JCI The Journal of Clinical Investigation

Abstracts

J Clin Invest. 1972;51(6):57a-107a. https://doi.org/10.1172/JCI106961.

186. Response of the Canine Gut to the Diarrhea Toxin of *Escherichia coli*. RUVEN LEVITAN, SHERWOOD L. GORBACH,* MICHAEL MADDOCK,* AND RICHARD D. COLEMAN,* Chicago, Ill.

There is growing evidence that "nonspecific" diarrhea in man may be caused by enterotoxin-producing strains of *Escherichia coli*. We studied the pathophysiology of such diarrhea in the dog using a whole cell lysate preparation of *E. coli* 410G, a strain isolated from the small bowel of a patient with acute diarrhea. Chronic Thiry-Vella loops of jejunum and ileum were exposed to an isotonic solution containing lysate. Net secretion of H_2O, Na^+, and K^+, and HCO_3^- started 20 min after exposure and peaked during the second hour postchallenge; the fluid output rapidly diminished during the subsequent 2 hr in both jejunum and ileum. Secretion was somewhat greater in the ileum than in the jejunum. Mean control and peak postchallenge values respectively were for jejunum, H_2O, 0.13 and -0.17 ml/min; Na^+, 0.022 and -0.033 mEq/min; and for ileum, H_2O, 0.21 and -0.31 ml/min; Na^+, 0.030 and -0.57 mEq/min. These differences were all significant as judged by the paired T test ($P < 0.01$). Perfusion of the colon with the toxin preparation showed no effect on salt and water transport. Thus, *E. coli* toxin is similar to cholera toxin in that the fluid effluent appears to originate in the small bowel. However, fluid secretion with *E. coli* peaked earlier and was of shorter duration than with cholera. This copious, but short-lived secretion is typical of the clinical picture of nonspecific diarrhea. The canine model should be useful in further studying the mechanisms of diarrheogenic toxins associated with nonspecific diarrhea. (These studies are supported by Army Contract DADA 17-70C-0110 and NIH Research Grant AM 13387.)

187. Some Unfavorable Effects of Using Organic Solvents to Fractionate Erythropoiesis Stimulating Factors (ESF's) from Human Urine. J. P. LEWIS,* EMILY T. WELCH,* W. A. NEAL,* C. M. DUBOSE, JR.,* C.-S. WRIGHT, W. G. LEWIS III,* AND LINDA L. SMITH,* Augusta, Ga.

Ethanol has been used to fractionate erythropoiesis stimulating factors (ESF's). The purpose of this report is to describe some of the unfavorable effects of organic solvents on ESF's. A urine concentrate of ESF, fraction II + III from a male patient with paroxysmal nocturnal hemoglobinuria (PNH), was the starting material. A relatively small ESF was removed from fraction II + III by selective membrane permeability. The retentate contained a relatively large ESF and an ESF-generating factor (EGF), which could be separated by selective membrane filtration. The ESF fractions were extracted with an ethanol-acetone mixture and the precipitate washed with ethanol and then ether. ESF and EGF activities were determined on all fractions before and after extractions and upon recombination of the extracts with the precipitates. The EGF was reversibly inactivated; the precipitate and the extract separately had no activity. When the two fractions were incubated together, however, an appreciable amount of activity was regenerated. In the latter respect the EGF resembled the renal erythropoietic factor (REF) studied extensively by Gordon et al. The relatively large ESF was irreversibly inactivated; however, the small ESF was not inactivated and was partially soluble in the organic solvents. Possibly the relatively large and small ESF fractions contained the same active moiety bound with different degrees of firmness. The relatively small ESF and the EGF were demonstrated previously in the urine of both sexes during two different anemias, however, the relatively large ESF was demonstrated only in the urine of the male PNH patient. (Aid from NIH grants HE-10591, 14886, and 12958.)

188. The Transmissibility of Canine Systemic Lupus Erythematosus (SLE). ROBERT M. LEWIS,* JANINE ANDRE-SCHWARTZ,* MARTIN C. HIRSCH,* PAUL BLACK, AND ROBERT S. SCHWARTZ, Boston, Mass.

