastrointestinal disease, i.e., chronic diarrhea, vague dietary intolerance, and negative gastroenterologic evaluation including normal jejunal biopsy were studied. In four there was no adaptive response to all the dietary sugars tested, and a poor response to folate. The high-carbohydrate diets exacerbated symptoms. Two of these patients were discovered to have a deficiency of formiminotransferase activity in the jejunum and red cells. In two other patients (brothers), oral glucose reproduced the "functional" symptoms, and there was no adaptive response to dietary glucose, but a normal adaptive response to fructose and folate. Treatment of all six with folic acid and appropriate carbohydrate restriction produced symptomatic improvement and a decrease in diarrhea. These studies of patients with a poor adaptive response of jejunal glycolytic enzyme activities to dietary sugars and folic acid demonstrate an "intestinal maladaptation syndrome," indicate a mechanism that may be responsible for symptoms in "functional" gastrointestinal disease, and suggest a possible approach to the therapy of this disorder.

259. Stimulation of Serum Hemopexin Levels by Lead Acetate. Are Hemopexin Induction and Heme Production Unrelated? Jean D. Ross* and Ursula Müller-Eberhard,* La Jolla, Calif. (introduced by Elisha Atkins**).

Synthesis of serum hemopexin, the heme-binding β-globulin, has been induced in rabbits by injection of the hepatic enzyme inducers 3-methylcholanthrene (3-MC), 3,4-benzpyrene (BP), and allylisopropylacetamide (AIA). Interre-

lations exist between biosynthesis of heme and of serum hemopexin and the pharmacological induction and function of heme enzymes. Preliminary results show that aminotriazole (AT) prevents induction of serum hemopexin by BP only inconstantly and partially, suggesting that the BP-induced rise in hemopexin is unrelated to heme production. AT inhibits the second step of heme synthesis, whereas AIA induces the first step and produces porphyrinuria coincident with the rise in serum hemopexin. Since lead is a porphyrogenic agent and an inhibitor of heme synthesis, the effect in rabbits of lead acetate on serum hemopexin levels (measured by radial immunodiffusion) was studied. Subcutaneous injection of lead acetate (150 mg/kg) caused striking elevation of serum hemopexin (mean % Δ hemopexin $= 184 \pm 43$, peaking at 2–5 days), which corresponded to increased urinary δ-aminolevulinic acid levels; porphobilinogenuria was absent. Saline controls were unremarkable. The stimulatory effect of lead acetate on serum hemopexin supports the interpretation, suggested by the AT results, that increased heme synthesis does not cause the previously noted drug-induced (3-MC, BP, AIA) rise in serum hemopexin levels. (Research supported by NIH grant HE-08660, NIH award AM-16923, and HD-4445.)

260. Left Ventricular Geometry and Sarcomere Structure in Acute and Chronic Cardiac Dilatation. JOHN ROSS, JR., EDMUND H. SONNENBLICK, ROGER TAYLOR,* HENRY M. SPOTNITZ,* AND JAMES W. COVELL,* Boston, Mass., and San Diego, Calif.

In chronic left ventricular (LV) dilatation, large increments in end-diastolic volume (EDV) occur, but whether or not the dilated ventricle operates on a descending limb of the sarcomere length-tension curve is unknown. Chronic shunts between aorta and vena cava were created in 10 dogs, leading to congestive failure with markedly elevated LV end-diastolic pressures (EDP) (average 27 mm Hg). Similar elevations in EDP were produced acutely in normal ventricles by volume overloading. Rapid diastolic cardiac arrest and fixation was performed at the EDP existing in vivo, casts of the LV cavities were made, and mid-wall sarcomere lengths were determined by electron microscopy. EDV of the chronically dilated LV was larger than that in acutely dilated LV (average 103 ml and 72 ml, respectively, $P < 0.01$) and the shape was more globular, but calculated diastolic wall stresses were no different. Sarcomere lengths averaged 2.19 ± 0.02 (SE) (range 2.11–2.27) microns, a value at the apex of the sarcomere length-tension curve, but not significantly different from sarcomere lengths in the acutely dilated ($2.25 \pm 0.03\,\mu$). Thus, there was a relation between sarcomere length and diastolic wall stress, but not with EDV. In the dilated LV, the lateral alignment of sarcomeres (register) was abnormal. Therefore, a slippage of fibrils within cells appeared to occur, but the sarcomeres remained optimally extended. This complete loss of Frank-Starling reserve mechanism implies, however, that in the dilated heart increased volume or systolic pressure can augment wall stress during ejection and thereby produce a descending limb of the heart's performance as a pump, without invoking a descending limb at the sarcomere level. (Supported by NIH grants HE-12373 and HE-11306.)

261. The Efficiency of Complement Reactions on Normal and Paroxysmal Nocturnal Hemoglobinuria Red Cells. WENDELL F. ROSSE AND GERALD L. LOGUE,* Durham, N. C.

Normal human red cells sensitized with antibody require high concentrations of complement (C) for lysis in vitro, whereas a proportion of the red cells (the complement-sensitive population) in paroxysmal nocturnal hemoglobinuria (PNH) is lysed by far lower concentrations. This implies either that more complement sequences are begun on the PNH cells or that initiated sequences are completed more efficiently. We have measured the fixation of the third (C3) and fourth (C4) components of complement to normal and PNH cells and have related this to lysis when the other components of complement are supplied. C4 was fixed to cells sensitized with rabbit antibody, and the number of molecules of C4 bound was estimated by the absorption of C2, using the Langmuir absorption isotherm. When the other complement components were added, the number of completed sequences was estimated, assuming that a single completed sequence was sufficient for lysis. With the same concentration of antibody and complement, equal amounts of C4 were bound to normal and PNH cells. However, when the other components of complement were added, the mean number of completed sequences per cell was 4–6 times greater on PNH cells. When purified, ^{125}I-labeled C3 (C3*) was incubated with C4-coated cells in the presence of C1 and C2, the same amount of C3* was bound to normal and PNH cells. When C3* was added to serum in the acidified serum lysis test, equal amounts fixed to normal and PNH cells, although only the complement-sensitive PNH cells were lysed. These findings indicate that the difference in susceptibility to complement lysis of PNH cells is not due to differences in the number of complement sequences which are initiated by immune reactions, but rather to differences in the efficiency with which they are completed. (NIH grant 5-RO1-CA-10267.)

262. Characterization of a Folic Acid–Binding Factor in Leukemic Cells. SHELDON P. ROTHENBERG* AND MARIA DA COSTA,* New York, N. Y. (introduced by Stanley Ulick**).

A folic acid (FA)-binding macromolecular factor recently identified in the leukemic cells of two patients with chronic myelogenous leukemia (CML) has now been identified in the serum of these patients, in the serum and cells of a third patient, and in the cells of a fourth patient with CML. Acute leukemic and chronic lymphocytic leukemic cell lysates, normal bone marrow lysate, and lysate of normal peripheral leukocytes did not contain this factor. Binder persisted in cells, but disappeared from serum in the one patient treated with busulfan. Binding of ^{3}H-FA by this factor prevented its reduction to tetrahydrofolate by folate reductase of cell lysates. Saturating factor with stable FA permitted determination of enzyme K_m, which appeared significantly higher than that of lysates which did not contain this binder. Filtration of binding lysate through Sephadex G-75 separated larger binder from smaller folate reductase. Sephadex filtration indicated a molecular weight between 100,000 and 200,000. Coated charcoal and Dowex 2-X8 re-

moved free but not bound ^{3}H-FA from solution. Binding of FA decreased sharply below pH 5.5 and partially after heating lysate at 56°C. Binder was in the supernatant after centrifugation of lysate at 105,000 *g*. Kinetic studies demonstrated two rates of association and dissociation, indicating heterogeneity of binding sites. Stable FA and dihydrofolate competitively inhibited binding of ^{3}H-FA, but there was no inhibition by tetrahydrofolate, methyltetrahydrofolate, or formyltetrahydrofolate. High concentrations of methotrexate inhibited binding. This folate-binding factor (1) may prove useful for the diagnosis and treatment of CML, (2) may be useful as a binder in a competitive inhibition radioassay for folic acid, (3) may play a role in resistance to methotrexate, since it can also bind this drug, and (4) may be a potential inhibitor of DNA synthesis, since it binds dihydrofolate, a folate coenzyme involved in the synthesis of thymidine.

263. The Effect of Thymidine Kinase on Tumor Growth. HENRY ROTHSCHILD* AND PAUL BLACK, Boston, Mass.

Uncontrolled DNA synthesis has been implicated in the growth of a tumor cell into a neoplastic mass. We have used a simian virus 40 (SV40)-transformed hamster kidney cell line and clones derived from this line which were made deficient in the enzyme thymidine kinase to test the possible role of this salvage pathway in tumorigenicity. Of 46 hamsters injected with transformed cells containing wild-type enzyme activity, 43 developed progressive tumors. In contrast, none of 49 animals injected with three different clonal cell lines lacking the enzyme developed tumors by the 8th week. However, several animals developed slowly growing tumors after 10 wk. Enzymatic determinations carried out on cultures established from two of three of the tumors initiated with cells lacking the enzyme indicated that a reversion to wild-type enzyme activity had occurred. Revertant cell lines were also isolated in vitro after chemical mutagenesis of cell lines lacking thymidine kinase. These revertant cell lines were of two types: one type contained levels of enzymatic activity similar to the wild type, and the other contained intermediate levels of thymidine kinase activity. Cells from a revertant with wild-type enzyme activity produced tumors in 7 of 12 animals; this suggests that a return of enzymatic activity was associated with the recovery of malignant potential. All cell lines were shown to contain the SV40-T antigen, and virus could be rescued by heterokaryon formation with indicator cells. The karyotypes of the wild-type and the enzyme-deficient cells were similar. The data indicate that thymidine kinase may play a rate-limiting role in tumor formation. (Research supported by grants from the NIH.)

264. Metabolism of Proinsulin and Insulin by the Liver. A. H. RUBENSTEIN,* L. POTTENGER,* M. MAKO,* F. MELANI,* AND D. F. STEINER,* Chicago, Ill. (introduced by T. N. Pullman**).

Measurements of endogenous serum proinsulin levels in man have suggested that proinsulin may be degraded more slowly than insulin. As the liver is quantitatively the most important organ for insulin degradation, we have compared the metabolism of bovine proinsulin and insulin in the isolated perfused rat liver. In each experiment, four livers (12–15 g) from fed rats (300–400 g) were perfused independently with 20% red cells in bicarbonate buffer (4% albumin) at flow rates of 23–25 ml/min. At high concentrations (above 80 ng/ml) the half-life ($t_{\frac{1}{2}}$) of insulin was 40 $\pm$ 4 min and the hepatic clearance (reaction velocity constant) was 1.35 ml/min. At lower levels (4–20 ng/ml) the $t_{\frac{1}{2}}$ was 14 $\pm$ 2 min and clearance 4.0 ml/min. In contrast, proinsulin was removed extremely slowly from the perfusate. At 200, 20, and 4 ng/ml, $t_{\frac{1}{2}}$ of proinsulin was 170, 150, and 130 min and the clearance 0.29, 0.34, and 0.46 ml/min respectively. Identity of the hormones was established by specific immunoassays and gel filtration of iodinated and unlabeled samples. Splitting of proinsulin to release insulin or C-peptide was not observed. Support for these data was obtained by measuring the conversion of trichloroacetic acid–precipitable ^{131}I-proinsulin and ^{131}I-insulin to trichloroacetic acid–soluble fragments by 2–8% homogenates of rat and bovine liver in the presence of unlabeled hormone (20–100 μg/ml). Insulin was degraded 10 times more rapidly than proinsulin, and was 2 to 5 times more effective in inhibiting the degradation of ^{131}I-insulin than equimolar concentrations of proinsulin. These findings are relevant to the consideration of the biological activity of insulin and proinsulin. A relative accumulation of proinsulin in the liver due to its slower degradation could account for the previously observed similar potency of the two hormones on hepatic metabolism.

265. Effects of Ethanol on Hepatic Mitochondrial Protein Synthesis. EMANUEL RUBIN,* KAREL JINDRAK,* ATTILA TOTH,* DIANA S. BEATTIE,* AND CHARLES S. LIEBER, New York, N. Y.

We have previously shown that chronic ingestion of ethanol with adequate diets interferes with hepatic mitochondrial functions, such as lipid and acetate oxidation. To assess the effects of ethanol on mitochondrial integrity, rats were fed ethanol (36% of total calories) with an adequate diet for 24 days. Volunteers were treated similarly (46% total calories as ethanol) for 12–18 days. After ethanol ingestion, electron microscope examination revealed enlarged, distorted, and clumped hepatic mitochondria, disorientation of cristae, and breaks in the outer membrane. In ethanol-treated rats (compared with pair-fed controls), after an overnight fast, incorporation of ^{14}C-labeled leucine and arginine was depressed in total mitochondrial protein, as well as in four major subfractions: water-soluble protein, cytochrome *c*, other cytochromes, and structural protein. The last showed the most striking reduction, 43% ($P < 0.02$). In vivo, mitochondrial protein labeling was not decreased by one dose (8 g/kg) of ethanol, whereas in vitro, 50–100 mM ethanol inhibited ^{14}C-leucine incorporation by 17% ($P < 0.001$). Washed mitochondria, either preincubated for 30 min with 100 mM ethanol, or obtained from rats 3 hr after one dose (8 g/kg) of ethanol, displayed unaltered in vitro incorporation. Thus inhibition of intramitochondrial protein synthesis, which occurs in the inner membrane, by concentrations of ethanol similar to those found in inebriated individuals is dependent on the presence of ethanol. By contrast, chronic ethanol administration results in a depression of amino acid incorpora-

tion into mitochondrial protein, demonstrable even in the absence of ethanol; this probably represents inhibition of transfer of protein from microsomes to mitochondria (inner and outer membranes), since at least 90% of in vivo incorporation reflects such transfer. These data may explain, at least in part, the structural and functional derangements in hepatic mitochondria produced by ethanol.

266. Hemolytic Titration of Ninth Complement Component in Serum and Other Body Fluids: Evidence for Completion of Complement Reaction Sequence In Vivo. S. RUDDY,* L. K. EVERSON,* D. P. BUDMAN,* AND K. F. AUSTEN, Boston, Mass.

The activity of the ninth component of complement in human serum has been measured by the lysis of a standard suspension of sheep erythrocytes pretreated with antibody and the first eight complement components, EAC1–8 cells. Target EAC1–8 cells were generated by reactng EAC14 cells with a DEAE-cellulose–derived serum fraction rich in C2, 3, 5, 6, 7, and 8, and devoid of C9. Incubation of EAC1–8 with purified C9 at 37°C demonstrated that both rate and, after 3 hr, extent of lysis were proportional to C9 input. Plots of calculated average numbers of damaged sites per cell versus amounts of C9 added yielded linear dose responses both for purified C9 and for C9 contained in human serum, synovial fluid (SF), or cerebrospinal fluid (CSF). The mean serum C9 for 36 normal adults was 52,000 ± 12,000 units/ml (± 1 SD). In lupus nephritis, serum C9 activities fall dramatically with exacerbations and return to normal with remissions; these changes are associated with similar alterations in the early components (C1, C4, C2, and C3). In osteoarthritis, SF values are approximately 65% of the corresponding serum values, and in CSF, C9 levels are approximately 1% of serum. Studies with rheumatoid SF and CSF from patients with CNS lupus are in progress to determine whether the complement sequence goes to completion in these fluids in which reductions in levels of early components have been observed. (Supported by NIH grant AI-07722.)

267. Pressure Flow Studies in Man: Effects of Respiration on Left Ventricular Stroke Volume. JEROME RUSKIN,* JUDITH C. REMBERT,* CHARLES L. CURRY,* HOWARD J. ZEFT,* AND JOSEPH C. GREENFIELD, JR., Durham, N. C.

The primary mechanism responsible for the inspiratory fall in systolic blood pressure in normal subjects and for pulsus paradoxus remains unknown. A phasic delay in the inspiratory increase in right ventricular stroke volume and/or the transmission of negative intrathoracic pressure to the aorta have been implicated in normals and in patients with airway obstruction. An inspiratory fall in left ventricular stroke volume has been postulated in pulsus paradoxus due to pericardial tamponade. Investigation in man has been limited by the inability to measure left ventricular stroke volume continuously. Using the pressure gradient technique, the effects of continuous or interrupted respiration on left ventricular stroke volume were studied in 11 control subjects, in nine patients with airway obstruction, and in three with pericardial tamponade. During quiet respiration, inspiration resulted in a fall in stroke volume of 7% in normal subjects ($P < 0.001$), 20% in those with airway obstruction ($P < 0.001$), and 34% in patients with pericardial tamponade ($P < 0.001$). An overshoot in left ventricular stroke volume three to four beats after inspiration of 2% both in normal subjects and in patients with airway obstruction ($P < 0.001$) and of 10% in patients with tamponade ($P < 0.001$) could be attributed to the respiratory pump mechanism. Similar results, though of slightly greater magnitude, were obtained after breath holding in expiration. From these data we conclude that an inspiratory reduction in left ventricular stroke volume, due to pooling of blood in the lungs, is the primary mechanism responsible both for the normal inspiratory decrease in blood pressure and for pulsus paradoxus seen in patients with either airway obstruction or tamponade. (Research supported by grant 5-RO1-HE-09711-05 from the NIH.)

268. Biological Activity of Newly Isolated Intestinal Peptide. SAMI I. SAID, VIKTOR MUTT,* AND TAKAHITO HIROSE,* Richmond, Va., and Stockholm, Sweden.

We recently reported the extraction from hog small intestine of a potent vasodilator peptide. Further investigation reveals this vasoactive intestinal peptide (VIP) to have a broad spectrum of actions on intact animals and isolated organs. On intravenous or intra-arterial infusion in anesthetized dogs, VIP induced systemic hypotension in doses of as little as 0.3 μg/kg. Larger doses (4 μg/kg) reduced mean arterial blood pressure by up to 50 mm Hg, for as long as 1 hr. Cardiac output increased by about 53% from an average dose of 2 μg/kg; the increase was mainly due to a greater stroke output, rather than to tachycardia. Femoral, intestinal, and hepatic arterial blood flow increased, but renal arterial flow usually decreased. Intra-aortic and right atrial infusions gave the same degree of hypotension, but portal vein infusions were without effect on the blood pressure. Respiratory changes included augmented minute ventilation and breathing frequency, without change in tidal volume. Blood sugar increased by about 13%. VIP relaxed five of the smooth muscle preparations tested: rat stomach strip, chick rectum and rectal cecum, and guinea pig trachea and gallbladder. The relaxant effects were unaltered by antagonism of catecholamines, acetylcholine, histamine, or 5-hydroxytryptamine. These findings show that VIP (1) can affect cardiovascular, respiratory, and metabolic functions, (2) relaxes a variety of smooth muscles by direct action, and (3) is inactivated in the liver but not in the lung. Possible physiologic roles are in the control of intestinal blood flow and of blood sugar. Intestinal ischemia and hepatic cirrhosis are two pathologic states in which the peptide might be an important mediator. (Supported by grants from the NIH, the American Heart Association, and the National Tuberculosis and Respiratory Diseases Association.)