Systemic lupus erythematosus (SLE) in dogs is remarkably similar to the disease in man. Affected animals usually have autoimmune hemolytic anemia, thrombocytopenia, nephritis, positive LE cell tests, and antinuclear antibodies (ANA). A colony of dogs with SLE has been established and is now in its fourth generation of inbreeding. Genetic analysis of the families suggests the presence of a vertically transmitted virus. Cell-free filtrates of tissues from seropositive dogs were injected into newborn mice. These animals developed ANA and, in some cases, lymphomas. The tumors are transplantable in mice and recipients surviving over 1 month have positive ANA tests as well as lymphomas. Passage of cells or filtrates from the tumors into normal newborn puppies resulted in ANA production or positive LE cell tests within 4 months. C-type RNA viruses were identified in the tumor by: (*a*) electron microscopy, (*b*) in vitro cytopathogenicity (XC) tests, (*c*) bioassay of oncogenicity, and (*d*) presence of antigenic determinants (gs-1) specific for murine leukemia viruses. Control mice did not have ANA, contained no detectable viruses, and failed to develop lymphomas. The results strongly suggest the presence in canine SLE of a filterable agent capable of activating latent oncogenic viruses in mice. Induction of positive ANA and LE cell tests in normal puppies by filtrates of the dog-induced mouse tumor could be due to either a murine or canine virus, possibilities which are now under study. These findings imply that both neoplasms and autoantibodies can be triggered by viruses capable of infecting across species barriers. (Research supported by grants from NIH.)

189. Erythrocytes Sphere Transformation in Stored Blood: Relationship to Plasma Lysolecithin and Erythrocyte ATP. MARSHALL A. LICHTMAN* AND GUIDO V. MARINETTI,* Rochester, N. Y. (introduced by Robert I. Weed).

Erythrocytes in plasma at 37°C change from disc to sphere when ATP falls to < 0.25 μmoles/ml RBC at ~ 16 hr. From 0 to 16 hr, intracellular calcium was zero but rose to 0.11 and 0.51×10^{-17} moles/cell at 24 and 48 hr. Membrane calcium (0.14×10^{-17} moles/cell) remained unchanged over 48 hr. Erythrocytes in autologous plasma at 37°C were nearly all ($> 85\%$) crenated spheres after 18 hr (ATP < 0.15 μmoles/ml) and were tightly crenated or smooth spheres after 36 hours (ATP < 0.08 μmoles/ml). However, erythrocytes were largely ($> 75\%$) discs at 18 hr and many were discoidal from 18 to 36 hours if examined

in fresh plasma indicating a plasma factor in spherogenicity although injury does become irreversible. Plasma incubated at 37°C could by ~ 10 hr convert autologous fresh erythrocytes to crenated spheres. Plasma spherogenic capacity was accompanied by a conversion of lecithin to lysolecithin and cholesterol to cholesterol-ester (plasma lecithin: cholesterol acyltransferase) (LCAT). Lysolecithin added to plasma in similar amounts produced morphologically identical crenated spherocytes. Incubated whole blood contained in its plasma spherogenic capacity for fresh autologous erythrocytes at a time when erythrocytes present in whole blood were not spherocytes (~ 10 hr). Spherocytosis did not ensue until ATP fell to < 0.25 μmoles/ml. Moreover, erythrocytes added to spherogenic plasma and instantaneously converted to spheres, returned to discs after 6 hr incubation in spherogenic plasma. Discs persisted if erythrocyte ATP remained > 0.25 μmoles/ml. Endogenous or exogenous increases in lysolecithin resulted in fusion of erythrocytes which persisted when fresh erythrocytes reverted to discs by further incubation. Hence, prelytic spherogenic and lytic capacity of stored plasma is due to lysolecithin accumulation. Lysolecithin may be detoxified (?acylated) by the erythrocyte if ATP levels are maintained. With severe ATP depletion, erythrocyte membrane injury ensues. Such injury may be due also to other changes, including cellular calcium accumulation.

190. Inhibition of Leukocytic Elastase from Purulent Sputum by α_1-Antitrypsin. JACK LIEBERMAN,* Duarte, Calif. (introduced by David E. Comings).

The elastic tissue of lung may be the primary site of damage during the development of emphysema in individuals with α_1-antitrypsin deficiency. Thus, the potential role of a leukocytic elastase in the pathogenesis of emphysema, and its inhibition by α_1-antitrypsin of human serum, is of major interest. Purulent sputum was found to contain an elastolytic enzyme that resists heating at 65°C for 1 hr in a low ionic strength medium at pH 5.5. Previous failure to demonstrate this enzymatic activity resulted from its inactivity when insoluble in a water homogenate. The enzyme was made soluble by 1 M NaCl, revealing an elastase activity with similar properties to those described by Janoff in leukocyte granules. Activity was inhibited by DFP, soybean trypsin inhibitor, EDTA, and salivary kallikrein inhibitor, but was not inhibited by NaCl. The enzyme from sputum was found to be active with denatured protein substrates, specifically with denatured collagen or hemoglobin, as well as with elastin substrates. Human serum strongly inhibited this elastase activity at a level directly proportional to the serum trypsin inhibitory capacity. Previous exposure of serum to trypsin blocked the inhibition of elastase, suggesting that the serum inhibitor may bind both enzymes at the same site. These observations provide further support for the concept that leukocytic proteases play a role in the destructive processes leading to pulmonary emphysema with α_1-antitrypsin deficiency. (Research supported by grant from NIH.)

191. Net Splanchnic Cyclic AMP (cAMP) Production in Normal and Diabetic Men: Effects of Glucagon. J. E. LILJENQUIST,* J. D. BOMBOY,* B. C. SINCLAIR-SMITH,* S. B. LEWIS,* P. W. FELTS,* W. W. LACY,* O. B. CROFFORD, AND G. W. LIDDLE,** Nashville, Tenn.

Glucagon activates cyclic AMP (cAMP) production in rat liver, increasing hepatic vein (HV) cAMP concentration. Insulin, however, antagonizes glucagon-mediated cAMP production, thus providing a hypothetical mechanism through which insulin corrects some of the metabolic abnormalities of diabetes. To study these hormonal interactions, net splanchnic cAMP production (NScAMPP) was investigated in four normal and five insulin-dependent diabetic men under basal conditions and in response to intravenous high-dose glucagon, 50 ng/kg per min for 2 hr. In normals, basal HV cAMP concentration was 21.4±1.3 nmoles/liter with NScAMPP of 3.2±0.89 nmoles/min. Glucagon stimulated NScAMPP to a peak of 102±42 nmoles/min at 25 min with subsequent fall to 12±5 nmoles/min by 90 min despite continuing glucagon infusion. Plasma immunoreactive insulin increased progressively during glucagon infusion, raising the the possibility that endogenous insulin might be responsible for the fall in NScAMPP that followed the initial spike. In diabetics, basal HC cAMP concentration was 24.3±0.96 nmoles/liter with no detectable NScAMPP. Glucagon stimulated NScAMPP to a peak of 170±43 nmoles/min with subsequent fall to 17±4 nmoles/min by 90 min even though there was no demonstrable insulin rise. Although the increase in mean NScAMPP was greater in diabetics, no significant difference was established. In all subjects, glucagon caused a rise in NScAMPP within 2.5 min, preceding or coinciding with rising HV glucose concentration. We conclude that in normal resting man, liver is a significant source of circulating cAMP. Diabetics do not have abnormally high NScAMPP under basal conditions. Glucagon markedly enhances hepatic cAMP release with a spike-plateau pattern in both normal and diabetic men. The decline in hepatic cAMP release despite continuing glucagon stimulation is due to factors other than insulin. (Supported by grants from NIH, Veterans Administration, and American Heart Association.)

192. Zinc Metabolism in Experimental Myocardial Infarction. ROBERT D. LINDEMAN,* ANIECE A. YUNICE,* DONALD J. BAXTER,* AND LEONARD R. MILLER,* Oklahoma City, Okla. (introduced by Stewart G. Wolf**).

Since zinc is the metal component or activator of numerous enzymes and influences the rates of protein synthesis and tissue repair, it was hypothesized that the fall in plasma zinc concentration after acute myocardial infarction might result from zinc mobilization to the site of tissue injury. Radiozinc uptake into and/or zinc concentrations (along with seven other cations) in central infarct and peri-infarct have been compared against normal myocardium in 38 dogs with documented infarctions of 3–7 days duration produced by coronary artery ligation. In the 16 animals with large infarctions, the mean±SD central infarct to normal myocardium ratios, based on concentrations per milligram ash weight, were: sodium 2.25±0.19, potassium 0.57±0.07, calcium 4.5±0.7, magnesium 0.55±0.08, zinc 0.77±0.06, copper, 0.93±0.04, manganese, 0.69±0.09, and cadmium 1.60±0.19. Radiozinc uptake and zinc concentrations were decreased

in intact myocardium and in the nuclear subcellular fraction in infarct compared against normal myocardium but were significantly increased ($P < 0.001$) in the mitochondrial, microsomal, and soluble protein subcellular fractions. Electron microscopic studies confirmed the specificity of these fractions and the similarities between central infarct and normal myocardium fractions. The increases in zinc uptake into the last three fractions apparently cannot be attributed to leukocyte infiltration into the infarct as most of the zinc in ultracentrifuged buffy coat is found in the nuclear fraction. Oral zinc supplementation in seven dogs failed to increase zinc concentrations in either infarcted or normal myocardium. Although no increase in zinc concentration or radiozinc uptake could be demonstrated in intact infarct, increases in both were observed in those subcellular fractions where protein (enzyme) synthesis occurs. (Grant support by VA and NHI [HE12882].)