269. Regulation of Intestinal Synthesis of Cholesterol in Patients with Hyperlipidemia. GERALD SALEN,* E. H. AHRENS, JR.,** AND SCOTT M. GRUNDY,* New York, N. Y.

Qualitative assessments of cholesterol synthesis in jejunal mucosa were carried out in seven patients with hyperlipidemia who were maintained at constant body weight on cholesterol-

free formula diets for 4–6 months. After pulse labeling with 4-^{14}C-cholesterol intravenously, specific activity–time curves were determined for plasma cholesterol and for mucosal cholesterol obtained by suction biopsy. Because diets were free of cholesterol and the ratio of specific activity of cholesterol in bile to that in plasma was always 1.0, any reduction in the mucosa/plasma specific activity ratio would indicate the presence of active mucosal cholesterol synthesis. In five patients with hypercholesterolemia (type II), specific activity ratios (mucosa/plasma) were 1.05 to 1.15 in each of four weekly biopsies. These higher ratios indicate a precursor-product relation between plasma and mucosa and are consistent with minimal rates of synthesis in mucosa. In contrast, two patients with endogenous hyperglyceridemia (types IV and V) on the same regimen consistently showed a specific activity ratio of 0.75 to 0.92. Cholesterol reabsorption was inhibited by feeding β-sitosterol to one hypercholesterolemic patient; this did not affect the mucosa/plasma specific activity ratio. However, in all five hypercholesterolemic patients, interruption of the enterohepatic circulation of bile acids by cholestyramine caused a significant decrease in the ratio (0.58 to 0.90). In the one adequately tested hyperglyceridemic patient and in the five hypercholesterolemic patients receiving cholestyramine, the administration of Atromid-S caused specific activities of mucosal cholesterol to rise to and remain at levels higher than that in plasma. These data suggest that a difference exists in cholesterol metabolism in the intestinal mucosa of hypercholesterolemic and hyperglyceridemic patients; they confirm the theory that mucosal synthesis of cholesterol in man is under feedback regulation by bile acids but not by cholesterol, as Dietschy has shown previously in rats and monkeys; and they provide the first direct evidence for suppression of cholesterol synthesis by Atromid-S in man. (This study was supported in part by USPHS grant HE-06222, National Heart Institute, and in part by USPHS grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources.)

270. Evidence for a Protein Renin Accelerator Originating from "Ischemic" Kidney in Renovascular Hypertension. MOHINDER SAMBHI* AND CHARLES WIEDEMAN,* Los Angeles, Calif. (introduced by Irving Gordon**).

Endogenous renin activity in bilateral renal venous plasma (RVP) of 20 patients with suspected unilateral renovascular hypertension was 8.6 and 4.8 ng/ml per hr, and with added excess of human renin, angiotensin generation rate (Ang GR) rose to 1485 and 978 ng/ml per hr. This difference between the maximal Ang GR in the RVP of two kidneys is not explained either by an unequal secretion of renin or by the concentration of renin substrate. A possible role of a lipid inhibitor of renin was studied by comparing the influence of lipid extraction on RVP with bilaterally unequal Ang GR with that on RVP with bilaterally equal Ang GR. The difference in Ang GR between the RVP from two kidneys when present was maintained after adequate lipid extraction; furthermore, addition of the contralateral lipid extract did not change Ang GR. These findings support the view that high Ang GR in the plasma from "ischemic" kidney is due to a protein renin accelerator. (Research supported by a grant from the NIH.)

271. Human Marrow Transplantation. GEORGE W. SANTOS,* LYLE L. SENSENBRENNER,* PHILIP J. BURKE,* O. MICHAEL COLVIN,* ALBERT H. OWENS, JR,* DANIEL C. HADLOCK,* WILMA B. BIAS,* AND RICHARD E. SLAVIN,* Baltimore, Md. (introduced by Dudley P. Jackson).

This report summarizes results in four adults with acute leukemia and one with Hodgkin's disease, using cyclophosphamide (CY) according to a rationale described previously. Recipients were given 500 ml donor blood. 1, 2, 3, and 4 days later, they were given equal doses of CY (50–60 mg/kg). About 10 billion marrow cells were given 24 hr after the last dose of CY. Lymphocyte typing and genetic (haplotype) analyses were completed in four cases. Two donor-recipient pairs inherited the same HLA antigens from one parent but not the other. Two donor-recipient pairs were serotype "identical," but only one proved to be haplotype identical. In the latter case the recipient was blood type A and the donor, O. In the other cases blood groups were similar. The grafted marrow functioned in all cases, and chimerism persisted without evidence of rejection. Severe graft-versus-host disease (GVH) was seen except after the haplotype identical graft. Patient survival ranged from 7 to 75 days after transplantation. GVH lesions were revealed at autopsy, but no leukemia was seen. Death was caused by infection in three cases, cerebral hemorrhage in one, and myocarditis (possibly due to CY) in the other. Thus, CY pretreatment permits successful marrow engraftment. Lymphocyte typing and haplotype analysis relate well to the occurrence of GVH. Blood group A does not appear to be a major antigenic stimulus for GVH. (Research supported by grant CA-06973 and contract PH-43-66-924 from the NIH.)

272. Inadequate Feedback Suppression of Renin Release by Angiotensin: A Possible Mechanism for Estrogen-Induced Hypertension. T. SARUTA,* G. A. SAADE,* AND N. M. KAPLAN, Dallas, Texas.

Hypertension appears or worsens in some women given oral estrogen for contraception (OC). Estrogens increase renin substrate levels, in turn increasing angiotensin formation per unit of renin. Skinner and associates suggest that the increased angiotensin formation is normally associated with a suppression of renin release, whereas failure to suppress renin secretion might account for the hypertensive effect of estrogen. We are examining the relations between plasma renin activity (PRA), plasma renin concentration (PRC), and plasma renin substrate levels in normotensive (NT) and hypertensive (HT) women given OC containing mestranol 0.05 mg + norethindrone 1.0 mg for 21 of every 28 days, starting 4 wk postpartum. Of the 56 NT who have been followed up to 30 wk, 10 have clearly become hypertensive (HT), and the blood pressure of one of the six HT has increased. Both in the 51 patients whose pressure is unchanged after OC and in the 11 whose pressure has increased, PRA rose similarly and significantly. Renin substrate increased in both groups, but to a greater degree in the 11 with increased pressure. Whereas PRC fell slightly

in the 51 with unchanged pressure, it increased in the 11 with increased pressure. It appears that women who become hypertensive on OC therapy fail to suppress renin secretion, as reflected in their higher PRC levels. The increased levels of angiotensin generated would be a factor in the induction of hypertension.

273. Physiologic Predictors of Death in Acute Myocardial Infarction. STEPHEN S. SCHEIDT,* SIDNEY J. FILLMORE,* AND THOMAS KILLIP, New York, N. Y.

Since the advent of the specialized monitoring unit, most deaths in hospitalized patients with acute infarction result from refractory heart failure or shock. Current supportive therapy is often unsatisfactory, and the mortality of cardiogenic shock is approximately 80%. Criteria are needed for the selection of patients at high risk in whom unconventional therapy such as mechanical circulatory support may be evaluated. 98 studies have been performed in 35 patients from 5 to 239 hr after acute infarction. Intracardiac pressures were monitored with indwelling nylon catheters. Mean heart rate was significantly higher ($P < 0.005$), and cardiac index, stroke volume index, and cardiac and stroke work were significantly lower ($P < 0.005$ for each) in patients who died as compared with those who survived. There was no significant difference in central blood volume or in pulmonary arterial or left ventricular end-diastolic pressure between the two groups. Analysis of data obtained during initial physiologic study after admission to hospital for acute infarction indicates that certain measurements are predictive of death. Thus, cardiac work exceeded 3.0 kg-m/min in all survivors but was above this value in only one of the patients who eventually died. Similarly, stroke work was above 30 g-m/beat in all but one survivor and below this value in all but two of those who died. Only six of the 14 patients who died were in shock at the time of the initial study. Time of death varied from 5 hr to 2 months (median 30 hr) after the first physiologic measurements. Cardiac and stroke work are derived from several variables and appear to be useful in identifying high-risk patients who may be candidates for unconventional therapy. (Supported by NIH contract PH-43-67-1439.)

274. Changes in Pulmonary Capillary Blood Volume during Menstrual Cycle and Oral Contraceptive Therapy. ANTHONY SEATON* AND N. LEROY LAPP,* Morgantown, W. Va. (introduced by Margaret Albrink**).

Pulmonary capillary blood volume (Vc) and membrane diffusing capacity (Dm) were calculated from duplicate measurements of single-breath transfer factor for carbon monoxide at varying oxygen tensions. 20 healthy young women were studied, of whom 13 were taking contraceptive pills (estrogen + progestogen) and seven were on no medication. These latter subjects were studied both in mid cycle and at or just before menstruation. The subjects taking no medication demonstrated a variation in Vc and Dm during the menstrual cycle, Vc becoming raised at the end of the cycle (mean 23.5 ± 3.68 [SD] ml/liter of total lung capacity [TLC]) and falling toward mid cycle (mean 19.8 ± 1.61 ml/liter). The Dm showed an alteration in the opposite direction (mean 14.1 ± 3.0 ml/min per mm Hg per liter of TLC at the menses, and 12.7 ± 2.1 ml/min per mm Hg per liter at mid cycle). The variation in Vc was significant ($t = 3.11$, $P < 0.05$), whereas that in Dm was not. The subjects taking contraceptive pills showed higher values for Vc than the controls (mean 25.05 ± 3.62 ml/liter), and this differed significantly from the findings in the controls at mid cycle ($t = 3.59$, $P < 0.05$). Dm in these subjects did not differ significantly from that in the controls (mean 12.7 ± 2.0 ml/min per mm Hg per liter). Three patients taking sequential pills have so far been studied, and these have shown an increase in Vc during the time when they take the progestogen-containing pill. These results suggest that progesterone has an effect on the pulmonary capillary bed, possibly causing vasodilatation. It is possible that increased Vc reduces the physiologic dead space, contributing to the lowered P_{CO_2} that occurs in the luteal phase of the cycle and in pregnancy.

275. Regulation of Cell-Free Hemoglobin Synthesis. DAVID SHAFRITZ,* JEFFREY GILBERT,* PHILLIP PRICHARD,* AND FRENCH ANDERSON,* Bethesda, Md. (introduced by Joseph E. Rall**).

A highly purified cell-free hemoglobin-synthesizing system from rabbit reticulocyte polysomes has been developed in our laboratory. This system is dependent on the addition of tRNA and three newly discovered initiation factors, M_1, M_2, and M_3, as well as on supernatant proteins (or on the known soluble transfer factors T_1 and T_2 when acylated tRNA is utilized). The system is highly active in the synthesis of complete new hemoglobin chains (3000 pmoles ^{14}C-valine per hr per mg ribosomes) which have been characterized by CM-cellulose chromatography, double-label tryptic peptide mapping, and N-terminal valine analysis. The ratio of α- to β-chain synthesis in this system can be reduced to 50% either by making tRNA rate limiting or by deleting a specific tRNA chromatographic fraction from the reaction mixture. The reticulocyte initiation factors were isolated by DEAE-cellulose and Sephadex G-200 chromatography from a 0.5 M KCl wash fraction of crude reticulocyte polysomes. Two of the factors, M_1 and M_2, can be assayed by their ability to stimulate poly U-dependent polyphenylalanine synthesis at low Mg^{++} concentration (5 mM). A third factor, M_3, in addition to M_1 and M_2, is required for natural initiation of new hemoglobin chains from endogenous mRNA in the cell-free system. In the presence of all three of these factors, hemoglobin synthesis is stimulated 10-fold. It is hoped that characterization of the individual initiation factors and of specific tRNA species will help to elucidate further the regulation of hemoglobin biosynthesis.

276. Acetylsalicylic Acid-Induced Hemolysis and Its Mechanism. N. T. SHAHIDI* AND D. W. WESTRING,* Madison, Wis. (introduced by R. F. Schilling**).

Acetylsalicylic acid (ASA) is known to induce severe hemolysis in some G6PD-deficient individuals. To study its mechanism, erythrocytes from a 7-yr-old boy with extreme

sensitivity to ASA were transfused to a normal compatible recipient. The administration of 150 mg/day of 2,5-dihydroxybenzoic (gentisic) acid, a known ASA metabolite with redox properties, to the recipient resulted in a 50% decrease in the survival of the patient's erythrocytes. Similar studies with red cells from individuals with A- and Mediterranean variant resulted in no alteration in the erythrocytes' survival. Incubation of the patient's erythrocytes with sodium gentisate (6 mM) resulted in 27% methemoglobin and a 60% decrease in GSH at the end of 2 hr. Similar studies with erythrocytes from an A- variant resulted in 12% methemoglobin and 40% decrease in GSH. Salicylate (10 mM) alone exhibited no oxidative properties; but it markedly enhanced the gentisate-induced hemoglobin and GSH oxidation in the patient's erythrocytes. Kinetic studies with partially purified G6PD from erythrocytes and in some instances fibroblasts from the patient, four normal individuals, two A- variant, and two Mediterranean variant revealed that in all instances sodium salicylate (10 mM) inhibited the G6PD by competing with NADP. Whereas, however, the change in $K_m(K_p)$ in individuals with A- and Mediterranean variant (refractory to therapeutic doses of ASA) did not significantly deviate from the normal, the K_m for NADP of the patient increased from 17.6 μM to the extremely high value of 123 μM. Gentisic acid was found in all instances to be a more potent inhibitor in that 5 mM produced an apparent change in K_m similar to that obtained with 10 mM of salicylate. The above studies suggested that enzyme inhibition by salicylate and gentisate play an important role in ASA-induced hemolysis.

277. Metabolic and Clinical Efficacy of Calcitonin in Paget's Disease. FLORENCE SHAI,* RICHARD K. BAKER,* AND STANLEY WALLACH, Brooklyn, N. Y.

The metabolic and therapeutic potential of calcitonin was studied in five patients with Paget's disease and in two patients with osteoporosis, using 2–8 MRC units per kg per day of porcine calcitonin (Armour) for 4–11 wk. Metabolic balance, hydroxyproline excretion, radiocalcium turnover, histology of iliac crest biopsies, and bone X-rays were assessed. In Paget's disease, calcitonin produced retention of calcium, with balance changing from approximately −150 to +50 mg/day. Urinary calcium decreased 25–50%, and therefore much of the retention resulted from increased gastrointestinal absorption of calcium. Calcitonin also caused natriuresis, phosphaturia, and increased gastrointestinal phosphorus absorption, summating in sodium loss and phosphorus retention. No significant effects on magnesium, potassium, and nitrogen balances were observed. Urinary hydroxyproline and radiocalcium turnover decreased 25–50%. Fasting serum calcium levels were unchanged. Elevated alkaline phosphatase values decreased 20–50% in three patients and were unchanged in two. Clinical effects of treatment included reduced bone pain and skin temperature over involved bones, improved cardiac compensation, and, most importantly, objective reversal of neurologic deficits due to vertebral column involvement. In osteoporosis, calcitonin caused more negative or minimally positive calcium balance, insignificant changes in other parameters, and questionable clinical improvement. No allergic reactions, hepatic or renal toxicity, bone marrow depression, electrocardiographic changes, or blood chemical or serologic abnormalities were encountered. These data indicate a beneficial effect of calcitonin on the metabolic and clinical parameters of patients with Paget's disease. The reversal of neurologic deficits suggests that pagetoid bone can remodel effectively under the influence of calcitonin, an inhibitor of bone resorption. Long-term therapeutic trials of calcitonin in patients with Paget's disease are justified by these findings.

278. Inhibition of *p*-Nitrophenyl Phosphatase Activity of the Microsomal Fraction of the Turtle Bladder Mucosal Cells. Y. E. SHAMOO,* W. N. SCOTT,* AND W. A. BRODSKY,** New York, N. Y.

Microsomes from epithelial cells of bladders of *Pseudemys scripta* turtles catalyze the hydrolysis of *p*-nitrophenyl phosphate (NPP; optimal pH 7.2–7.3). This activity (NPPase) requires Mg^{++} and K^+, and is inhibited by ouabain. The degree of ouabain inhibition is reduced in a competitive way by increasing K concentration of the medium. Thus, 5×10^{-8} M ouabain is required for 50% inhibition when K = 1.9 mM; and 5×10^{-7} M ouabain is required when K = 10 mM, which indicates that the ouabain sensitivity of the NPPase reaction in these microsomes is greater than that reported for other tissues. The ouabain-induced inhibition was independent of the time of preincubation of the microsomes with ouabain over the range of 5 to 30 min. Sodium also inhibits NPPase in a manner similar to that induced by ouabain; i.e., the degree of Na-induced inhibition is reduced in a competitive way in increasing K concentration of the medium. Complete inhibition of NPPase was achieved by 100 mM Na in the presence of 10 mM K (the optimal Na and K concentrations for ATPase activity of the same microsomes). On the other hand, *N*-ethylmaleimide (NEM)-induced inhibition of ATPase, which is independent of K concentration, is dependent upon the time of preincubation of microsomes with NEM. For example, 50% inhibition can be achieved after 32 min of incubation with 5×10^{-3} M NEM at 37°C, and after 47 min of incubation with 10^{-3} M NEM. ATP, 0.5–1.2 mM, inhibits NPPase activity by 30–50% in the presence of NPP, 4.0 mM. Apparently ATP competes with NPP as the substrate. (Supported by grants from the NIH, the NSF, and the National Aeronautics and Space Administration).

279. Congenital Factor X Dysproteinemia: A Structural Disorder of Coagulation Factor X. SANDOR S. SHAPIRO,* BRUCE T. CHODOSH,* AND DAVID L. ARONSON,* Philadelphia, Pa., and Bethesda, Md. (introduced by Allan J. Erslev**).