193. Pattern of Loss of Membrane Immunoglobulin in Variable Immunodeficiency with Thymoma, and Other Immunodeficiencies: Studies by Mixed Antiglobulin Reaction. S. D. LITWIN,* H. J. MEUWISSEN,* AND B. POLLARA,* New York (introduced by Hartwig Cleve).

Membrane immunoglobulin (Ig) can be found on up to 10% of normal human peripheral lymphocytes by the mixed antiglobulin reaction. In 30 normal subjects Ig antigens were as follows: γ, 4.1%; μ, 3.1%; α, 1.2%; κ, 5.7%; λ, 2.3%. 15 cases of primary immune deficiency were analyzed. Four cases of variable immunodeficiency with thymoma had differing patterns of findings. One subject had all Ig heavy-chain class and light-chain type antigens on his peripheral lymphocytes. Two other cases possessed less than 0.5% μ (+) cells whereas, γ, α, and κ were normal or only partially decreased. A last patient lacked all membrane Ig's on her cells. The clinical presentations of the patients were compared and discussed. The variability of the findings in this clinical group is consistent with heterogeneity of the disease, or may suggest that membrane Ig can vary at different stages in the natural history of this disorder. The lymphocytes of a child with combined immunodeficiency were analyzed at 11 months at which time the patient appeared to be producing small amounts of Ig's. No membrane Igs were found on peripheral lymphocytes. Four subjects lacking serum IgA associated with different immune problems were investigated. All were found to have IgA on 1–2% of peripheral blood lymphocytes. Five cases of variable immunodeficiency without thymoma were found to possess all membrane Ig's although often at decreased levels. (Supported by NIH Grant AI 09239.)

194. DNA Synthesizing Units in Normal Human Serum: Relation to Australia Antigen. LAWRENCE A. LOEB,* RICHARD O. WILLIAMS,* ALTON I. SUTNICK,* AND BARUCH S. BLUMBERG,** Philadelphia, Pa.

We report for the first time evidence of DNA-synthesizing activity in serum from individuals without apparent disease. It is assayed by measuring incorporation of radioactive deoxynucleotide triphosphates into a product digestable with deoxyribonuclease. Small amounts of activity are present in most individuals (0.1–0.4 pmoles $dTM^{32}P$/0.025 ml serum per hr); in 3 of 120 normal individuals activity was as great as 0.7–1.5 pmoles. All four deoxynucleotides and Mg^{2+} are required for activity. The activity is not increased by adding DNA, RNA, or synthetic homopolymers suggesting that a polynucleotide template is already present, intimately associated with a polymerase. Sedimentation through sucrose gradients suggests that this activity is associated with low-density substances, presumably lipids. The activity is cold labile and not RNA-dependent in that we have not yet been able to abolish it with ribonuclease. We have examined Australia antigen (Au(1)), a particle associated with viral hepatitis which may contain RNA, for the presence of a "reverse transcriptase." We have detected an activity that is not copying RNA and is similar to the one present in normal serum in one of four preparations of purified Au(1). Comparison of serum and particulate fractions of serum from age- and sex-matched patients with and without Au(1) demonstrated a statistical association between amount of activity in serum and presence of Au(1) in one of two studies ($P = 0.04$). The distribution of this activity in serum of patients with hepatitis is similar to that found in the normal population. These findings suggest that this activity may be statistically associated with Au(1), but is not unique to it. (Supported by NIH.)

195. The Specificity of IgM Rheumatoid Factors for Native IgG. MALCOLM R. MACKENZIE,* Davis, Calif. (introduced by P. D. Hoeprich**).

The specificity of IgM rheumatoid factors for antigenic sites on native IgG or "buried" determinants on altered IgG remains unresolved. A monoclonal IgM_{Brd} kappa protein which precipitates antigen-antibody complexes of human and rabbit origin or aggregated IgG but not native IgG in fluid systems was used. Its reaction with native IgG was studied by: (*a*) analytical ultracentrifugation (UC), and (*b*) precipitation of ^{125}I-labeled IgG in free solution. UC studies showed clear evidence of binding of IgG by IgM_{Brd}. ^{125}I-labeled IgG was not precipitated by IgM_{Brd}. However the addition of either anti-IgG or anti-IgM antiserums precipitated equal amounts of the ^{125}I-IgG indicating the presence of soluble IgG-IgM_{Brd} complexes. IgM_{Brd} rheumatoid factor combines with unaltered IgG to form a soluble antigen-antibody complex confirming the presence of a binding site on native IgG. Precipitation of the complex occurs with introduction of lattice-forming agents, either an anti-immunoglobulin, an aggregated immunoglobulin, or an antigen. It is postulated that the conformational changes in IgG induced when it reacts with antigen may promote lattice formation, thus creating a complex which will precipitate with the addition of IgM. The studies suggest it may not be necessary to invoke a buried antigen hypothesis to explain the in vitro behavior of rheumatoid factors. These data do not exclude the development of a new antigenic site on IgG in vivo when antibody-antigen complexes stimulate the production of rheumatoid factors. (Research supported by Grant IC-11A from ACS.)