A new one-stage assay for plasma factor X activity has been developed, based on the activation of purified human prothrombin by Russell viper venom. The range (±2SD) of plasma factor X in 20 normal individuals is 54–146%. Excellent agreement was found between this assay and one based on correction of a congenitally deficient substrate. An immunoassay has been developed utilizing an antiserum, produced in rabbits, to a highly purified human factor X

preparation. The range (±SD) of immunoreactive factor X in 12 normal plasmas is 45–155%. Five patients with congenital factor X deficiency, from four unrelated families, were studied with these techniques. In four patients severe deficiency of factor X (<2%) was demonstrated both by bioassay and by immunoassay. The fifth patient, a young boy whose parents both have half normal levels of factor X activity, had a bioassayable level of 4%, but an immunoassayable level of 30%. The immunoreactive factor X was insensitive to activation by tissue thromboplastin as well as by Russell viper venom. It has an electrophoretic mobility indistinguishable from that of normal factor X, and shows a line of identity with its normal counterpart on Ouchterlony double diffusion. Factor X levels in the father are similar by bio- and immunoassay, whereas the patient's mother has an immunoreactive factor X level considerably higher than that obtained by bioassay. It thus appears that the patient is doubly heterozygous for factor X deficiency, having one gene producing no recognizable protein and a second producing an immunologically identifiable but biologically inactive factor X molecule. (Research supported in part by grants from the NIH.)

280. Synthesis, Storage, and Secretion of Parathyroid Hormone. LOUIS M. SHERWOOD,* JOHN S. RODMAN,* WALTER B. LUNDBERG,* AND JEROME TARGOVNIK,* Boston, Mass. (introduced by Irving H. Goldberg).

Although it is known that the secretion of parathyroid hormone (PTH) is regulated by Ca^{++} and Mg^{++}, the detailed effects of these cations on hormone synthesis, storage, and secretion have not been elucidated. Bovine parathyroid glands were incubated in organ culture for 24–72 hr with ^{14}C-leucine in varying concentrations of divalent cation (Ca^{++} plus Mg^{++}). The medium and a tissue extract were chromatographed on Sephadex G-100; simultaneous measurements of radioactive protein and immunoreactive PTH in each fraction were made. Leucine was rapidly incorporated into PTH, but labeled hormone was released into the medium only after 3–6 hr. Hormone synthesized in the tissue (peak of radioactivity corresponding to immunoreactive PTH) was inversely proportional to cation and varied between 2210 and 882 cpm/mg tissue protein as cation was increased from 1.5 to 3.5 mM. Tissue storage of hormone, on the other hand, was diminished in highly stimulated tissue (4410 mμg PTH per mg at 1.5 mM as compared with 7150 mμg at 3.5 mM). The release of labeled and immunoreactive unlabeled PTH was inversely proportional to cation concentration and thus paralleled changes in hormone synthesis. PTH secretion was independent of the Ca^{++}/Mg^{++} ratio and depended only on the sum of the two. In contrast to earlier observations in vivo, which suggested a strictly proportional relation between calcium and PTH release, the current data show a first-order relation, with a 0.5 mM increase in cation causing a 50% fall in hormone secretion. These studies provide direct evidence for parallel changes in the synthesis and secretion of PTH in response to variations in cation concentration, and suggest the existence of a reversible metal ion-ligand interaction in the gland. (Research supported by grants from the NIH and The Medical Foundation.)

281. Transacylation of Fatty Acid from Phosphatidylcholine to Phosphatidylethanolamine: A Stage in the Catabolism of Erythrocyte Phospholipid. STEPHEN B. SHOHET,* Boston, Mass. (introduced by Park S. Gerald**).

The relative concentration of phosphatidylcholine (PC) in an artificial membrane influences its permeability characteristics; moreover, increased RBC-PC has been associated with hemolytic anemia. Two independent paths exist for regulation of RBC-PC levels: (1) passive balanced exchange of preformed membrane PC with plasma PC, and (2) active assembly of PC within the membrane from plasma fatty acid (FA) and lyso-PC. Since RBC-PC does not increase during circulation, some means of release of actively assembled PC-FA must exist. In fact, we have previously shown that FA is released to fresh serum from RBC-PC labeled by this route. To define the mechanism of this release, human RBC phosphatides were actively prelabeled with ^{14}C-FA. Upon reincubation in fresh serum, RBC radioactivity was observed to fall in PC and rise in phosphatidylethanolamine (PE) and neutral lipids. These data suggested FA transesterification from PC to PE before release to serum. To rule out possible conversion of PC to PE by either demethylation or base exchange, which would simulate transacylation, cells containing PC actively labeled with both ^{14}C-choline and ^{3}H-FA were reincubated. A major drop in ^{3}H-FA in PC and a rise in ^{3}H-FA in PE occurred without any appearance of ^{14}C in PE. Similar experiments with cells reincubated with ^{14}C-ethanolamine showed no ^{14}C-ethanolamine incorporation into cell PE, although FA transfer was again demonstrated. These data indicate a pathway for the catabolism of RBC-PC-FA via FA transfer to PE before release to serum. Neither degradative demethylation nor base exchange–mediated conversion of PC to PE occurs. Abnormalities in this pathway can modify cell phospholipid composition and, in turn, may affect cell membrane permeability. (Research supported by NIH grant HD-02777.)

282. The Induction of Human Muscle Mitochondrial Proliferation and Increased Glycogen and Triglyceride Synthesis by Long-Term Exercise. FLOYD A. SHORT,* RUSSELL ROSS,* LEONARD A. COBB,* AND THOMAS E. MORGAN, Seattle, Wash.

10 male human volunteers exercised one leg by 2 hr of submaximal bicycle pedaling daily for 1 month. This exercise produced an average 2.3 cm increase in thigh girth and increased capacity for exercise on the bicycle ergometer. At the end of the month, both quadriceps femoris muscles were biopsied under local anesthesia. Mitochondria isolated from both exercised and contralateral nonexercised muscles were shown to have normal respiratory control and tightly coupled oxidative phosphorylation. Mitochondrial oxygen utilization increased 52% in response to exercise training and was paralleled by highly significant increases in mitochondrial protein, oxidative enzymes, and phospholipid. Morphometric analyses of mitochondria were made on electron micrographs; there were significant increases ($P < 0.05$) in mitochondrial number and cross-sectional area and a decrease in surface-to-volume ratio. The increased ability of trained

muscle to oxidize substrates is accompanied by a 2-fold increase in resting intracellular glycogen and significant increases in glycogen synthetase (glucose-6-phosphate dependent) and hexokinase ($P < 0.025$). Other glycolytic enzymes were not increased in the exercised muscle. In vitro studies of fatty acid utilization showed increased incorporation of fatty acid into intracellular triglyceride in response to exercise training. Fatty acid entry into muscle, triglyceride synthesis, and fatty acid oxidation were stimulated 3- to 5-fold by addition of glucose. Carnitine and acyl carnitine transferase, present in large amounts, did not respond to training. We conclude that exercise training increases muscle mitochondria, the capacity to oxidize substrates, and the synthesis of two intracellular energy sources, glycogen and triglyceride.

283. Cell Cultures of Chondrocytes and the Metabolism of Cartilage. HERBERT J. SHULMAN* AND KARL MEYER,* New York, N. Y. (introduced by John Sandson).

Chondrocytes produce an altered cartilaginous matrix with aging and in disease states such as osteoarthritis. This matrix contains collagen and protein polysaccharides, the latter being macromolecular with chondroitin-4-sulfate, chondroitin-6-sulfate, and/or keratan sulfate chains attached covalently to a protein core. Keratan sulfate is essentially absent at birth and increases with advancing age. Because chondrocytes are embedded in this tough, insoluble matrix, the study of the regulatory mechanisms involved in matrix metabolism has been difficult. To approach this problem we have studied a cell culture system of chicken embryonic chondrocytes which can produce a cartilaginous matrix in culture. Primary cultures were established and grown in the presence of $Na_2{}^{35}O_4$, ^{14}C-glucose, or ^{3}H-glucosamine. The matrix was analyzed and found to contain a protein polysaccharide with chondroitin-4-sulfate and keratan sulfate chains. This macromolecule was similar to one purified and characterized from adult chicken cartilage, but its composition was unlike that of chicken embryonic cartilage, which contained essentially no keratan sulfate. Embryonic chondrocytes in culture thus produce an "older" matrix than they do in vivo. Factors which influence the differentiated state of chondrocytes in culture and thus the character of the matrix produced have been the subject of continuing study. It is suggested that chondrocyte cultures, including human articular chondrocytes, represent a promising approach to elucidating the molecular basis for maturation, aging, and disease processes in cartilage. (Supported by grants from the NIH and the John A. Hartford Foundation.)

284. Treatment of Paget's Disease with Salmon Calcitonin. FREDERICK R. SINGER,* ROBERT M. NEER,* JOHN A. PARSONS,* STEPHEN M. KRANE, AND JOHN T. POTTS, JR., Boston, Mass.

Several recent studies have indicated that calcitonin may be useful in the treatment of generalized Paget's disease. These clinical trials utilized porcine calcitonin (PCT). We have evaluated salmon calcitonin (SCT) because we have found that it is much more potent, on a molar basis, than PCT in man. In three patients the comparative fall in plasma calcium produced by the two hormones indicated that SCT was 50- to 150-fold more potent than PCT. In one patient as little as 0.2 μg SCT i.v. lowered urinary hydroxyproline excretion progressively from 677 to 418 μg/mg creatinine by 6 hr. Greater and more prolonged effects were found with increasing doses of 0.6 μg, 4 μg, and 40 μg SCT. With 40 μg SCT the values reached 220 μg/mg creatinine. A total of six patients with Paget's disease have been treated for periods of up to 11 wk with calcitonin. Hydroxyproline excretion decreased as much as 60%. Effective doses were small enough to permit not only intramuscular but also subcutaneous administration. This suggested the feasibility of self-treatment at home; in one patient this was confirmed in a preliminary study of several weeks' duration. There was no evidence of toxicity, antibody formation, or tachyphylaxis in any patient. The reason for the extraordinary potency of SCT in man has not yet been established. Since the amino acid sequence of SCT differs markedly from that of human and other mammalian calcitonins, resistance of the salmon hormone to degradation after injection may account for its greater potency.

285. Pathogenesis of Hyperparathyroidism in Chronic Renal Disease. E. SLATOPOLSKY,* S. CAGLAR,* J. P. PENNELL,* D. TAGGART,* J. M. CANTERBURY,* E. REISS, AND N. S. BRICKER, St. Louis, Mo., and Chicago, Ill.

We have recently proposed a mechanism for the pathogenesis of hyperparathyroidism in advancing renal disease which (1) gives central importance to phosphate retention as an initiating event, and (2) depicts hyperparathyroidism as a continuum beginning with the earliest loss of nephrons and progressing throughout the course of chronic renal disease. The hypothesis states that with nephron loss, transient phosphate retention occurs and ionized calcium falls reciprocally. PTH secretion thereby increases and phosphate excretion per nephron rises, restoring plasma phosphate and then calcium levels toward normal. But PTH levels must remain elevated, increasing with each wave of nephron loss. The hypothesis has been subjected to experimental examination. The nephron population of dogs was reduced sequentially by ligating branches of the renal arteries. Studies were performed serially. PTH was measured by radioimmunoassay using an antiserum prepared against bovine PTH. Hyperparathyroid serum (from a nephrectomized dog) served as a reference standard. One group of animals received normal phosphate intake. With each period of nephron reduction, GFR fell, TRP fell, and PTH levels rose from normal to values 10 to 30 times normal. These extremely high values are comparable to those observed in uremic man. Identical studies were performed on dogs receiving a phosphate-deficient diet. Although GFR decreased progressively, TRP remained high throughout, and, of great importance. PTH levels did not rise above normal even with high-grade uremia. When phosphate was given to these uremic dogs, TRP fell markedly and PTH levels rose strikingly. Thus, phosphate retention does appear to play a central role in the pathogenesis of secondary hyperparathyroidism; and when phosphate is provided, secondary hyperparathyroidism does begin early and increase in magnitude throughout the course of chronic renal disease.

286. The Source of Abnormal Bile in Patients with Cholesterol Gallstones. DONALD M. SMALL AND SEPPO RAPO,* Boston, Mass.

Cholesterol gallstones are formed when excess cholesterol precipitates from bile supersaturated with cholesterol. Either the gallbladder, by alteration of the composition of normal hepatic bile, or the liver by production of an abnormal bile, could be the source of the bile supersaturated by cholesterol. Since cholesterol gallstones are very prevalent among American Indians of the Southwest, we have analyzed their gallbladder and hepatic bile to determine whether the liver or the gallbladder is responsible for the disease. Among 30 American Indians undergoing cholecystectomy, 29 had cholesterol and one had pigment gallstones. We analyzed 19 gallbladder, 23 hepatic, and 6 T-tube biles obtained from these patients. A small sample of T-tube bile was collected immediately after the tube had been clamped for 24 hr to reestablish normal enterohepatic circulation. The mean relative composition of gallbladder bile in moles/100 moles of total bile salts, phospholipid, and cholesterol was: bile salts 71.5%, phospholipids 19.5%, cholesterol 9%; while simultaneously collected hepatic bile was: bile salt 58%, phospholipids 25%, cholesterol 17%. When plotted on triangular coordinates, the composition of the gallbladder bile indicated that it was saturated with cholesterol, whereas the composition and physical state of hepatic bile indicated that it was a liquid highly supersaturated with cholesterol. Five of six T-tube biles collected 7–12 days after operation were also supersaturated with cholesterol. Thus, hepatic bile collected at surgery and T-tube bile collected after reestablishment of a normal enterohepatic circulation are supersaturated with cholesterol, but the gallbladder bile in contact with the cholesterol stones is only saturated. These findings suggest that in American Indians with cholesterol gallstones the liver secretes a supersaturated bile which precipitates its excess cholesterol in the gallbladder. (Research supported by grant AM-11453 from the NIH.)

287. Fetal Bile Salt Absorption. RICHARD A. SMALLWOOD,* ROGER LESTER, ANNE S. BROWN,* GEORGE J. PIASECKI,* AND BENJAMIN T. JACKSON,* Boston, Mass.

We have previously established that bile salt is readily excreted by the fetal liver, concentrated by the gall bladder, and delivered into the intestine. It is not known, however, whether the fetal intestine can reabsorb bile salt and thus complete the enterohepatic circulation. Fetal taurocholate absorption was therefore investigated in vivo. Near-term fetal dogs were prepared with bile duct catheters. ^{14}C-taurocholate was instilled into, or perfused through, isolated 10 cm segments of bowel. Studies were performed under light anesthesia with the fetus *in utero,* and with fetal and uterine incisions closed. Absorption was quantitated by disappearance of ^{14}C label from the perfusate, and by its appearance in fetal bile. Within 3 hr, 80–85% of the radiolabel instilled into the ileum, and 60–70% of that instilled into the jejunum, appeared in fetal bile. Over nine-tenths of the excreted label was identified as ^{14}C-taurocholate. Ileal segments retained 0–2% of the dose; jejunal segments, 8–12%; fetal liver, 0–9%; total recovery, 70–95%. Maximal rates of ileal absorption were twice jejunal rates (0.6 vs. 0.3 μmole/hour) and were attained 20 min earlier. Perfusion experiments showed 0.1–0.2 μmole/hour of taurocholate absorbed from the ileum, and 0.05–0.15 μmole/hour from the jejunum. This study provides the first direct evidence of a fetal enterohepatic circulation of bile salt. Unlike that in the adult, however, the fetal mechanism for ileal bile salt transport is only partially developed, and absorption from the jejunum is nearly as efficient as absorption from the ileum. Thus the enterohepatic circulation in the fetus may partially, or largely, bypass the ileum. We conclude that, although the near-term fetal liver is remarkably mature in its handling of bile salt, the fetal intestine has not developed an adult pattern of absorption.

288. The Role of Polyribophosphate in Immunity to *Hemophilus influenzae* b. DAVID H. SMITH,* PORTER ANDERSON,* RICHARD B. JOHNSTON, JR.,* A. IZZET BERKEL,* AND JANE PITT,* Boston, Mass. (introduced by Charles A. Janeway**).

All *Hemophilus influenzae* causing systemic infections are encapsulated strains, of which 95% or more are type b. The capsule of *H. influenzae* b is a polymer of ribose phosphate (PRP), and antibodies producing passive protection are absorbable from antiserum by PRP. Therefore, PRP has been purified for consideration as an immunogen. Methods have been devised which give a high yield of a preparation that contains <0.5% nucleic acid and <0.02% protein and that is homogeneous in size (molecular weight ≥200,000), resistant to degradation by shear and heat, nontoxic for mice and guinea pigs, and nonpyrogenic for rabbits in doses immunogenic for man. The distribution of anti-*H. influenzae* b antibodies has been assayed by passive hemagglutination, bactericidal capacity, and opsonization in the sera of individuals who are either normal, convalescent from *H. influenzae* b infection, or immunized with purified PRP. Titers of the three antibody activities vary in randomly selected subjects, but all increase markedly during convalescence and in adults immunized with as little as 10 μg of PRP. Hemagglutinating antibody is primarily 19S globulin; type-specific bactericidal activity is mediated primarily by 7S globulin and can be adsorbed from serum by PRP. Both bactericidal and opsonizing activities require complement. Immune subjects vaccinated intradermally have a local tuberculin-type reaction which is maximum at 36–48 hr, and is apparently not related to antibody titers. These observations prompted tests of the in vitro mitogenic activity of PRP. In cultures of lymphocytes from "sensitized" persons, PRP at concentrations as low as 0.001 μg/ml stimulates ^{3}H-thymidine incorporation into DNA. It thus appears that PRP is important for both humoral and cell-mediated immune responses to *H. influenzae* b.

289. Metabolism of Plasma Retinol-Binding Protein in Man. FRANK REES SMITH* AND DEWITT S GOODMAN, New York, N. Y.

Vitamin A is transported in human plasma by a specific protein, retinol-binding protein (RBP). RBP has a molecular weight of about 21,000, has a single binding site for one

molecule of retinol, and circulates as a protein-protein complex together with prealbumin. RBP was purified 1500- to 2000-fold by procedures described previously. Pure RBP was injected into rabbits and an anti-human RBP antiserum prepared. A radioimmunoassay for RBP was developed using ^{131}I-RBP and a double-antibody precipitation technique. In the immunoassay the standard curve obtained with normal plasma is identical with that with pure RBP. Duplicate samples differ from their mean by $5 \pm 5\%$ ($\pm$ 1SD). The immunoassay accurately measures RBP in the amounts of 0.01–0.1 μg per assay tube. The mean plasma values $\pm$ SEM of RBP in a group of normal subjects were 47 ± 2 μg/ml for 39 males and 41 ± 2 μg/ml for 37 females. Plasma RBP levels were markedly depressed (15 ± 2.3 μg/ml) in 14 patients with acute hepatitis. There was a significant correlation between plasma RBP and vitamin A levels in both normal and diseased subjects. In these subjects RBP was generally saturated with retinol; the data suggest that RBP circulates almost exclusively as the holoprotein. The turnover of plasma RBP was studied in vivo by injecting purified ^{131}I-RBP intravenously into normal volunteers. The plasma disappearance curves conformed to a simple two-term exponential equation with a final half-time of disappearance of 11.7 hr. The metabolic clearance rate (MCR) of RBP in 11 normal subjects was 8.1 ± 0.5 liter/day per m^2 (mean $\pm$ SEM), and the production rate was 321 ± 22 mg/day per m^2. The PR of RBP was significantly correlated with the plasma vitamin A level. In molar terms, the turnover of plasma RBP appears to be at least as rapid as that of vitamin A. (Supported by NIH grant AM-05968.)

290. Production of Septic Survival by Administration of Lymphocytes Stimulated In Vitro with Phytohemagglutinin. HENRY D. SOLTYS* AND JEROME I. BRODY, Philadelphia, Pa.

The purpose of this investigation was to determine whether, after the in vitro stimulation of lymphocytes with phytohemagglutinin (PHA), these cells, or their biosynthetic products, might be effective anti-infectious agents capable of protecting rabbits against lethal pneumococcal bacteremia. The present study extends completed work in this laboratory which has shown that PHA given to rabbits directly elicits high-titer serum pneumococcal agglutinins, facilitates neutrophilic phagocytosis of bacteria, and enhances reticuloendothelial clearance of these microorganisms. Since PHA is potentially toxic, an alternate approach was designed. This experimental protocol consisted of harvesting peripheral blood lymphocytes from two groups of rabbits, A and B, by arterial bleeding and leukapheresis. Subsequently, neutrophil-free lymphocytes were obtained by dextran sedimentation and differential centrifugation and placed in cell culture. Lymphocytes from rabbits in group A were stimulated with PHA while those from group B remained unprovoked. After 72 hr, cells and culture supernatants were separated. After resuspension, A and B cells were returned to their original donors, and culture supernatants, presumably containing released cellular products, were infused into two additional, homologous animal groups. All rabbits, including a third untreated set, then received intravenous virulent pneumococci. Within 72 hr all control rabbits and those given unstimulated culture components developed fever and positive blood cultures, and ultimately died. Importantly, the two rabbit groups that received either cells or supernatants from PHA-stimulated cultures remained healthy and survived. These observations indicate that the biologic effects of PHA may be indirectly mediated by cells or their products after exposure to this mitogen and that mammalian host defense may be augmented in this unique manner. (Supported by NIH grants.)

291. Differences in Carbohydrate Content between Myeloma and Normal Immunoglobulin Light Chains. HAROLD C. SOX,* Bethesda, Md. (introduced by John L. Fahey).

Light chains of 15% of serum myeloma protein contain a carbohydrate prosthetic group. Myeloma proteins have appeared to be representative of normal serum immunoglobulins. Thus, it seemed likely that a comparable proportion of light chains from normal serum immunoglobulins would also contain carbohydrate. Normal serum immunoglobulin light chains isolated from a pool of normal humans and from three normal individuals were analyzed for carbohydrate by two methods. Glycopeptides were isolated from light chains by enzymatic digestion followed by ion exchange and gel filtration chromatography. In addition, light chains were acid hydrolyzed for quantitative analysis of glucosamine. Both approaches showed that the amount of carbohydrate peptide was at most 1 mole per 100 moles of light chain above the level of heavy-chain contamination (about 0.5 mole/100 moles as determined by acrylamide gel electrophoresis). Thus, although 12–15% of light chains from serum myeloma proteins contained carbohydrate, no more than 1% of normal light chains contained carbohydrate. These data indicate that a large sample of myeloma proteins differs significantly from normal serum immunoglobulins by the presence of a carbohydrate prosthetic group on light chains. The cell population subject to the myeloma carcinogenic process is different from that responding to antigenic stimulation.

292. Hypoxemia-Induced Pulmonary Edema. J. RICHARD SPEARS,* K. GOPINATHAN,* D. SAROJA,* JACKSON H. STUCKEY,* AND GEORGE EMMANUEL,* Brooklyn, N. Y. (introduced by Ludwig W. Eichna**).

The role of acute hypoxemia in the production of pulmonary edema and its possible mediation through humoral mechanisms were studied in five dogs under pentobarbital anesthesia. Extravascular lung water volumes were calculated from indicator dilution measurements of labeled red cells, albumin, and water during air breathing and during 1 hr of 5% oxygen breathing. Serial blood samples were analyzed for whole-blood histamine and plasma serotonin levels. Pulmonary arterial, pulmonary artery wedge, left atrial, and systemic pressures, pulmonary vascular resistance, pulmonary compliance, and airway resistance were determined throughout the experiments. Wet/dry lung weight ratios and histological findings were compared. The volume of extravascular lung water increased in two dogs from 3.2 to 4.8 ml/kg body wt and 2.9 to 4.5 ml/kg body wt respectively, and did not change in three. Pulmonary artery pressure increased in all

dogs from an average of 35/15 and 57/23 mm Hg; the largest increase in extravascular lung water was observed in the dog with the highest elevation of pulmonary artery pressure. Pulmonary capillary pressure remained unchanged. Pulmonary vascular resistance averaged 215 dynes·sec·cm^{-5} during air breathing and 222 dynes·sec·cm^{-5} during hypoxemia. Histamine and serotonin levels decreased during hypoxemia from 0.029 to 0.022 μg/ml and from 0.12 to 0.09 μg/ml respectively. In the absence of pulmonary capillary hypertension, hypoxic vasoconstriction of pulmonary arterioles may be a mechanism of production of acute pulmonary edema. (Supported by USPHS grant HE-11840.)

293. Ribosomal Ribonucleic Acid Metabolism in Erythroid Differentiation. JERRY L. SPIVAK,* C. JANE MARMOR,* AND HERBERT W. DICKERMAN,* Baltimore, Md. (introduced by C. Lockard Conley**).

Induced erythropoiesis in the mouse spleen was employed as a model system for study of differentiation of erythroid cells. Normally not an erythropoietic organ, the spleen of the C57B1/6J mouse becomes the major site of red cell production when hematopoiesis is activated. During response to phenylhydrazine-induced hemolytic anemia there was a 5-fold increase in spleen weight and an increase in hemoglobin-containing nucleated cells to 70% of total splenic cell population. Previous studies have shown a parallel increase in the activities of porphyrin biosynthetic enzymes. At the peak of splenic erythropoiesis, there was a 2-fold increase in splenic RNA concentration (mg/g wet weight) as compared with controls. A similar change in DNA concentration occurred a day after peak erythropoiesis. The bulk of the accumulated RNA was ribosomal RNA (rRNA). Methods employing polyacrylamide gel electrophoresis were developed to study the in vitro incorporation of precursors into rRNA during the cycle of erythroid differentiation. The specific activities of 28S and 18S rRNAs increased 15-fold 3 days before peak erythropoiesis and diminished abruptly on the day after maximal incorporation. This increase in rRNA specific activity was tissue specific and sensitive to inhibition at low concentrations of actinomycin D. The increased incorporation of precursors into rRNA was accompanied by an increased nucleolar RNA content of erythroblast nuclei, demonstrated by histochemical techniques, and the RNA content of nucleoli diminished in parallel with the decrease in rRNA incorporation despite the marked accumulation of cytoplasmic rRNA. These data indicate that the control of ribosome formation is an early event in the differentiation of erythroid cells. (Research supported by grants from the NIH.)

294. Genetic Heterogeneity of the Mediterranean Glucose-6-Phosphate Dehydrogenase Deficiency. GEORGE STAMATOYANNOPOULOS,* VOLKER VOIGTLANDER,* P. KOTSAKIS,* AND A. AKRIVAKIS,* Seattle, Wash., and Athens, Greece (introduced by Arno G. Motulsky**).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is common in persons of Mediterranean ancestry and is usually supposed to be due to a mutant enzyme called the Mediterranean variant. In this investigation the hypothesis of genetic heterogeneity of this condition was tested by studying 79 Greek males whose red cells showed severe G6PD deficiency. Partially purified G6PD preparations were examined by electrophoresis, substrate analogue utilization, K_m for G6P and NADP, and pH-dependent activity. G6PD electrophoresis revealed two variants: one fast, in three persons, kinetically similar to G6PD Union or G6PD Markham and called G6PD U-M; one slow, in eight persons, kinetically different from all described G6PD mutants and called G6PD Orchomenos. Deamino-NADP (dNADP) utilization (expressed as percentage of NADP utilization) subdivided the remaining 68 cases into two nonoverlapping groups: 12 samples with low dNADP utilization (152.7 ± 12) were considered to have an "Athens-like" G6PD; 56 samples with high dNADP utilization (312.9 ± 27) were considered to have the Mediterranean mutant. 2-Deoxyglucose-6-phosphate utilization (expressed as percentage of G6P utilization) separated the 79 samples into four nonoverlapping ($P < 0.0001$) groups with the following mean values: G6PD Athens-like 1.54 ± 4, G6PD Mediterranean 50.3 ± 6, G6PD Orchomenos 104.8 ± 9, G6PD U-M 200.3 ± 24. The 79 cases included 26 pairs of brothers, and in 22 of these pairs the brothers had the same G6PD deficiency gene, because their mother was a Gd^+/Gd^- heterozygote. There was a high degree of concordance in G6PD characterization between the two members of each of these 22 pairs. These data suggest that Mediterranean G6PD deficiency is heterogeneous and this group comprises at least four G6PD mutants. Their relative frequency among the males examined was G6PD U-M 5%, Orchomenos 7%, Athens-like 18%, Mediterranean 70%.

295. Translational Control of Protein Synthesis during the Immune Response. JASON STARR AND DAWN WILLIS,* Memphis, Tenn.

Protein synthesis is regulated by both the rate of transcription of mRNA and the rate of translation of ribosome-bound messenger. Transcriptional control, as defined in the Jacob-Monod theory, is predominant in microorganisms. In mammalian cells, translational control, by a variety of mechanisms, is equally important. We have demonstrated previously that primary immunization of rats with *Salmonella* flagella elicits a burst of synthesis of all forms of RNA, including unstable and relatively stable mRNA. Coincidentally, the efficiency for translation of endogenous and exogenous template by spleen microsomes and ribosomes is increased transiently, peaking at 3 days after immunization. This period coincides with the maximal cell proliferation, but precedes the detectability of circulating antibody. One factor in the increased efficiency of immunized microsomes was shown to be the inactivation of a membrane-bound inhibitor. Soluble factors were also shown to be involved, since control supernatant preparations were essentially inactive with NH_4Cl-washed liver ribosomes, whereas preparations from immunized spleens approximated liver supernatants in promoting amino acid incorporation. Spleen and liver pH 5 supernatants were resolved by published methods into fractions containing transferase I (aminoacyl-tRNA–binding fac-

tor) and transferase II (translocase). By appropriate supplementation experiments, unimmunized spleens were shown to contain normal amounts of transferase II, but little or no transferase I, whereas immunized spleen contained both. Extensively washed ribosomes from control and immunized spleens were indistinguishable in translational efficiency when supplemented with both transfer factors. These results indicate that protein synthesis by spleen after immunization is stimulated by at least two mechanisms, namely, inactivation of a microsome-bound inhibitor, and activation, synthesis, or stabilization of transferase I. (This research was supported by grant E-458 from the American Cancer Society.)

296. The Measurement of Cyclic Nucleotides by Radioimmunoassay. ALTON L. STEINER,* CHARLES W. PARKER, AND DAVID M. KIPNIS, St. Louis, Mo.

We have described a sensitive and specific radioimmunoassay for cyclic AMP (cAMP) which allows measurement of the nucleotide in 5–10 mg of tissue and eliminates the need for chromatographic separation of cAMP from other tissue nucleotides. This assay is based upon competition of the cyclic nucleotide with isotopically labeled cyclic nucleotide derivative for binding sites on specific antibody. Specific radioimmunoassays have now been developed for the purine nucleotides cGMP and cIMP and the pyrimidine nucleotide cUMP. Antibodies to cGMP, cIMP, and cUMP were obtained by immunizing rabbits with an antigen prepared by conjugating the 2′-*O*-succinyl derivative of the cyclic nucleotide with keyhole limpet hemocyanin. High specific activity derivatives of the cyclic nucleotides (>150 c/mmole) were prepared by iodinating (^{125}I) the tyrosine methyl ester derivative of the succinylated cyclic nucleotide. Free and antibody-bound ^{125}I-labeled cyclic nucleotides were separated by the "second antibody" technique. Binding equilibrium is reached in 24 hr, but sensitive and reproducible assays can be obtained after 4–6 hr incubation. Sensitivity and range of the various radioimmunoassays are (picomoles per tube): cAMP, 0.2–20; cGMP, 0.03–2.0; cIMP, 0.2–20.0; and cUMP, 0.1–10.0. Structurally related purine and pyrimidine nucleotides and nucleosides exhibited <0.005% competitive binding in all immunoassays except cIMP, 0.5% in cGMP assay; cGMP, 1% in cIMP assay; and cTMP, 1% in cUMP assay. cAMP levels in rat adipose tissue, liver, kidney, skeletal muscle, and pituitary are 20- to 100-fold greater than cGMP and can be increased independently of concomitant changes in cGMP (e.g., ACTH and epinephrine–adipose tissue; anoxia–cerebral cortex; glucagon-liver). cGMP (0.02–0.05 μmole/kg) in mouse cerebral cortex, thalamus, and hypothalamus is 2–5% of cAMP, but is 5- to 10-fold elevated in cerebellum (0.2–0.4 μmole/kg). Preliminary studies indicate that cIMP and cUMP in rat liver, if present, are less than 0.01 μmole/kg.

297. Porcine Insulin, Proinsulin, and Connecting Peptide Response to Various Stimuli. RALPH W. STOLL,* JAN L. TOUBER,* JOHN W. ENSINCK, AND ROBERT H. WILLIAMS,** Seattle, Wash.

Using a radioimmunoassay for proinsulin (PI), another for the connecting peptide linking the A and B chains (CP), and a third for insulin (I), we have studied their changes in peripheral swine plasma in response to various stimuli. The PI immunoassay was specific for proinsulin, the CP antibody reacted on an equimolar basis with proinsulin and the connecting peptide but not with insulin, and the I antibody reacted three times more effectively with insulin than with proinsulin. Results are expressed in moles $\times 10^{-13}$/ml ± SEM above control values at the time (t) in minutes of the maximal insulin response. The following tests in five or six pigs were performed after an overnight fast: i.v. glucose, I = 4.7 ± 0.7, CP = 6.2 ± 1.1 (t = 2); i.v. glucagon, I = 4.9 ± 1.1, CP = 5.1 ± 1.2 (t = 5); i.v. arginine, I = 2.0 ± 0.4, CP = 2.5 ± 1.0 (t = 2); i.v. glucose, tolbutamide, and glucagon, I = 12.0 ± 3.0, CP = 12.6 ± 2.1 (t = 20); oral glucose, I = 5.7 ± 1.5, CP = 5.2 ± 1.5 (t = 30). In a repeat oral glucose test after a 4 day fast, I = 3.4 ± 1.0, CP = 4.4 ± 3.2 (t = 30). At no time during these tests did PI change significantly from the fasting level of 2.1 ± 0.6. The qualitative equimolar response and time course of I and CP suggest that insulin and the connecting peptide are stored together in the secretion granule. However, porcine proinsulin release was not influenced by factors that increased the secretion of its connecting peptide and insulin. (Research supported by grants from the NIH.)

298. The Potentiality of Out-of-Cycle Acute Leukemic Cells to Synthesize DNA. PIERRE STRYCKMANS,* GUY DELALIEUX,* AND JOS MANASTER,* Brussels, Belgium (introduced by H. J. Tagnon**).

In human acute leukemia a large fraction of marrow and blood blasts are nonproliferating. The problem whether all the nonproliferating blasts are "dormant cells" retaining a latent potential for division, or rather "end cells" which have lost forever the capacity to proliferate and therefore are destined to die, is the subject of this study. Blood lymphocytes which represent dormant cells capable of dividing on appropriate stimulation are known to synthesize DNA after irradiation of their DNA with UV light. 16 leukemic patients (1 chronic myeloid, 5 acute lymphoblastic, and 10 acute myeloblastic) were studied. Venous blood white cells mixed with TC-199 were exposed to a UV source (2537 A) for 30 sec (11 ergs/mm^2 per sec). Calf serum and tritiated thymidine (^{3}H-Tdr) (8 μc/ml) were added, and this mixture was incubated for 2 hr at 37°C. Cell smears were processed for radioautography. The blood of CML contained myeloid cells at different stages of maturity: end cells (segmented granulocytes and metamyelocytes) and proliferating cells (myelocytes). After UV irradiation an uptake of ^{3}H-Tdr comparable to the uptake by the blood lymphocytes was found in 100% of the myelocytes, whereas only some metamyelocytes showed a very low uptake among the nondividing myeloid cells. In acute leukemia the percentage of blasts labeled after UV ranged from 95 to 100% in 14 untreated patients. It reached only 55% in one patient treated by 6-MP. In human acute leukemia the majority of nonproliferating blasts seem therefore only out of cycle and still able to synthesize DNA. All leukemic blasts should therefore be eradicated to cure this disease.

299. Nature of the Iodoproteins Formed after Injection of Labeled Thyroid Hormones. MARTIN I. SURKS* AND JACK H. OPPENHEIMER, Bronx, N. Y.