196. Bile Acids Inhibit Cholecystokinin (CCK) Release in Man. JUAN R. MALAGELADA,* VAY L. W. GO,* EUGENE

P. DIMAGNO,* AND WILLIAM H. J. SUMMERSKILL,* Rochester, Minn. (introduced by E. E. Wollaeger**).

Interaction between bile acids and digestive products on CCK release from the small intestine was studied in 41 healthy volunteers using a validated bioassay which relates CCK secretion to pancreatic enzyme output in response to intraluminal stimuli. Trypsin outputs were quantified during randomized, steady-state intraduodenal perfusions of normal saline (NS) with or without CCK by vein (0.25 CHRU/kg per min), and physiologic concentrations of essential amino acids (EAA, 78 mM) or fat (1-monolein, 10 mM) in micellar form (TCF) with sodium taurocholate (TC, 10 mM) or emulsified (F) with 1 mM TC. Trypsin output was 5.7±1.6 (KU/hr; mean±SE) with NS, higher with EAA (20.6±2.1; $P < 0.01$), and even greater with F (39.1±4.4; $P < 0.002$). Outputs with F or with EAA + F (40.1±4.4), accorded with maximal response to intravenous CCK (41.4 ±2.3). TC alone was inert, outputs (7.3±0.4) being similar to NS. TC added to EAA (10.9±3.3) or F (10.7±0.6) produced outputs less ($P < 0.02$) than EAA or F alone. TCF added to EAA (12.3±3.7) also yielded outputs lower than EAA or F ($P < 0.05$). Gall bladder contraction occurred with EAA and F, but not with solutions containing TC (10 mM); nevertheless intraluminal bile acid concentrations were higher ($P < 0.001$) with TC (9.2±0.4 mM) than without it (3.3 ±0.4 mM). Since simultaneous perfusion of TC and EAA at different intestinal sites containing CCK caused trypsin outputs (18.5±0.7) identical with EAA alone, the inhibitory effect of TC is local, and as intravenous-CCK given with TCF + EAA yielded outputs (39.8±4.3) similar to intravenous CCK alone, a direct effect of TC on the pancreas can be excluded. Thus, CCK release in response to the potent stimuli of intraluminal EAA or F is inhibited by bile acids and this relationship demonstrates a feedback mechanism regulating gall bladder contraction and pancreatic secretion during digestion. (Supported by NIH Grant AM 6908.)

197. Penicillin Binding by Intraleukocytic Bacteria. GERALD L. MANDELL,* Charlottesville, Va. (introduced by Edward W. Hook**).

Bacteria that survive intracellularly after phagocytosis by polymorphonuclear neutrophils (PMN) are protected from the bactericidal action of penicillin (Pcn). In order to determine if PMN membranes are permeable to Pcn, experiments were performed quantitating Pcn bound to extraleukocytic and intraleukocytic bacteria. 5×10^8 Pcn sensitive *Staphylococcus aureus* were incubated with 0.1 μg of benzyl Pcn-^{14}C (121 μCi/mg) for 2 hr at 37°C. Unbound Pcn was washed off and bound Pcn was extracted and counted in a liquid scintillation counter. Live bacteria bound 424±19 pg Pcn at 37°C while bacteria killed by heat bound only 11.1 ±3.7 pg Pcn ($P < 0.001$). Despite a much larger cellular mass, 1×10^8 PMN bound only 55.9±7.4 pg Pcn. Studies were carried out to ascertain if Pcn could bind to living bacteria inside phagosomes of intact PMN. Staphylococci were incubated with PMN at a ratio of 10:1. After 30 min, extracellular staphylococci were removed by washing, and viable intracellular staphylococci were quantitated by lysis of PMN, serial dilution, and plate counts. Live intraleukocytic bacteria bound only 17% as much Pcn as did equal numbers of live bacteria incubated with killed PMN. The oxygen consumption of intraleukocytic and extraleukocytic bacteria was measured as an index of bacterial metabolism. PMN, containing viable intraleukocytic bacteria, were placed in the chambers of a polarographic oxygen monitor and PMN respiration and bactericidal activity was suppressed with large doses of hydrocortisone (10 mg/ml). Extraleukocytic bacterial oxygen consumption was abolished by the addition of the enzyme lysostaphin. Intraleukocytic bacteria consumed oxygen at the same rate as extraleukocytic bacteria. Intraleukocytic staphylococci bind only 17% as much Pcn as extraleukocytic staphylococci, but intraleukocytic bacteria consume oxygen normally. These studies suggest that Pcn does not freely enter the phagocytic vacuole; this may be the explanation for the survival of phagocytized bacteria in the presence of high concentrations of this antibiotic. (Research supported by grant from NIH.)