Injection of phenolic-ring iodine-labeled L-thyroxine ($T_{4\beta}$) and L-triiodothyronine ($T_{3\beta}$) into human subjects and rats results in the formation of small quantities of labeled plasma and tissue iodoproteins which cannot be extracted with 95% ethanol (nonextractable iodine, NEI). Experiments were performed to define the chemical nature of the iodinated constituents of these products. (1) Groups of rats were injected with a combined dose of ^{131}I-$T_{4\beta}$ and nonphenolic (α)-ring ^{125}I-L-thyroxine or ^{14}C-$T_{4\alpha}$ alone. After 4 days, labeled components from both rings of L-thyroxine were found in the iodoproteins of plasma, liver, kidney, heart, and skeletal muscle. (2) Pronase hydrolysates of rat liver and plasma NE^{125}I 3 days after injection of ^{125}I-$T_{3\beta}$ or 7 days after ^{125}I-$T_{4\beta}$ were analyzed by paper chromatography. Addition of ^{131}I-iodothyronines to all samples before extraction enabled the calculation of residual extractable ^{125}I-iodothyronines in the NE^{125}I hydrolysates. 30–40% of the net NEI radioactivity was recovered at T_3 and T_4, an equivalent amount as 3-iodotyrosine, and the remainder as ^{125}I-iodide ($^{125}I^-$). Similar data were obtained in rats in which the NE^{125}I was labeled endogenously by multiple injections of $^{125}I^-$. The hormonal ^{125}I in the NE^{125}I hydrolysates greatly exceeded the contamination from residual extractable ^{125}I-iodothyronine. These data showed that NEI is composed, in part, of a hormone-protein complex. In other experiments, the terminal $t_{\frac{1}{2}}$ of hepatic and renal NE^{125}I from $T_{4\beta}$ or $T_{3\beta}$ ranged between 4 and 6 days. The slow fractional turnover of NEI in comparison with that of exchangeable hormone suggests the possibility that a hormone-protein complex may act as a biochemical mediator of hormonal activity and account for the delayed onset and prolonged duration of the biological effects of the thyroid hormones. (Supported by a NIH grant and a United States Army contract.)

300. Cerebrotendinous Xanthomatosis: The Distribution of Cholestanol in Cerebral Subcellular Fractions. PHILLIP D. SWANSON,* S. M. SUMI,* AND WILLIAM L. STAHL,* Seattle, Wash. (introduced by Seymour J. Klebanoff).

Cerebrotendinous xanthomatosis, a rare recessively inherited degenerative disease characterized by xanthoma deposits in tendons, cerebellar white matter, and lung, has recently been shown to be associated with storage in the brain of dihydrocholesterol (cholestanol). Cholestanol was present throughout the brain, even in regions which lacked histopathology. A sample of frontal lobe was obtained at autopsy from an additional patient with this condition. The frozen tissue was thawed, dispersed in 0.32 M sucrose, and fractionated into subcellular fractions by methods which included density gradient centrifugation of the crude mitochondrial fraction. Fractions were assayed for succinate dehydrogenase and acetylcholinesterase activities and also were examined with the electron microscope. Cholesterol and cholestanol were separated by thin-layer chromatography on $AgNO_3$-impregnated silica gel and quantitated by the method of Marsh and Weinstein. Cholestanol accounted for 26% of the free sterols in the tissue homogenate. In the various subcellular fractions which were morphologically and enzymatically distinguishable, cholestanol contributed the following percentages of the free sterol: nuclei 17, microsomes 19, myelin 23, nerve endings and other membranes 19, mitochondria 10. Cholestanol storage is thus a property not only of myelin, which is metabolically stable, but also of other membrane structures in brain. (Research supported by a grant from the National Institute of Nervous Diseases and Stroke, NIH.)

301. Effects of Nephrectomy and Uremia on Plasma Glucose and Insulin Homeostasis. ROBERT S. SWENSON,* ABRAHAM SILVERS,* DANIEL T. PETERSON,* SHOICHI KOHATSU,* AND GERALD M. REAVEN, Stanford, Calif.

Patients with chronic renal failure have decreased rates of removal of plasma insulin. To define the respective roles of loss of renal tissue and uremia, studies were performed serially in seven fasting dogs (1) after sham operation (control), (2) immediately post nephrectomy, and (3) after 4–6 days of uremia (mean S creatinine 15.3 mg/100 ml). ^{125}I-insulin was rapidly injected i.v., and the rate of disappearance of immunoprecipitable radioactivity was determined (fractional insulin loss rate, λ_e, min^{-1}). From λ_e and endogenous plasma insulin the total irreversible loss rate of plasma insulin was calculated (ILR, μU/min). Since insulin concentrations were stable, ILR is the delivery rate of insulin into the systemic circulation (DR). Nephrectomy resulted in a 65% decrease in ILR (1695 $\rightarrow$ 687 μU/min, $P < 0.01$). With development of uremia post nephrectomy, there was no further decrease in ILR (687 $\rightarrow$ 764 μU/min, NS); however, uremia had significant effects on glucose and insulin homeostatis. As compared with the control, uremia was associated with fasting hyperglycemia (111 $\rightarrow$ 143 mg/100 ml, $P < 0.05$) and hyperinsulinemia (17 $\rightarrow$ 31 μU/ml, $P < 0.05$). Despite hyperglycemia, DR did not increase, but remained lower than in the normoglycemic controls. In both control and anephric studies, a direct correlation was seen between insulin concentration and DR ($r = 0.58$, $P < 0.05$), but for any given insulin concentration DR was much lower in anephric subjects. Elevated insulin concentrations in renal failure cannot be interpreted as indicating increased insulin secretion rates. We have found that the kidney is responsible for 65% of insulin removal from the systemic circulation, and that uremia per se does not affect insulin removal rates. In uremia, resistance to insulin action and subnormal DR of insulin in response to hyperglycemia are seen. (Research supported by grants from the Veterans Administration.)

302. Gynecomastia of Hemodialysis: Considerations of Pathogenesis. RONALD S. SWERDLOFF,* GARY KANTOR,* AND STANLEY G. KORENMAN,* Torrance, Calif. (introduced by Joseph St. Geme, Jr.).

Gynecomastia, occasionally seen in untreated renal failure, is common after chronic hemodialysis (HD). This process has been attributed to absolute or relative estrogen excess and to increased secretion of a pituitary mammotropic hormone. The plasma concentrations of LH, FSH, testosterone (T), and 17β-estradiol (E_2) were compared in nine untreated

renal failure patients without gynecomastia, in eight HD patients without gynecomastia, and in seven HD patients with gynecomastia. FSH and LH were measured by radioimmunoassay; T and E_2 by radioligand binding assay. E_2 levels ranged from 9 to 37 pg/ml (normal male values). T levels were either normal or low, and the FSH, with one exception, was low-normal or undetectable (normal >35 mIU/ml). The presence of gynecomastia did not correlate with E_2, T, or FSH. LH was normal (>35 mIU/ml) in eight of nine untreated renal failure patients and in seven of eight HD patients without gynecomastia. In contrast, all patients with gynecomastia had elevated plasma LH levels (47–176 mIU/ml). Only one of the latter patients had an associated FSH elevation (78 mIU/ml). Conclusions: Gynecomastia in patients on HD was caused neither by an absolute increase in the plasma E_2, nor by a dissociation between plasma E_2 and T. The plasma LH was invariably elevated in those patients with gynecomastia. The mechanism by which HD results in this unique dissociation of LH and FSH (in males) is unknown. These data, demonstrating an elevation of LH without an associated increase of testicular steroid secretion, strongly suggests that LH itself may be a mammotropic hormone in this setting. (Supported in part by Population Council grant M69.6.)

303. Variable Fructose Diphosphate Activation of Red Cell Pyruvate Kinase: Further Evidence for Variant Forms. KOUICHI R. TANAKA, Torrance, Calif.

Genetic heterogeneity in pyruvate kinase (PK) deficiency hemolytic anemia is apparent from the few reports of PK deficiency with either an increased or a decreased Michaelis constant (K_m) for phosphoenolpyruvate (PEP). The effect of fructose diphosphate (FDP) on PK activity in hemolysates of normal RBC, the usual type of deficient RBC PK, deficient variant forms of RBC PK, and homogenates of WBC was investigated. In normal RBC, FDP increased PK activity at low concentrations of the substrate PEP, but the effect was slight. The maximum PK activity, achieved at 3 mM PEP, was not increased by FDP. There was no effect of FDP on leukocyte PK. PK kinetics was studied in 16 cases of homozygous PK deficiency; 11 cases were found to have PK kinetics similar to that of normal RBC. FDP activated slightly at low PEP concentrations as in normal RBC; maximum PK activity was not increased at 3 mM PEP. In five patients the K_m for PEP was increased; in these, FDP at a concentration of 0.3 or 0.5 mM had an activating effect at low concentrations of PEP. At 3 mM PEP, the effect of FDP was variable; the PK activity in units without and with FDP being, respectively, 1.35 and 2.83, 4.94 and 9.49, 1.61 and 3.11, 2.74 and 3.43, and 3.88 and 4.63, in the five cases. The normal range for PK activity under the conditions of assay employed is 4.03 to 6.93 units. Thus, the maximum activity achieved was increased into the normal or higher than normal range in two instances. These data support in part the hypothesis of Koler and associates that PK consists of two identical protomers, each containing a catalytic site, which may be occupied by either PEP or FDP. These results also suggest that further heterogeneity exists in PK deficiency hemolytic anemia. Additional studies with FDP should be useful in increasing our understanding of PK kinetics and PK heterogeneity. (Research supported by a grant from the NIH.)

304. Estimation of Myocardial Cell Permeability to Potassium in the Intact Heart. R. G. TANCREDI,* T. YIPINTSOI,* D. R. RICHMOND,* AND J. B. BASSINGTHWAIGHTE,* Rochester, Minn. (introduced by C. F. Code**).

The beating Langendorff blood-perfused dog heart was used to evaluate ^{42}K exchanges between the intracellular and intravascular compartments. 33 bolus injections of a mixture of ^{42}K- and ^{131}I-albumin were made into the coronary artery inflow of six hearts, permitting calculation of maximum fractional extractions, E_{max}, and permeability-surface area products, PS, of the capillary membrane: $PS_{cap} = -F_p \ln(1 - E_{max})$, where F_p is plasma flow (ml/min per g). Values for PS_{cap} ranged from 0.3 to 1.2 ml/min per g, approximately doubling as F_p quadrupled. External gamma detection over four hearts after impulse injection provided the residue functions, $C^*(t)/C^*(0)$, and the emergence functions, $\eta(t)$, which are $-d \ln[C^*(t)/C^*(0)]dt$. The $\eta(t)$ were constant at 0.008–0.010/min from 2 to 5 hr after injection. Since F_p was high, the escape was considered to be limited by the low conductance, PS_T, between the large cellular volume of distribution, V_{dist}, and the outflowing blood, i.e., $\eta(t)$ was PS_T/V_{dist}. Assuming negligible diffusional resistances within cells or interstitium, $1/PS_T$ is the sum of resistances of capillary membrane, $1/PS_{cap}$, and myocardial cell wall, $1/PS_{cw}$. Taking V_{dist} for potassium to be 13 ml/g, values for PS_{cw} were 0.13–0.16 ml/min per g, giving PS_{cw}/PS_{cap} ratios of 0.11–0.26. Assuming (from Niedergerke) cell wall surface area to be 1.1×10^4 cm^2/g, cell permeability is estimated at about 2×10^{-7} cm/sec, which is about 0.5% of that of the capillary membrane. (Supported by grants from the NIH [HE-9719 and FR-7] and the American Heart Association.)

305. Effect of Phenobarbital on Microsomal Enzyme Induction and Biliary Excretion in Man. M. MICHAEL THALER,* PETER R. DALLMAN,* AND JOSEPH R. GOODMAN,* San Francisco, Calif. (introduced by Melvin M. Grumbach).

Although phenobarbital (PB) accelerates processes of biotransformation and stimulates biliary flow in animals, direct evidence for these effects in humans is not available, nor is their relationship understood. Microsomal enzyme induction by PB was studied in jaundiced children using assays of NADPH–cytochrome *c* reductase in liver tissue obtained by biopsy before and during PB therapy in each patient; quantitative analyses of biliary excretion into feces were performed with intravenously administered ^{131}I-Rose Bengal, a cholephil whose excretion does not require biotransformation. After 5 days of PB therapy (10 mg/kg per day p.o.) enzyme activity increased 250% in two subjects with relatively normal parenchymal functions, and 50% in a young infant with extrahepatic biliary atresia; two children with advanced cirrhosis and one with recurrent cholestasis failed to respond. Ultrastructural changes in hepatic smooth endoplasmic reticulum corresponded to the enzymatic

findings. In jaundiced patients with intact extrahepatic bile ducts, PB therapy caused prompt reduction in conjugated bilirubin levels and increased the 72 hr fecal excretion of ^{131}I-Rose Bengal. Label appeared within 6 hr in feces of treated patients, as compared with 20 hr in untreated. Excretion of label increased during therapy from 78% to 99% in a normal subject, from 10% to 71% in cholestasis, and from 2% to 8% in intrahepatic atresia. Our results show that PB induces an enzyme essential to drug and steroid metabolism in human liver. PB also stimulates hepatic excretion of inert dyes and endogenous cholephils, e.g., Rose Bengal and conjugated bilirubin, respectively. Enzyme induction and excretory stimulation can occur independently. These effects are greatest in patients with relatively well preserved hepatic functions, suggesting diagnostic and therapeutic applications of PB in several cholestatic conditions. (Supported by NIH grant HD-03148.)

306. Selection of Patients for Surgical Treatment of Hypertension. GURDARSHAN S. THIND,* WILLIAM S. BLAKEMORE,* AND HARRY F. ZINSSER,** Philadelphia, Pa.

Selective renal venous and peripheral renin and urinary aldosterone determinations were performed in 60 hypertensive patients in addition to the usual tests. Arteriography showed main renal artery stenosis in 22, renal infarction in 2, renal parenchymal disease in 13, and essential hypertension in 23 patients. Renin activity in 170 plasma samples was bioassayed by the technique of Thind and associates. Renal artery repair was performed in eight and nephrectomy in two patients with renal artery stenosis. Surgical cure of hypertension was obtained in only three of 10 patients, and this was predictable from the selective renal venous renin determinations in all patients. Other tests such as IVP, renogram, renal scan, split renal function, and arteriography were less sensitive indicators of functional impairment. Of the 60 hypertensive patients, 27 had features suggestive of primary aldosteronism. Low normal renin activity was present in 11 (18.3%), and hypokalemia or diabetes mellitus or both were present in 16 patients (26.6%). But a negative adrenal venography in three patients and a normal response to renin and aldosterone stimulation tests excluded primary aldosteronism. We suggest that selective renal venous renin should be determined preoperatively in all patients considered for renovascular surgery. A ratio greater than 2 of renal renin of the involved to that of the uninvolved kidney was indicative of favorable surgical results. Although primary aldosteronism is a curable form of hypertension, no cases were found in this series. (This investigation was supported in part by NIH grant 1-MO1-FR-00322; USPHS HE-07762; and N ONR-551 [54].)

307. Myosin from Human Papillary Muscle. PER T. THYRUM,* EVE M. KRITCHER,* AND ROBERT J. LUCHI, Iowa City, Iowa.

Physiochemical properties of myosin from human papillary muscle were compared with data from canine ventricular tissue in the hope that this information could contribute to an understanding of the relation between the chemical and structural properties of myosin. Human papillary muscle was obtained during prosthetic replacement of mitral valves in patients with rheumatic heart disease. Canine ventricular tissue was obtained from healthy mongrel dogs. Cardiac myosin, extracted with a phosphate salt solution, was purified by repeated fractionation with $(NH_4)_2SO_4$ in the presence of 2 M LiCl. The myosin samples sedimented as a single component in the analytical ultracentrifuge. The following data were obtained for human and canine cardiac myosin respectively: ATPase activity (10^{-7} mole P per mg protein·min), 5.9 ± 0.2 and 8.9 ± 0.1 ($P < 0.001$); helical content (%), 49 ± 3 and 60 ± 1 ($P < 0.003$); sulfhydryl groups (groups per mole), 41 ± 1 and 42 ± 1; intrinsic viscosity, (dl/g), 2.0 ± 0.1 and 2.0 ± 0.1; sedimentation coefficient ($S_{20,w}$), 6.4 ± 0.2 and 6.0 ± 0.1; molecular weight, $\times 10^{-5}$, 5.3 ± 0.1 and 5.1 ± 0.2. Acid hydrolysis of myosin was carried out at $110 \pm 1°C$ for 24, 48, and 72 hr. The amino acid content of the protein hydrolysates was determined utilizing a Beckman 120C amino acid analyzer. The human cardiac myosin had a higher content ($P < 0.001$) than canine cardiac myosin of the helix-disturbing amino acids threonine, serine, proline, and valine. The differences noted between human cardiac myosin and canine cardiac myosin in ATPase activity, helical content, and amino acid composition may be a function of both pathology and species difference. The low helical content of human papillary myosin, however, can be interpreted as the result of its relatively high content of helix-disturbing amino acids. (This research was supported in part by grant HE-08805 from the USPHS and grant HE-06352 from the National Heart Institute.)

308. Demonstration of Tyrosinase Activity in Melanosomes Contained within Keratinocytes. K. TODA,* J. MATSUMOTO,* AND T. B. FITZPATRICK,** Boston, Mass.

Melanosomes in melanocytes show demonstrable tyrosinase activity, but melanosomes that have been transferred to keratinocytes have been reported to be without tyrosinase activity. Experiments to be described demonstrate by electron microscopic histochemical methods the presence of tyrosinase activity in melanosomes contained within keratinocytes of hair bulbs obtained from Mongoloid and Caucasoid subjects. This demonstration of tyrosinase activity was made possible by eliminating the prefixation of the specimen with glutaraldehyde. Glutaraldehyde is shown to be an inhibitor of tyrosinase activity, and therefore, previous investigators using glutaraldehyde have not been able to detect the relatively low levels of tyrosinase activity present in the melanosomes contained within keratinocytes. The fact that melanin formation may occur on melanosomes in keratinocytes after the melanosomes have been transferred from melanocytes to keratinocytes has important implications in understanding the process of melanin pigmentation.

309. Distribution and Synthesis of Secretory Immunoglobulin A. THOMAS B. TOMASI, JR., ANTHONY YURCHAK,* DAVID BULL,* AND DONALD TOURVILLE,* Buffalo, N. Y.

The distribution and sites of synthesis of the component parts of the secretory antibody molecule (IgA and secretory "piece" [SP]) were studied in sera and various human tis-

sues using four different techniques: (1) precipitation in agar gels using antisera specific for IgA and SP, (2) the fluorescent antibody technique, (3) in vitro organ culture, and (4) *ex vivo* perfusion involving the incorporation of labeled amino acids by isolated loops of human bowel. The tissues examined included: respiratory, GI, biliary, genital, thymus, parathyroid, skin, fetal membranes, and others. Fluids bathing the mucous surfaces from many of these sites, including human tubal and amniotic fluids, were also studied. The results indicate that SP is ubiquitously distributed and seems to represent a marker for epithelial cells of endodermal origin. Though IgA and SP usually occur together, in certain tissues SP is found in the apparent absence of IgA. A striking localization of SP was found in the Hassall's corpuscles of the thymus gland, and synthesis of SP as well as immunoglobulins could be demonstrated by culture techniques. The perfusion experiments show the incorporation of amino acids into secretory IgA synthesized locally and secreted into the intestinal lumen. Moreover, significant amounts of labeled IgA were also found in the perfusion fluid or "venous return." Approximately 70% of the IgA in the perfusion fluid was 10S, and the majority of this lacked SP. These results suggest that the locally formed polymeric molecule may be a significant source of the IgA normally found in serum. Very small amounts of secretory IgA were detected in all concentrated normal sera, and larger amounts are regularly found in the unconcentrated sera of patients with certain diseases such as regional ileitis and malabsorption syndromes.

310. Gonadotropin Therapy of Infertile Males. PHILIP TROEN, TAKUMI YANAIHARA,* HOWARD NANKIN,* TOSHIRO TOMINAGA,* and HARRY LEVER,* Pittsburgh, Pa.

Eleven infertile men with a wide range of testicular histology and function were treated with human menopausal gonadotropin (HMG) for periods up to 2 yr. Four groups of patients were separated. Two hypogonadotropic eunuchoid patients showed maturation of testis on HMG + HCG (human chorionic gonadotropin), but remained azospermic. Three normogonadotropic patients with disorderly spermatogenesis or spermatogenic arrest had increased sperm counts on HMG, but remained oligospermic. Four normogonadotropic patients showed no increase in sperm counts. They were not distinguishable from the previous three patients by histology of testis or by response to therapy of serum gonadotropin, plasma testosterone (T), urinary estrogen excretion, and production rates. Two hypergonadotropic patients showed no response. Five of the patients without clinical androgen deficiency had T levels at or below 400 ng/100 ml. On HMG, serum FSH increased, but remained within normal range; serum LH showed no consistent change. There was no clinical LH effect of administered HMG in hypogonadotropic patients, and no evidence for testis stimulation in other patients as measured by estrogen excretion or production. Plasma T had a variable course without increase above mid-normal levels until HCG was added. In some patients, a correlation appeared present between changing LH and T levels. Organ culture using 3–6 mg of testicular biopsy tissue demonstrated conversion of radioactive pregnenolone and dehydroepiandrosterone to testosterone. Intermediates were identified to obtain information on pathways; comparison with tissue without spermatogenesis difficulty is underway. This comprehensive approach is being used to develop criteria for therapy of infertile males. Evaluation of dose-response relations for gonadotropins by following gonadotropin levels is suggested, as well as study of interaction of circulating testosterone and testicular steroid biosynthesis with spermatogenesis. (Supported in part by a NIH grant.)

311. Nucleic Acid Metabolism in Proliferating and Differentiating Colonic Cells of Man. FRANK TRONCALE* AND MARTIN LIPKIN, New York, N. Y.

Enzymes active in the metabolism of nucleotide precursors of DNA and RNA have been measured on man in proliferating and maturing epithelial cells in the colon. Normal cells and those in benign and malignant growth lesions were studied. Cells were separated from superficial and deeper layers of mucosa by a new tissue-planing instrument. Gradients of thymidine kinase (TK), AMP and IMP pyrophosphate phosphoribosyl transferases (APRT and HPRT, respectively), and thymidine phosphorylase (TP) were found to characterize different stages of cell differentiation. TK and TP were highest in young proliferating cells, and decreased during differentiation and migration of the cells to the mucosal surface. APRT and HPRT levels were lowest in young proliferating cells and increased markedly during differentiation. Uridine and purine nucleoside phosphorylases had similar gradients. Patterns of enzyme activity characteristic of young proliferating cells were found in cells lining the surfaces of the lesions. In hyperplastic excrescences, adenomatous polyps, villous papillomas, and carcinomas, amounts of TK increased progressively. Corresponding decreases in APRT and HPRT were found in these lesions. Levels of these enzymes in villous papillomas and carcinomas were similar to those found in immature proliferative cells of normal mucosa. Highest levels of TK were found in a polyp from a young woman who had Gardner's syndrome with polyposis and multiple carcinomas of the colon; APRT levels were low. The findings identify the development of nucleic acid metabolic pathways during the normal differentiation of colonic cells in man. They also demonstrate lack of expression of specific enzyme activities in colonic cells having increased proliferative activity, impaired maturation, and increased malignant potential, thus defining more precisely the antecedent mucosa that gives rise to colonic cancer. (Research supported by NIH grants CA-08921, 1-FO3-AM-44662, and AM-04468.)

312. Measurements and Considerations of Serum Gastrin Levels and Gastric Hydrochloric Acid Secretion. WALTER L. TRUDEAU* AND JAMES E. MCGUIGAN, Gainesville, Fla.

The pathogenesis of the Zollinger-Ellison (ZE) syndrome has been recently clarified by demonstration of increased levels of gastrin in the sera of patients by radioimmunoassay. This study was directed to the question whether elevated rates of gastric acid secretion in other gastrointestinal diseases also reflect elevated levels of serum gastrin. In 72

patients with various gastrointestinal illnesses (including duodenal and gastric ulcer, reflux esophagitis, varieties of gastritis, and gastric atrophy), fasting sera were obtained for gastrin radioimmunoassay. Then immediately under fluoroscopic control gastric tubes were placed and 1 hr basal and histamine (0.04 mg/kg)-stimulated gastric acid secretory rates were determined. The mean fasting serum gastrin level was 131 ± 7.5 (SEM) pg/ml. The mean rates of basal and histamine-stimulated acid secretion were 2.36 ± 0.3 and 17.74 ± 1.37 mEq/hr, respectively. As opposed to the ZE syndrome, there was no direct positive correlation of serum gastrin levels with rates of basal or histamine-stimulated acid secretion. Conversely, those patients with reduced rates of histamine-stimulated acid secretion (<5 mEq/hr) exhibited a mean serum gastrin level (169 ± 25.9) which was significantly greater ($P < 0.005$) than the mean gastrin level (101 ± 11.8) for those with increased rates of histamine-stimulated acid secretion, i.e. >30 mEq/hr. This study (1) for the first time demonstrates the absence of elevated serum gastrin concentrations in patients with higher rates of gastric acid secretion, and (2) is consistent with our prior studies demonstrating (a) significant fasting hypergastrinemia in pernicious anemia, which is characterized by absent gastric acid secretion, and (b) absence of fasting hypergastrinemia in patients with peptic ulcer disease. This study emphasizes the value of demonstration of fasting hypergastrinemia in patients with increased rates of gastric acid secretion in establishment of the diagnosis of the ZE syndrome. (Research supported by grants from the NIH and the American Cancer Society.)

313. New Species of Hybridizable Nuclear RNA in Breast Cancer Cells. ROGER W. TURKINGTON,* Durham, N. C. (introduced by R. Wayne Rundles**).

In order to determine how the regulation of gene expression may be altered in cancer cells, the rapidly labeled nuclear RNAs of normal and neoplastic breast cells were compared by the techniques of RNA-DNA hybridization and hybridization-competition. The RNAs from normal and neoplastic cells of mice and rats hybridized with DNAs from various other species in proportion to their known genetic relatedness. The specificity of the hybridization-competition reactions was indicated by the observation that heterologous RNAs from various species competed in proportion to the known relatedness of the DNAs from which they were transcribed. Whereas the unlabeled breast cancer RNAs competed with ^{3}H-RNA from breast cancer cells to 94% of the theoretical value for identical RNA populations, unlabeled RNA from normal cells competed to only 50–65% of this value. Artifacts arising from differences in precursor pool sizes or differences in RNA degradation were ruled out, since similar results were obtained using reciprocal labeling and double-isotope labeling of combined samples. The greater diversity of nuclear RNA species in the cancer cells did not relate to cell proliferation per se, since this greater diversity was not manifested by rapidly proliferating breast cells of pregnancy. The findings indicate that these breast cancer cells synthesize species of nuclear RNA which either are not formed by normal breast cells or are present in nonneoplastic breast cells in undetectably low concentration. The results support the concept that altered regulation of gene transcription may be a fundamental feature of cancer cells. (Research supported by grant CA-10268 from the NIH.)

314. The Anemia of Chronic Trauma. C. ROBERT VALERI* AND IRMA O. SZYMANSKI,* Chelsea, Mass. (introduced by Mark D. Altschule**).

Red cell mass deficits have been found in patients 1 to 2 months after severe disabling trauma to the extremities. The venous hematocrit and hemoglobin values were normal or slightly reduced, but the ^{51}Cr red cell volumes were markedly reduced. ^{51}Cr survival of the patient's own red cells was normal or shortened, as was the survival of transfused cells measured by an automated differential agglutination procedure. In several cases the survival curves of transfused red cell populations were biphasic, showing an initial rapid rate of destruction followed by an almost normal rate of destruction. The shortened survival of both recipient and donor red blood cells suggested an extracorpuscular hemolytic factor. A normocytic normochromic blood picture without reticulocytosis was observed. Serum iron levels and iron-binding capacities were normal or slightly reduced. ^{59}Fe kinetic studies showed rapid plasma clearance and prompt incorporation of iron into the peripheral red cells. Decreased rate of red cell production was calculated by serial determinations of both the red cell mass and the rate of donor red blood cell destruction. Destruction of the transfused red cells was not associated with increased serum bilirubin levels and could not be accounted for by fecal urobilinogen excretion. In vitro measurements showed abnormal hemolysis during incubation of the blood with adrenochrome, an epinephrine metabolite. With clinical improvement the adrenochrome susceptibility returned toward normal. The in vitro hemolysis produced nonheme compounds, suggesting removal of iron from the porphyrin ring. The anemia of trauma consists of both a hemolytic component and a decrease in red cell production.

315. Lipoprotein Glyceride Uptake by Perfused Rabbit Aorta. ALAN VOST,* Montreal, Canada (introduced by C. H. Hollenberg).

Rabbit abdominal aortas were perfused, in situ, with albumen buffer or blood-buffer containing ^{3}H-labeled chylomicrons. Chylomicrons were prepared by feeding triglycerides labeled with ^{3}H-palmitic or oleic acid to rats or rabbits with cannulated thoracic ducts; >92% of ^{3}H-labeled lipid in chylomicrons was triglyceride. Aortas were perfused for up to 2 hr at triglyceride concentrations of 0.1–8.0 mM, and in the final 15 min the radioactive perfusate was replaced with perfusate containing unlabeled chylomicrons. In intima and media, influx of lipoprotein triglyceride was 25 times greater than previously demonstrated rates of aortic triglyceride synthesis from perfusate glucose and free fatty acids. Though triglyceride influxes in both aortic segments increased with increasing perfusate triglyceride concentrations, a maximal rate of influx into intima was reached at low physiological concentrations of perfusate triglyceride. Triglyceride entered aorta without hydrolysis, and radioautography showed that, after 30 min perfusion, most ^{3}H-

triglyceride in aorta was in the aortic media. These experiments suggest that there is a process limiting the rate of entry of lipoprotein glyceride into aortic intima and inner media, and that, after entry without hydrolysis, triglyceride is rapidly transported across the aortic intima. (Research supported by a grant from the Quebec Heart Foundation.)

316. Further Characterization of Purified Fragments of Type 12 Streptococcal M Protein. KENNETH L. VOSTI, RUDOLPH H. JOHNSON,* AND MICHAEL F. DILLON,* Stanford, Calif.

Although the biological importance of M protein has been recognized for over 40 years, purified preparations suitable for analysis of structure-function relations have only recently become available. We have previously described the isolation of two apparently homogeneous fragments of type 12 M protein which contained all the serologically active M determinants present in the initial crude acid extract. Both fragments migrated as single broad bands with different R_f on electrophoresis in polyacrylamide gels; moved as narrow zones with different sedimentation coefficients in the analytical ultracentrifuge; lacked methylpentoses, group A serologic reactivity, and nucleic acids; and contained similar two-dimensional peptide maps. The present studies assess further the homogeneity of these two purified fragments and their relation to each other. Electrophoresis in SDS polyacrylamide gels revealed three bands in the "a" and a single band in the "b" fraction with estimated molecular weights of 69,000, 50,500, 32,500, and 29,000, respectively. Five amino acids (glu, lys, leu, ala, and asp) were the most common in each fraction. N-terminal analysis revealed ala in each fraction, whereas C-terminal analysis revealed primarily lys and arg in "a" and leu in "b." Both fractions raised type-specific precipitating and bactericidal antibodies in rabbits. These antisera contained antibodies for at least two antigenic determinants that were not recognized with antisera raised against whole bacterial cells. These studies suggest that M protein molecules vary by a constant unit of approximately 20,000 molecular weight. Whether this is their native form or the result of the extraction method is not known. Additional support for the latter hypothesis is provided by the detection of new antigenic determinants only with antisera against the purified fractions. (This research was supported by a grant from the NIH, National Institute of Allergy and Infectious Diseases.)

317. The Importance of Creatine Phosphokinase in Diagnosis of Acute Myocardial Infarction. GALEN S. WAGNER,* DAVID O. CLARK,* AMOS H. GRABER,* ROBERT A. ROSATI,* AND ANDREW G. WALLACE, Durham, N. C. (introduced by Eugene A. Stead, Jr.**).

This study was performed to discover whether monitoring of serial serum creatine phosphokinase (CPK) would reveal instances of acute myocardial infarction (AMI) not diagnosed by EKG or other enzymes. Serum CPK was determined at admission and three times daily for 5 days on all patients with a history compatible with AMI. Daily SGOT, LDH, SGPT, and EKG were obtained. The study includes 210 consecutive patients who survived long enough to allow evaluation by EKG and serum enzymes, and in whom one or both of these parameters were diagnostic of AMI. Patients with obvious skeletal muscle trauma were eliminated. Serial CPK levels were diagnostic of AMI in 206 patients. CPK elevation was the only enzymal indication of AMI in 77 patients. In 28 of these the diagnosis was confirmed by QRS changes on EKG. CPK measurements every 8 hr detected only three patients with elevations not shown on once daily determinations. Thus, the improved diagnostic capability of CPK determinations resulted from enzyme sensitivity and specificity and not from multiple sampling. Over-all mortality for these 210 patients was 14%. In 157 patients in whom CPK diagnosis was confirmed by EKG or other enzymes, mortality was 15%. In 49 "CPK infarctions" mortality was 8%. An important difference in mortality was seen when this group was divided into those with normal SGOT and LDH (3%) and those in whom elevated SGPT prevented diagnostic interpretations of elevated SGOT and LDH (19%). Serial monitoring of serum CPK was a useful method of diagnosis of AMI in 98% of 210 patients. It was the only positive indicator of AMI in 23%. Mortality was remarkably low in patients in whom CPK was the only elevated serum enzyme. (Research supported by a grant from the NIH.)

318. Disorders of Serum Protein Metabolism in Renal Disease. THOMAS A. WALDMANN, WARREN STROBER,* AND R. PETER MOGIELNICKI,* Bethesda, Md.

Serum protein metabolism in various renal disorders and controls was evaluated using radioiodinated IgG (mol wt 160,000) and L chains of immunoglobulins (mol wt 22,000). Controls metabolized 0.28%/hr of the circulating IgG and 22.3%/hr of the circulating L chain. All the IgG and 98% of the L chain was catabolized, with the remaining L chain lost unchanged into the urine. Three distinct patterns of protein metabolic disorder were noted in patients with nephrosis, tubular disease, and uremia with loss of nephrons, respectively. In patients with nephrosis, L chain survival was slightly increased; in contrast, IgG survival was markedly decreased and the fraction lost as proteinuria markedly increased. Here abnormal glomerular permeability to large serum proteins is the basic abnormality. Patients with proximal renal tubular disease (cystinosis, adult Fanconi syndrome) had a 20- to 100-fold increase in daily urinary excretion of such 15,000–40,000 molecular weight proteins as lysozyme, ribonuclease, and L chains. Serum IgG and L chain survivals were normal. However, the fraction of overall L chain metabolism accounted for by proteinuria was increased 10- to 60-fold, and endogenous catabolism was correspondingly decreased. Thus "tubular proteinuria" results from failure of proximal tubule uptake and catabolism of small proteins that normally are filtered through the glomerulus. Patients with uremia and nephron loss have normal IgG survival, but 4–10 times prolonged L chain survival with increased serum concentration of this protein and ribonuclease. These studies indicate that the kidney is a major catabolic site for small serum proteins. In tubular disease, loss of this catabolic mechanism results in "tubular proteinuria." Uremia with loss of entire nephrons results in decreased catabolism and excretion with resultant increased

serum levels of small, biologically active proteins. This may be of importance in the pathogenesis of the uremic syndrome.

319. Cerebral Energy Metabolism in Short-Chain Fatty Acid–Induced Coma. C. O. WALKER,* D. W. MCCANDLESS,* J. D. MCGARRY,* AND STEVEN SCHENKER, Dallas, Texas.

Short-chain fatty acids (SCFA) have recently been given as a cause of hepatic coma, and in vitro studies have suggested that the coma may be due to impaired cerebral energy metabolism. In this study, cerebral energy metabolism was assessed in vivo in rapidly frozen cortex and brainstem of rats with SCFA-induced coma. The brainstem was evaluated separately because (1) EEG changes in experimental SCFA-induced coma suggest brainstem involvement, and (2) the brainstem contributes importantly to the maintenance of consciousness. Reversible coma of 38–67 min was reproducibly induced in rats in 5–12 min by butyrate, valerate, or octanoate given i.p. Alert controls received equimolar sodium acetate or saline. Normal ATP and phosphocreatine concentrations were found in both cerebral cortex and brainstem of rats at onset of and during established SCFA-induced coma. Energy utilization rates in both brain areas, at onset of coma, were also normal (response to ischemia-Lowry technique), indirectly suggesting normal rate of cerebral energy synthesis. At onset of coma, cerebral lactate and lactate/pyruvate ratios were consistent with normal oxidative metabolism of the brain. Changes in serum osmolarity, pH, glucose, ketone concentrations, or cerebral catecholamines were not responsible for onset of coma. Average blood octanoate levels at onset of coma were 8 μmoles/ml and fell to 3 μmoles/ml before awakening. Brain octanoate at onset of coma was about 2.6 μmoles/g, with similar values in cortex and brainstem; it fell below 0.25 μmole/g before awakening. The normal cerebral energy metabolism in our in vivo studies of SCFA-induced coma, contrary to prior in vitro studies, likely resides in higher concentrations of SCFA and the protein-free medium employed in vitro. In conclusion, these direct in vivo studies suggest that altered regional cerebral energy metabolism is not responsible for SCFA-induced acute experimental coma. (NIH-supported research.)