198. The Oxyhemoglobin Dissociation Curve in Patients with Chronic Obstructive Lung Disease and Hypoxemia. FELICE MANFREDI,* MARK O. FARBER,* AND KENNETH F. ATKINSON,* Indianapolis, Ind. (Introduced by Paul J. Fouts**).

Blood [H^+], RBC[H^+], plasma lactate, MCHC, COHb, RBC 2,3-diphosphoglycerate (DPG), and P_{50} were compared in a group of 10 male patients with chronic obstructive lung disease (COLD) and chronic hypoxemia (Pa_{O_2} 58±3 mm Hg SEM) and in a group of 10 age-matched males without cardiopulmonary disorders. The only significant differences observed in the COLD group were: (*a*) MCHC, from 26.3 to 34.6, $\bar{m} = 30.8\%$; (*b*) COHb from 0 to 8.9, $\bar{m} = 2.5\%$; (*c*) DPG from 11.6 to 27.2, $\bar{m} = 16.6$ μmoles/g Hb (controls = 10.3 to 16.9, $\bar{m} = 13.2$); (*d*) P_{50} from 21.5 to 32.5, $\bar{m} = 26.0$ mm Hg. It is generally accepted that in the absence of other factors displacing the position of the HbO_2 curve, the relation between P_{50} and DPG is defined by the linear regression $\hat{Y} = 17.6 + 0.7X$ (1971. *J. Clin. Invest.* **50:** 700; 1970. *J. Pediat.* **77:** 941). In the COLD group this relation was found to be $\hat{Y} = 19.6 + 0.4X$, r 0.7, $P < 0.025$, clearly indicating that the P_{50}'s were low for the observed DPG's. When P_{50}'s were calculated using correction factors for the various parameters which displace the HbO_2 curve to the right [+ 0.7 mm Hg/μmole increase in DPG per g Hb] and to the left [− 0.3 mm Hg% increase in COHb (1968. *Science (Washington).* **162:** 1352); and − 0.5 mm Hg/1% decrease in MCHC (1971. *J. Clin. Invest.* **50:** 700)] an excellent correlation was observed between the calculated and the measured P_{50}'s ($\hat{Y} = 5.1 + 0.84X$, r 0.88, $P < 0.001$). These data indicate that the apparent discrepancies between P_{50}'s and DPG's observed in patients with COLD and hypoxemia can be explained by considering both the right shift of the HbO_2 curve induced by the elevated DPG and the left shift induced by decreased MCHC and increased COHb. (Supported by NIH Grant He12248.)

199. An Effect of Dexamethasone on the Cyclic AMP Content of Human Fibroblasts Stimulated by Catecholamines and Prostaglandin E_1. VINCENT MANGANIELLO,* JAN BRESLOW,* AND MARTHA VAUGHAN, Bethesda, Md.

Human fibroblasts were cultured from foreskins of apparently normal children less than 1 wk old. Subcultures were grown to confluency, then incubated for 2–3 days with or without 1 μM dexamethasone. They were exposed to theophylline, catecholamines, or prostaglandin E_1 (PGE_1) for 15 min before harvesting for determination of cyclic AMP content using a protein-binding assay. In cells not treated with dexamethasone, maximally effective concentrations of epinephrine (27 μM) and isoproterenol (2 μM) increased the cyclic AMP content 4- to 10-fold and 10- to 20-fold, respectively. The effect of isoproterenol was completely prevented by 1 μM propranolol. PGE_1 produced a several hundredfold increase in cyclic AMP to levels as high as 4 nmoles/mg protein. In the presence of 1.5 mM theophylline (which itself raised the cyclic AMP content only slightly) effects of catecholamines and PGE_1 were enhanced by 50–100%. Incubation with dexamethasone did not consistently alter basal cyclic AMP content but the effects of PGE_1 and catecholamine (with or without theophylline) were markedly increased, often 100%, in steroid-treated cells. We have found that in an established cell line (HTC-hepatoma) dexamethasone decreases cyclic AMP phosphodiesterase activity. It seems probable that the steroid has a similar effect in the normal human fibroblasts. We have grown the latter cells for about 60 generations, and during that period the cyclic AMP responses of one line to dexamethasone, catecholamines, and PGE_1 have been repeatedly evaluated. Thus far, no alterations associated with aging have been observed. However, the period of senescence which we have not yet been able to study may be of the greatest interest.