320. Changes of Action Potential Shape after Chronic Cardiac Denervation. ANDREW WALLACE, JAMES DORSEY,* AND JOSEPH MILLER,* Durham, N. C.

Experiments were designed to determine whether chronic surgical denervation alters the relation between the shape of the action potential (AP) and the pattern of stimulation in canine ventricular muscle and Purkinje fibers. Papillary muscles with attached false tendons were studied at 37.8°C utilizing standard microelectrode techniques. AP area was obtained by electronic integration and used as an index of changes in shape. Resting membrane potential and AP amplitude were not different in normal and denervated preparations. In normal tissues, at any given frequency of stimulation, AP duration and area were greater in Purkinje fibers than in muscle. These differences were absent in denervated tissues. In normal tissues, an abrupt decrease in frequency resulted in an increased duration and area of the first Purkinje AP, and a decrease of duration and area of the first muscle AP. These responses to abrupt changes of frequency were absent in denervated tissues. Our observations demonstrate that chronic denervation essentially eliminates the discrepancy between AP duration in Purkinje fibers and that in ventricular muscle. Denervation also eliminates discordant behavior of AP shape in Purkinje fibers and muscle during abrupt changes of frequency. These differences between normal and denervated tissues may contribute to antiarrhythmic influence of denervation. They also should be considered in the design of experiments to explain the "intrinsic" properties of the cardiac membrane which are responsible for the generation of the AP. (Research supported by a grant from the NIH.)

321. Quantal Transport of Sodium and Chloride in the Toad Bladder. MACKENZIE WALSER, Baltimore, Md.

It is generally held that epithelia which transport sodium between identical media against an electrical gradient (J Na→) permit passive movement of other ions, including sodium backflux (J Na←), along this gradient. The present data indicate that in toad bladders mounted without edge damage, in which resistance is high (10,000–50,000 Ω cm²), transepithelial potential difference (PD) has little or no effect on any fluxes except J Na→ itself. Despite spontaneous PD averaging 80 mv, a comparison of J Na←, J Cl←, J Cl→, J K←, and J K→ during open vs. short circuiting, measured isotopically between well stirred normal media in 120 experiments, showed no significant change in these fluxes with PD in about one-fourth of the bladders, a small change in the expected direction in about one-half, and a significant change in the reverse direction in about one-fourth. J Na→, by contrast, regularly changed about 3-fold. These results suggest that only J Na→ moves along a continuous electrochemical gradient; the other fluxes occur along different paths, in which the gradient is discontinuous (quantal transport); alternatively, the moving ions may be bound in an electrically neutral form. Further, transport numbers (t) for Na and Cl estimated by an equation which takes into account interactions between tracer and nontracer flows (Meares) are less than 0.3 even in bladders in which fluxes did respond to PD. Hyperpolarization or reversal of PD also altered J Na→ far more than J Na←, J Cl←, or J Cl→. When J Na→ was nearly obliterated by cooling (4°C) or by ouabain and dinitrophenol, bladder conductance fell farther, t rose, and applied currents now affected all four fluxes. Thus energy is required to maintain the quantal character of the "passive" fluxes. In the undisturbed bladder, the only flux which can carry current is that which generates the PD, J Na→.

322. A Fifth Complement Component Cleaving Enzyme and Leukotactic Factors in Rheumatoid and Non-rheumatoid Synovial Fluids. PETER A. WARD* AND NATHAN J. ZVAIFLER,* Washington, D. C. (introduced by Laurence H. Kyle**).

Polymorphonuclear leukocytes are thought to play an important role in joint inflammation and destruction, but the

factors responsible for their accumulation in the articular cavity are unknown. Synovial fluids from patients with rheumatoid arthritis and other inflammatory joint diseases have been studied for the presence of factors chemotactic in vitro for rabbit neutrophilic granulocytes. 70% of rheumatoid fluids contained complement-derived chemotactic factors, consisting of a macromolecular complex, $\overline{C567}$, and/or a factor of low molecular weight. On the basis of studies using specific antibody, ultracentrifugation, and gel filtration, this latter factor (C5a) has been identified as a cleavage product of the fifth component (C5) of human complement. In half of the rheumatoid fluids there also exists an enzyme capable of producing a chemotactically active small molecular weight cleavage product (C5a) when incubated with isolated human C5 but not C3. This enzyme is active at a neutral pH and can be suppressed by soybean trypsin inhibitor and ε-amino caproic acid and by esters bearing basic but not aromatic amino acids. It has a molecular weight close to that of bovine albumin. A similar, if not identical, enzyme can be extracted from lysosomal granules of leukocytes obtained from rheumatoid synovial fluid exudates and lysosomal granules of rabbit neutrophils. In inflammatory nonrheumatoid joint effusions, chemotactic activity is present in half of the fluids tested, and C5 cleaving enzyme is present in one-third of such fluids. No enzyme or preformed factors have been found in synovial fluids from patients with osteoarthritis (five cases) or systemic lupus erythematosus (four cases). The presence in synovial fluids of chemotactic factors and a generator of chemotactic activity may be related to the pathogenesis of joint inflammation. (Research supported by grants AI-07291, AM-05042, and AM-05140 from the NIH.)

323. Independent Effects of Salt Depletion and Oncotic Pressure in the Proximal Tubule. EDWARD J. WEINMAN,* JOHN P. HAYSLETT,* MICHAEL WEINER,* MICHAEL KASHGARIAN,* AND FRANKLIN H. EPSTEIN, New Haven, Conn.

Contraction of extracellular volume is accompanied by renal retention of water and sodium. In order to study the associated changes in reabsorption in the proximal tubule, micropuncture studies, using the Gertz technique, were performed in rats volume depleted with furosamide and sodium deprivation. In these hypovolemic animals a greatly accelerated reabsorptive rate constant of 8.1×10^{-5} mm³/mm per sec was found, compared with the normal control values of 6.5×10^{-5} mm³/mm per sec. This effect was shown to be independent of filtration rate, tubular fluid velocity, and aldosterone. The possible contribution to this effect of the observed increase in plasma protein was then examined. Serum protein levels were reduced 15–25% in normal and volume-depleted rats by replacing approximately one-third of the blood volume with donor red cells suspended in Ringer's bicarbonate solution, adjusted to the hematocrit of the experimental animal. This procedure did not alter filtration fraction in either group of animals. When peritubular oncotic pressure was reduced to less than the normal value of 48.9 mm Hg, the resorptive rate constant was reduced in normal rats to 5.06×10^{-5} mm³/mm per sec but remained elevated at 7.84×10^{-5} in the hypovolemic group. The findings suggest that although the rate of tubular sodium transport is responsive to changes in peritubular capillary oncotic pressure, volume depletion has a distinct and independent action that enhances the reabsorption of sodium by the proximal tubule.

324. Histone Cleavage by Lysosomal Protease: A Mechanism for Augmented Transcription. GERALD WEISSMANN, PHILIP DAVIES,* AND KATHRIN KRAKAUER,* New York, N. Y.

Although lysosomes have been implicated in widespread gene activation as in the promotion of tumors by phorbol esters, no specific enzyme system has been identified. Since neutral proteases (e.g., trypsin) hydrolyze histones, and increase nuclear template activity for RNA polymerase (rabbit liver, human lymphocytes), such an enzyme was sought in lysosomes. Using calf thymus histone as substrate, and measuring release of acid-soluble arginine (Sakaguchi) or tyrosine (Folin), a histonase was identified in rabbit leukocyte lysosomes. The histonase had a pH optimum of 7.2–7.4; lysosomes (azurophil granules) contained 97.2 ± 3.1% of total enzyme; nuclei, debris, and postgranule supernatants (0.34 M sucrose homogenates) had negligible activity. The enzyme was partially inhibited by 0.05 M epsilon amino caproic acid (25–30%) and totally inhibited by a substance present in the cytosol but absent in nuclei. Inhibition was overcome in high salt solutions (>2.5 M KCl) or by polyanions (e.g. heparin). Previous failure to detect this enzyme was due to its narrow substrate specificity: it did not hydrolyze denatured hemoglobin at neutral pH, synthetic substrates for trypsin and chymotrypsin (benzoyl-L-arginine paranitroanilide, glutaryl-L-phenylalanine paranitroanilide, succinyl-L-phenylalanine paranitroanilide, *N*-benzoyl-L-phenylalanine-2-naphthyl ester); nor was it a leucyl naphthylamidase. The histonase was partially resolved by gel filtration on Sephadex G-75 (mol wt <70,000, specific activity = 28 μg arginine released per mg protein per hr). After isolated rabbit liver nuclei were incubated with histonase-rich leukocyte lysosome fractions, the nuclear template activity for exogenous RNA polymerase (*Micrococcus lysodeikticus*) was enhanced 5-fold. Since the histonase (also present in other cells) is inhibited by cytosol, but not by nuclei, it can selectively cleave nucleohistones. Tumor-promoting agents may thereby preempt normal transcriptive controls by releasing the neutral protease from lysosomes. (Supported by grants from the NIH.)

325. A Gamma Aminobutyric Acid "Shunt" in Kidney: Role in Acidosis. DONALD T. WHELAN,* CHARLES R. SCRIVER, AND FAZL MOHYUDDIN,* Montreal, Canada.

Gamma aminobutyric acid (GABA), an important metabolite in brain, also occurs in kidney of man and other mammals, where we have shown that it is formed from glutamic acid by a pyridoxal phosphate–dependent decarboxylase (GAD) whose kinetics resembles that of brain GAD. Renal GABA is removed by an aminotransferase (GABA-T)

and later converted to succinate at rates equivalent to those of its formation. The steady-state GABA concentration and GAD activity in kidney homogenates are each about one-fifth those in brain and are the highest for any tissue outside the CNS. The GABA pathway is absent in rat kidney papilla, suggesting that it participates in renal oxidative metabolism. GABA (20 mM) stimulates respiration in rat kidney cortex slices by 45%; this effect is completely blocked by hydroxylamine, an inhibitor of GAD and GABA-T, and by aminooxyacetic acid (AOAA), a specific inhibitor of GABA-T. The GABA "shunt" oxidizes glutamate at rates equivalent to those of its oxidation via alpha ketoglutarate. Chronic acidosis lowers the steady-state renal GABA concentration in rat to half of normal ($P < 0.01$). Renal GABA in AOAA-treated acidotic rats is 6-fold greater than in acidotic controls ($P < 0.01$), and 2.5-fold greater than in AOAA-treated nonacidotic controls ($P < 0.01$), indicating enhanced activity of the GABA shunt in acidosis. Brain GABA is not similarly influenced by acidosis. We propose that glutamate, formed from glutamine during renal ammoniogenesis, can be oxidized efficiently by the GABA shunt. (Supported by grants from the Medical Research Council and the Q. E. II Fund, Canada.)

326. Location of Intrarenal Antibiotic Concentration in Hydropenia. ANDREW WHELTON,* DANIEL G. SAPIR,* GORDON G. CARTER,* JULIAN KRAMER,* AND W. GORDON WALKER, Baltimore, Md., and Washington, D. C.

After a 4 hr i.v. infusion of an antibiotic, tissue concentrations measured within the cortex, medulla, and papilla of the dog kidney show considerable variation depending upon the type of antibiotic and the state of hydration of the experimental animal. In the hydropenic state, a significant gradient of increasing concentration from cortex to papilla occurred with penicillin (PEN) and cephalothin (KEF), whereas ampicillin (AMP), oxytetracycline (O-T), and kanamycin (KAN) concentrations did not differ as between cortex, medulla, and papilla. Hydration effectively dissipates this concentration for all antibiotics studied. Papillary:cortex antibiotic ratios in hydropenia were: PEN, 3.9 ± 0.3; KEF, 3.0 ± 0.2; AMP, 2.1 ± 0.6; O-T, 0.9 ± 0.07; and KAN, 0.9 ± 0.02. Corresponding values in the hydrated state were: PEN, 0.78 ± 0.12; KEF, 0.72 ± 0.07; AMP, 0.4 ± 0.1; O-T, 0.4 ± 0.01; and KAN, 0.7 ± 0.08. To further delineate the interstitial or intratubular location of antibiotic concentration in hydropenia, these data were compared with the distribution of a "glomerular" substance and a "tubular" substance. ^{14}C-inulin ($n = 11$) and ^{3}H-PAH ($n = 7$) studies yielded papillary:cortex ratios of 2.6 ± 0.3 and 2.1 ± 0.3 respectively when the urine osmolality averaged 1655 mOsm/kg with an average urine flow of 0.06 ml/min per kidney. These ratios are less than the ratios for PEN and KEF but greater than those for AMP, O-T, and KAN. These data indicate that PEN is concentrated in the medullary and papillary interstitial tissue, but AMP as well as O-T and KAN are not. Data on KEF are inconclusive. This approach provides a means for selecting antibiotics most effectively concentrated in the papillary and medullary tissue and yields information of potential therapeutic importance.

327. Polymers of Hemoglobin. JAMES G. WHITE* AND BEATRICE HEAGAN,* Minneapolis, Minn. (introduced by Robert A. Ulstrom**).

Three different structural arrangements of sickled hemoglobin (HbS) have been described from electron microscope observations, including empty hexagonal crystals (Stetson), microtubules (Murayama), and solid rods (White). Recent studies in this laboratory have indicated that rods are the characteristic polymers of sickled HbS, but the origin of hollow tubules and crystals observed by others remained obscure. In this investigation a method was found which regularly produced gels in normal and abnormal hemoglobin solutions. Cell-free solutions of HbAA, HbAS, and HbSS combined with potassium phosphate buffer, or a reducing agent and buffer, yielded firm gels in minutes or hours. Chemical analysis revealed no denaturation or conversion to methemoglobin during gel formation. Gels of oxy-HbSS, oxy-HbAS, and oxy- or reduced HbAA contained masses of 240 A hollow polymers resembling microtubules. The hollow polymers were often aligned in parallel bundles with individual microtubules in wall-to-wall contact, an arrangement similar to some observed by other workers in intact sickled cells and sickled hemolysates. Gels of reduced HbSS or HbAS, however, did not contain hollow polymers resembling microtubules, or arrangements in which polymers were in wall-to-wall contact. Instead, they consisted of 170–190 A solid rods in radial configurations or bundles with rods parallel to and equidistant from each other, identical with our findings in intact sickled erythrocytes. Whole-mount studies of gels and sickled cells indicated that each rod of HbS is a composite of seven stacks of molecules rather than six monofilaments twisted around an empty central core. Hollow polymers are interesting hemoglobin structures, but they are not related to the sickling phenomenon.

328. Studies on the Hypertension of Chronic Renal Failure: The Role of Bilateral Nephrectomy. R. WILKINSON,* D. F. SCOTT,* P. R. ULDALL,* J. SWINNEY,* D. N. S. KERR,* AND V. ROBSON,* Newcastle, England (introduced by Roy H. Maffly).

The role of the kidneys in the hypertension of chronic renal failure (CRF) and the value of bilateral nephrectomy (BNx) in the management of this hypertension is under dispute. 45 patients on regular dialysis treatment have been studied, measuring plasma renin (PR), exchangeable sodium (Na_E), and blood pressure (BP). 14 hypertensive patients were studied after BNx performed in preparation for renal transplantation. Before BNx, BP did not correlate with Na_E, a group remaining hypertensive despite reduction of Na_E to normal or subnormal levels. After BNx there was a significant correlation, $r = +0.52$, $P < 0.01$, between BP and Na_E. Before BNx there was a highly significant correlation between BP and PR, $r = +0.63$ (diastolic) and $r = +0.51$ (systolic), $P < 0.001$ in both. BNx consistently reduced BP when PR was inappropriate to Na_E. Mean duration of dialysis before BNx was 5 months. After BNx, fluid replacement of up to 6 liters in the first 24 hr was required to maintain BP. Considerable morbidity and two deaths followed BNx. Unless transplantation is contemplated, we

recommend BNx only if conservative measures fail and PR is elevated to a level inappropriate to Na_E. It may be possible to predict the necessity for Bnx without a preliminary trial of salt restriction.

329. Primary Renal Potassium Wasting in a Patient with Juxtaglomerular Hyperplasia (Bartter's Syndrome). GORDON H. WILLIAMS,* STUART HANDWERGER,* ROGER B. HICKLER, AND JOHN F. CRIGLER, JR.,* Boston, Mass.

The mechanism for potassium wasting in children with juxtaglomerular hyperplasia has been attributed to secondary hyperaldosteronism. Supine plasma renin activity (PRA) and aldosterone secretion rate (ASR) were measured in an 11-yr-old Negro female with the classical clinical and pathological features of this syndrome under varying conditions. On an ad lib. diet containing 60–75 mEq sodium (Na) and 35–40 mEq potassium (K), her PRA was 12,800 ng/100 ml; ASR, 181 μg/day; and serum K, 1.8 mEq/liter. In response to sodium restriction (10–15 mEq/day) without change of K intake, her ASR rose to 351 μg/day with an unchanged serum K (1.7 mEq/liter). With K supplementation (150–160 mEq/day), her PRA fell to 5356 ng/100 ml, her ASR rose to 2096 μg/day, and serum K to 3.4 mEq/liter. In response to ACTH infusion (80 units/24 hr), her ASR increased to 3027 μg/day without a significant change in either Na or K excretion. DOCA 10 mg i.m. daily (×2) also failed to alter Na or K excretion. In response to i.v. saline 200 mEq/m² for 2 days, PRA fell from 12,500 to 6500 ng/100 ml, ASR from 1911 to 629 μg/day, with no significant increase in K excretion. It is concluded that the ASR in this patient was appropriate for the respective level of K balance and PRA. There was no increased kaliuresis in response to an acute increase in ASR (secondary to ACTH stimulation), DOCA, or saline loading. Contrary to previous suggestions, the hypokalemia in this case appears not to be secondary to either hyperaldosteronism or decreased proximal tubular Na reabsorption, but to be due to primary renal K wasting.

330. Identical Idiotypic Determinants in Two Paraproteins (Immunoglobulins M and G) from the Same Patient. S. K. WILSON,* J. E. HOPPER,* H. H. FUDENBERG, A. C. WANG,* AND A. NISONOFF,* Chicago, Ill., and San Francisco, Calif.