200. Reactivity of Antisera Detecting Acute Leukemia-Associated Antigen(s). DEAN MANN,* ROGER HALTERMAN,* AND BRIGID LEVENTHAL,* Bethesda, Md. (introduced by William Terry).

Peripheral white blood cells (PWBC) from patients with acute lymphocytic leukemia and acute myelocytic leukemia appear to contain an antigen(s) not detectable on PWBC from normal individuals. The antigen is detected in a cytotoxic assay using antisera prepared in rabbits against a water-soluble cell membrane component from the cultured lymphoid cell line (RAJI). Positive tests were found with PWBC from 21 patients with ALL and 14 patients with AML with active disease. 10 patients (AML, ALL), followed from diagnosis to drug-induced remission, were found to lose detectable antigen in remission. Negative tests were also obtained with PWBC from 527 normal individuals, 5 patients with Hodgkin's disease, 3 with infectious mononucleosis, 5 with chronic myelogenous leukemia, and 4 with chronic lymphocytic leukemia and with bone marrow cells from 2 normal donors. Nonlymphoid tissue culture cells, derived from tumors from patients with a variety of neoplastic diseases (cervical carcinoma, bronchiogenic carcinoma, mammary carcinoma, osteogenic sarcoma) were tested for the presence of this antigen and found to be negative. However, nine cultured lymphoid cell lines were found to be antigen positive. Other investigators have tested four of these cell lines and found them to contain a virus or viral genome. The antisera were cytotoxic to human embryonic kidney cell lines (HEK) infected with the murine Rauscher leukemia virus and not cytotoxic to noninfected HEK cells. The antisera demonstrated cross-reactivity with Rauscher virus-infected HEK cells, cultured lymphoid cells, and the ALL and AML cells. The finding of antigen(s) common to human cells infected with virus and AML and ALL cells may indicate that a common mechanism is responsible for the expression of the antigen.

201. Effects of Calcium on Renal Ammoniagenesis. O. MANOS,* G. SCHREINER,** AND H. PREUSS,* Washington, D. C.

Hypercalcemia is associated with decreased ammonia excretions (1963. *Metab. (Clin. Exp.)* **12**: 792.). To determine why, we followed ammoniagenesis in rat kidney slices incubating in varying calcium concentrations. In bicarbonate-buffered medium (pH 7.4) without calcium, glutamine ammoniagenesis was 39.9±2.3 μmoles/g per 90 min. Adding 1 mM or 2 mM calcium significantly enhanced production, 49.4 ±1.9 and 49.2±2.6 μmoles/g per 90 min. However, further increases to 3 mM and 4 mM significantly decreased ammonia production from these levels, 43.4±2.0 and 42.7±1.9 μmoles/g per 90 min. In phosphate-buffered medium, ammoniagenesis at calcium 0 mM (56.9±3.0), 1 mM (66.4±1.2), 2 mM (67.2 ±3.5), and 3 mM (57.0±2.8) again showed significantly less production at 0 and 3 mM calcium. In slices from NH_4Cl-drinking rats, ammoniagenesis was: at calcium 0 mM = 108.2 ±4.5, 1 mM = 153.7±8.0, 2 mM = 141.7±7.4, 3 mM = 123.2 ±2.8, and 4 mM = 127.7±5.0 μmoles/g per 90 min. Despite enhanced ammoniagenesis by acidosis, significantly less ammoniagenesis was seen at low (0 mM) and high (3 mM and 4 mM) calcium concentrations. To study this further, glutamate was substituted as substrate. The trends in ammoniagenesis were similar, i.e., in bicarbonate buffer, 0 mM calcium = 22.9±2.2, 1 mM = 38.3±1.8, 2 mM = 36.1±2.3, 3 mM = 24.2±2.8, and 4 mM = 30.2±2.3; and in phosphate buffer, 0 mM = 27.7±1.2, 1 mM = 38.5±1.6, 2 mM = 35.2±1.5, and 3 mM = 26.9±1.7 μmoles/g per 90 min. While ammoniagenesis from glutamine and glutamate was significantly lower at 0 mM, 3 mM, and 4 mM calcium than at 1 mM and 2 mM, gluconeogenesis was decreased only at 0 mM. Since glutamate deamination is an oxidative process, Q O_2 was followed. At 0 mM and 3 mM, Q O_2 was significantly lower, 2.60±0.30 and 2.70±0.07 μl/mg per hr, than at 1 mM and 2 mM, 3.13 ±0.10 and 3.42±17 μl/mg per hr. We conclude that high concentrations decrease ammoniagenesis but not gluconeogenesis in kidney tissue by the following means: decreased Q O_2 → decreased glutamate, deamination → increased glutamate, concentrations → decreased glutamine deamidation. (Supported by NIH Grant.)