A patient (Til.) was previously shown to have two monoclonal paraproteins (IgM-K and IgG-K) with identical L chains by peptide mapping, circular dichroism, etc. Rabbit antiserum was prepared against each paraprotein and absorbed with both normal IgG and IgM. Each absorbed antiserum reacted strongly with Til. IgG and IgM, but not at all with a large array of other paraproteins. (The degree of reaction of non-Til. paraproteins by quantitative radioimmunoassay was less than 0.3%.) Thus these antisera define "idiotypic" determinants. Isolated L chains from Til. IgG or IgM failed to react with either anti-idiotype antiserum, indicating that these idiotypic determinants are dependent on both H and L chains. The idiotypic determinants appear identical in the two paraproteins by several criteria. The results strongly suggest that not only the L chains but also portions of the H chains are identical in amino acid sequence in the Til. IgG and IgM paraproteins. The results therefore suggest that the same gene controls the variable segment of the heavy polypeptide chain of both IgG and IgM. The implications with respect to the "switchover" from IgM to IgG antibody synthesis will be discussed. (Supported by grants from the NIH and the American Cancer Society.)

331. Human Cutaneous Vascular Smooth Muscle Responses to Catecholamines, Histamine, Serotonin, Bradykinin, Angiotensin, and Prostaglandins. R. K. WINKELMANN,* W. MITCHELL SAMS, JR.,* AND JOAN H. KING,* Rochester, Minn. (introduced by Ward S. Fowler**).

Human breast skin vessels, 200 to 400 μ outer diameter, obtained at surgery were dissected into continuous helical strips and studied in oxygenated physiologic salt solution at pH 7.4 for tension changes induced by natural pharmacologic agents. All 36 strips from eight patients responded with contraction to 0.01 M KCl, and strips from all eight patients responded with contraction to epinephrine and norepinephrine. No responses were observed to acetylcholine or histamine in concentrations up to 10^{-3} g/ml. Serotonin caused a contraction in 22 of 36 strips tested (seven of eight patients). Angiotensin and prostaglandins (PGE_{2a}, PGE_2, PGA_2) produced contraction of strips from all patients tested, but bradykinin produced no vascular response in 22 strips tested. Cold (5° and 20° to 22°C) physiologic salt solution caused contraction of the strips. Blocking experiments with phentolamine and propranolol revealed an alpha receptor activity. Serotonin, prostaglandins, angiotensin, and catecholamines are mediators of importance for human skin resistance vessels; histamine, bradykinin, and acetylcholine (which cause contraction of rabbit skin, dog skin, and pulmonary and cardiac vascular muscle, respectively) play no direct role in human skin vessels.

332. Stimulation of Insulin Secretion by Adipose Tissue: Effect of Fat Cell Size. JONATHAN K. WISE* AND LESTER B. SALANS,* Hanover, N. H. (introduced by Thomas P. Almy**).

Hyperinsulinemia occurs in obesity in association with enlarged insulin-resistant fat cells. The observation that this occurs in spite of normal glucose concentration suggests that adipose tissue may play a role in regulation of insulin secretion. The influence of adipose cell size (μg lipid per cell) upon insulin secretion from islets of Langerhans was examined to determine the mechanisms by which large insulin-resistant fat cells could lead to hyperinsulinemia. Equal amounts of epididymal fat from six obese and six nonobese rats (mean fat cell size 0.563 μg, 0.133 μg respectively) were incubated in bicarbonate-buffered medium. The medium was then filtered free of tissue and cells, and glucose concentration was adjusted to range from 0.5 to 5.0 mg/ml. Islets from nonobese rats were placed in flasks containing media previously incubated with large or small fat cells or control bicarbonate buffer (no preincubation); thus, islets from each rat were exposed to the full range of glucose con-

centration in all three incubation media. Insulin content of the medium was measured immunochemically after 90 min incubations. Insulin secretion from islets exposed to medium preincubated with enlarged fat cells was significantly greater, at all levels of glucose tested, than insulin secretion from islets exposed either to medium preincubated with small cells (+36–90%) or to control buffer (+34–81%). Similar but more pronounced (+100–300%) results were obtained when islets of obese were substituted for islets of nonobese rats. Neither glycerol, free fatty acid, nor triglyceride concentration differed in media preincubated with large or small cells. Thus, the hyperinsulinemia of obesity may be due to a factor(s) present in large rather than small adipose cells. (Supported by a NIH grant.)

333. Familial Lupus Erythematosus: Abnormal Immunoglobulin G Metabolism in Apparently Healthy Family Members. R. Dean Wochner* and Clifford J. Harris,* St. Louis, Mo., and Mesa, Ariz. (introduced by Gerald T. Perkoff).

Familial occurrence of lupus erythematosus (SLE) and other connective tissue diseases has been reported previously, often associated with laboratory abnormalities in healthy relatives. We have observed two such kindreds and found in addition abnormal ^{125}I-IgG metabolism in apparently unaffected family members. Hypercatabolism of normal IgG is frequent in patients with connective tissue disorders. In 42 patients we previously studied, the fractional catabolic rates (FCR) averaged 11.5% of circulating IgG per day, as compared with 6.76 ± 1.24%/day in normals and 7.07 ± 1.38%/day in patients with other inflammatory diseases. This hypercatabolism was masked by increased IgG synthesis, resulting in normal or elevated serum IgG concentrations. The first kindred totaled 15 members; two had SLE, and two had polyarthralgias without a specific diagnosis. ^{125}I-IgG metabolism was studied in nine. Abnormally high FCR (>2SD above the normal mean) were found in one with SLE, one with arthralgias, and four who were normal by all clinical and laboratory criteria; these six FCR averaged 10.0%/day. Three studies in other healthy members were normal. In the second kindred (36 members), there were three with SLE, two with polymyositis, and two with Raynaud's phenomenon. 19 had ^{125}I-IgG studies; one with SLE, one with polymyositis, one asymptomatic woman who had once had an episode of thrombocytopenia, and another with minor arthralgias and a positive ANA had abnormal FCR averaging 10.8%/day. Normal results were obtained in the two women with Raynaud's phenomenon and in 13 asymptomatic relatives. These observations are consistent with a postulated genetic predisposition toward connective tissue diseases. The degree of abnormality found in a given subject may vary from inapparent abnormalities to fully expressed clinical disease.

334. Effects of Ethanol on Splanchnic Metabolism in Healthy Men. B. M. Wolfe,* R. J. Havel, E. B. Marliss,* J. P. Kane,* and J. Seymour,* San Francisco, Calif., and Boston, Mass.

Arterial and hepatic venous blood samples were obtained from four young men fasted 15 hr and three fasted 69 hr and given constant intravenous infusion of 1-^{14}C-palmitate and indocyanine green during a 2-hr control period followed by 2 hr of intravenous ethanol maintained at 4 mM in plasma. Samples were analyzed chemically and radiochemically for various metabolites. Ethanol had no effect on O_2 consumption (1200 and 1900 μmoles/min · m^2 after 15 and 69 hr fasts, respectively). After 15 hr fast, FFA uptake (130 μmoles/min · m^2) decreased 27% with ethanol while oxidation of FFA to ketones fell 70% and to CO_2 fell 32%. After 69 hr, FFA uptake (270) increased 37% with ethanol while oxidation to ketones decreased 4% and to CO_2 decreased 44%. Oxidation of ethanol to acetate compensated for reduced FFA oxidation. Production of plasma triglyceride fatty acids (18 μmoles/min · m^2 after both 15 and 69 hr) increased 37% and 66%, respectively; with ethanol and storage of lipid in liver increased greatly after 69 hr. Glycerol could account for 25, lactate 40, pyruvate 7, alanine 19, and other amino acids 35% of control glucose output after 69 hr fast, changing to 50, 6, 7, 25, and 9% after ethanol. We conclude that substitution of ethanol oxidation for that of FFA with resultant increased storage and secretion of triglycerides contributes materially to fatty liver and hyperlipemia after ethanol. This is especially prominent after 69 hr fast because ethanol fails to inhibit peripheral fat mobilization. Ethanol also causes marked shifts in substrates for gluconeogenesis. (Research supported by USPHS grants HE-06285 and 2-MO1-RR-00079-08.)

335. The Role of Cyclic Adenosine-3′,5′-Monophosphate and Prostaglandin in Platelet Aggregation. Sidney M. Wolfe,* Joseph Muenzer,* and N. Raphael Shulman, Cleveland, Ohio, and Bethesda, Md.

Thrombin induces platelet aggregation accompanied by stimulation of glycolysis and release of adenine nucleotides and calcium. We found that thrombin-induced platelet aggregation and lactate production were inhibited by theophylline (3 mM) and prostaglandin (10^{-6} M). Inhibition decreased with increasing amounts of thrombin. When suboptimal levels of prostaglandin (PGE_1) and theophylline were added simultaneously, potentiation was observed. Inhibitors had to be present before addition of thrombin to be most effective, for release of nucleotides and calcium was measurable as early as 10 sec after addition of thrombin. Known inhibitors of glycolysis have previously been shown to inhibit the release reaction. We found that PGE_1 has a similar inhibitory effect. Since PGE_1 increases intracellular production of cyclic AMP (cAMP) and theophylline inhibits cAMP breakdown, it appears that inhibition of glycolysis, release reaction, and aggregation are mediated by high cAMP. Dibutyryl cAMP (3 mM), which penetrates cells, inhibits the glycolysis necessary for the release reaction and also inhibits platelet aggregation. These studies suggest that cAMP is the key to regulation of platelet aggregation and that PGE_1, which is present in all tissues and known to modify responses to hormones such as insulin and epinephrine through its effects on adenyl cyclase, may play a similar role in regulating platelet aggregation.

336. Proenzyme Components of the Plasma Kinin System. KIRK D. WUEPPER,* THOMAS G. LAWRENCE,* AND CHARLES G. COCHRANE, La Jolla, Calif.

The sequence of activation of the kinin-forming system of rabbits was assessed directly by isolation of components of this system in precursor form. Precursor proteins were salted out with neutral $(NH_4)_2SO_4$ and separated by ion exchange chromatography, gel filtration, and electrophoresis. Four separate molecular entities have been identified that interact in sequence: a clot-promoting factor (CPF), an activator of prokininogenase (PKA), prokininogenase, and kininogen. Activation of CPF leads to sequential proenzyme-enzyme activation steps to release kinin from kininogen. A single kininogen was identified and isolated (3.8S, Ve/Vo 1.75, pI 5.7, α mobility). This substrate was cleaved by kininogenase or trypsin, and a vasoactive polypeptide indistinguishable from bradykinin was recovered. A single prokininogenase (4.5S, Ve/Vo 1.65, pI 5.9, γ mobility) was selectively activated by the PKA isolated from rabbit plasma or trypsin. PKA-activated kininogenase differed from the precursor in several physiochemical parameters (4.0S, Ve/Vo 1.70, pI 5.4, β mobility), suggesting that activation occurred by limited proteolysis. Kininogenase caused increased vascular permeability, hydrolyzed synthetic ester substrates (BAEe, TAMe, BAPN), and released kinin from kininogen. Kininogenase was inhibited by DFP, SBTI, and Trasylol, but not by LBTI, OMTI, heparin, or hydrocortisone. A precursor of PKA (5.5S, γ mobility) was activated by preparations containing purified CPF. CPF-activated PKA (3.0S, Ve/Vo 2.1, pI 4.6, prealbumin mobility) is apparently a fragmentation product of its precursor. PKA caused increased vascular permeability, but failed to hydrolyze BAEe or release kinin directly from kininogen. CPF in precursor form eluted from DEAE-cellulose at low ionic strength. CPF was activated by diatomaceous earth (DE), desorbed from DE, and isolated from disc gel electrophoresis. Treatment of prokininogenase with isolated CPF failed to activate this proenzyme. However, DE-activated CPF accelerated clotting in human plasma deficient in factor XII or XI (but not IX), and activated PKA from its precursor. (Supported by grants from the NIH, the National Multiple Sclerosis Society, and the American Heart Association.)

337. Potentiation of Human Lymphocyte Transformation by Autologous Red Blood Cells and Platelets. STANLEY YACHNIN, Chicago, Ill.

The isolation of high-titer hemagglutinating (H-PHAP) and low-titer hemagglutinating (L-PHAP) mitogens from the phytohemagglutinin derived from *Phaseolus vulgaris* has helped define the role of cell-cell and membrane-membrane interaction in the potentiation of lymphocyte transformation as measured by ^{14}C-thymidine incorporation. Red blood cells (RBC) lead to a marked increase in lymphocyte transformation when H-PHAP is used as mitogen, whereas lymphocyte transformation by L-PHAP is unaffected by the presence of RBC. Potentiation of lymphocyte transformation by H-PHAP is also effected by RBC stromal particles. Potentiation has been shown to depend upon the formation of a mixed agglutination lattice between RBC and lymphocytes by H-PHAP, a property not shared by L-PHAP. To extend these observations further, studies were performed on the effects of autologous RBC and platelets upon lymphocyte transformation by a variety of mitogens, utilizing human lymphocytes free of RBC and platelets as the starting cell preparation. The mitogenic effects of pokeweed mitogen (PWM) are potentiated to a maximum of 300% by a RBC:lymphocyte ratio of 0.5–20:1; potentiation of lymphocyte transformation by RBC using concanavallin A (CON-A) is highly sensitive to RBC concentration, inhibition occurring at RBC:lymphocyte ratios of 10:1. The mitogenic activities of L-PHAP, H-PHAP, PWM, and CON-A are all potentiated (200–400%) by the presence of platelets in a platelet:lymphocyte ratio of 3–10:1; higher doses of platelets are less stimulatory and may actually cause inhibition of lymphocyte transformation. In contrast to these findings, lymphocyte transformation induced by antigen (PPD) or by one-way mixed lymphocyte culture (MLC) is not potentiated by the presence of RBC or platelets, and in certain MLC experiments their addition markedly inhibits transformation.

338. Change in Subcapsular Pressure and Renal Volume in the Experimentally Diseased Dog Kidney versus a Contralateral Control Kidney after Extracellular Fluid Volume Expansion. STEPHEN W ZIMMERMAN,* STEPHEN W. FLAX,* JON P. WAGNILD,* STUART J. UPDIKE,* FRANK D. GUTMANN,* AND RICHARD E. RIESELBACH,* Madison, Wis. (introduced by Edwin C. Albright**).

Acute ECF volume expansion (VE) induces a disproportionate increase in fractional excretion of sodium (FE_{Na}) in the experimentally induced pyelonephritic dog kidney (DK) as compared with a simultaneously studied contralateral control kidney (CK). To determine whether this response is dependent upon a disproportionate increase in DK interstitial pressure and/or interstitial volume, Δ subcapsular pressure (SCP) and Δ renal volume (RV) induced by VE with 75 ml/kg normal saline were determined in DK and CK, and related to ΔFE_{Na}. ΔSCP was measured with bilateral subcapsular microtransducers in 14 anesthetized dogs, and ΔRV with bilateral chronically implanted plethysmometers (CIP) in six unanesthesized, split-bladder dogs. The CIP is a mercury-filled strain gauge implanted around the kidney. A model of kidney geometry indicates that its Δ voltage output can be reliably converted to Δ volume. Mean base-line DK/CK SCP was 18.00/18.1 mm mercury, with a mean DK/CK GFR of 7.3/27.6 ml/min and FE_{Na} of 1.33/0.35%. Whereas VE induced a markedly disproportionate absolute increase in FE_{Na} ($\Delta DK/\Delta CK = 14.8/5.4\%$), ΔSCP did not differ ($\Delta DK/\Delta CK = 27.7/30.0$ mm mercury; $P > 0.5$). In subsequent 10 min periods after VE, FE_{Na} and SCP underwent parallel decreases toward control levels. In the six split-bladder dogs, a similar pattern was observed for ΔFE_{Na} vs. ΔRV. Mean base-line DK/CK was 14.2/56.2 and FE_{Na} 0.59/0.31. The VE-induced $\Delta DK/\Delta CK$ FE_{Na} was 12.3/5.8, whereas ΔRV of DK vs. CK did not significantly differ ($\Delta DK/\Delta CK = 17.2/27.7\%$; $P > 0.01$). Thus, if SCP and RV reflect interstitial pressure and interstitial volume respectively, these data do not support an exaggerated change of these determinants of intrarenal environment in DK as

the basis for its disproportionately increased FE_{Na} following VE.

339. The Immunologic Differentiation of the Antihemophilic Factor (Factor VIII) Abnormalities in Classic Hemophilia and von Willebrand's Disease. THEODORE S. ZIMMERMAN,* OSCAR D. RATNOFF,** AND A. E. POWELL,* Cleveland, Ohio.

Using naturally occurring circulating anticoagulants as antibodies against antihemophilic factor (AHF, factor VIII), several investigators have detected functionally inactive material antigenically related to AHF in only a small proportion of patients with classic hemophilia. In contrast, our studies with heterologous antibody now demonstrate material antigenically resembling AHF in plasma in each of 10 patients with classic hemophilia, in quantities comparable with that in normal plasma. In seven patients with von Willebrand's disease, however, the quantities of this material directly paralleled the degree of AHF deficiency measured in procoagulant assays. Antibody was prepared in rabbits against human AHF (8000 to 16,000-fold purified by a modification of Johnson's technique and stabilized in 2 M glycine hydrochloride). This antiserum, absorbed with AHF-depleted plasma fractions, formed a single line upon gel diffusion against normal plasma, crude AHF concentrates, or highly purified AHF. Normal and hemophilic plasma gave reactions identical with each other upon gel diffusion against absorbed rabbit anti-AHF. Measured by semiquantitative immunoelectrophoresis, AHF-like material was found in comparable amounts in hemophilic and normal plasmas, but in von Willebrand's plasma, it was found in proportion to functional AHF activity. Similarly, ethanol-precipitated fractions of hemophilic plasma, lacking AHF activity, inhibited the antibody's anti-AHF activity to the same degree as similar fractions of normal plasma, as measured in clotting assays, whereas von Willebrand's fractions blocked antibody in proportion to their functional AHF activity. Thus, classic hemophilic plasma contains functionally inactive AHF-like material, whereas plasma of patients with von Willebrand's disease contains functionally active AHF, but usually in reduced amount. Transfusion of hemophilic plasma into patients with von Willebrand's disease induces a rise in plasma antihemophilic activity. Whether the nonfunctional AHF in classic hemophilia is the agent responsible for this rise in AHF activity is unresolved. (Research supported by grants from the NIH and the American Heart Association.)