202. Exertional Syncope in Aortic Stenosis: Possible Implication of Ventricular Baroreceptors. ALLYN L. MARK,* J. MICHAEL KIOSCHOS,* PHILLIP G. SCHMID,* DONALD D. HEISTAD,* AND FRANCOIS M. ABBOUD, Iowa City, Iowa.

Left ventricular outflow obstruction in dogs produces reflex vasodilatation by activating left ventricular baroreceptors. This study was done to test the hypothesis that increases in left ventricular pressure and activation of ventricular baroreceptors during leg exercise in patients with aortic stenosis inhibits the normal reflex forearm vasoconstrictor response

to exercise. Forearm blood flow (plethysmograph), left ventricular pressure, arterial pressure, and cardiac output were measured before and during exercise (100–250 kg/min) in seven patients with aortic stenosis and six patients with mitral stenosis. Forearm blood flow increased 1.1±0.4 ml/min per 100 ml arm volume (mean±SE) in aortic stenosis and decreased −0.8±0.3 in mitral stenosis during exercise ($P < 0.05$). Arterial pressure did not change significantly in either group, but systolic left ventricular pressure increased 13±4 mm Hg in aortic stenosis ($P < 0.05$). Forearm vascular resistance tended to decrease (−6±4 U) in aortic stenosis in contrast to a significant increase (16±8 U) in mitral stenosis. Resting values for cardiac output and increases during exercise were greater in aortic stenosis than in mitral stenosis, but resting and exercise heart rates were similar in both groups. Reflex forearm vasodilatation during exercise in one patient with aortic stenosis and syncope converted to vasoconstriction after valve replacement. The results indicate that forearm vasoconstrictor responses to leg exercise are inhibited or reversed in patients with aortic stenosis, possibly because of increases in left ventricular pressure and activation of ventricular baroreceptors. These observations suggest that reflex vasodilatation resulting from activation of left ventricular baroreceptors may contribute to syncope during exertion in patients with aortic stenosis.

203. Attachment of Immune Complexes to Platelet Membranes in Tyrode's Solution via Determinants on the Fc Fragment of IgG. S. R. MARNEY, JR.,* D. G. COLLEY,* AND R. M. DES PREZ, Nashville, Tenn.

Exposure of rabbit platelet-rich plasma to an antigen and specific IgG antibody results in platelet aggregation, release of platelet amines, and coagulation acceleration. The reaction is complement-dependent, but many other details of the mechanism are unknown. The present studies began with the observation that platelets incubated with antigen and antibody in EDTA-plasma (in which no amine release occurs), washed, and resuspended in nonchelated plasma aggregated and released amines just as when antigen and antibody were added directly to nonchelated platelet-rich plasma. This suggested that the platelets were sensitized by incubation with antigen and antibody in EDTA-plasma, presumably by the attachment of immune complexes. We have utilized radiolabeled antibody to establish this mechanism. Purified radiolabeled rabbit antiovalbumin IgG fixes onto washed platelets in EDTA-Tyrode's solution in the presence of ovalbumin but not in its absence. Antigen-antibody ratios near equivalence are necessary for optimum fixation. Prior pepsin digestion of antibody prevents immune complex fixation, indicating a requirement for an intact Fc fragment. Since the medium used is Tyrode's solution, it follows that complement is not necessary for immune complex-platelet interaction and that complement fixation and platelet membrane injury are secondary events. These findings suggest that the platelet membrane has receptor sites for determinants on the Fc portion of IgG which are exposed or activated as a result of IgG-antigen interaction. This mechanism, which has not previously been described for platelets, may have relevance to human diseases such as post-infectious, neonatal, and possibly idiopathic thrombocytopenic purpura. (Research supported by NIH grant HE 08399 and the Veterans Administration.)

204. Hereditary Hypodysfibrinogenemia Characterized by Fibrinogen Hypercatabolism. JOSE MARTINEZ,* RUTH R. HOLBURN,* SANDOR S. SHAPIRO, AND ALLAN J. ERSLEV,** Philadelphia, Pa.

Several members of a family found to have a low plasma fibrinogen concentration were studied. The inheritance pattern was autosomal. Plasma fibrinogen measured by the Ellis-Stransky method and by quantitative immunodiffusion revealed a fibrinogen level ranging from 60 to 90 mg/100 ml in three members, while in several others the concentration was normal. Patients' plasma and purified fibrinogen had markedly prolonged thrombin and reptilase times. The clots were insoluble in 5 M urea, and euglobulin lysis times were normal. Immunoelectrophoresis and immunodiffusion showed no difference from normal when either plasma or fibrinogen was tested. Polyacrylamide gel electrophoresis at different pH values revealed a band with identical mobility to that of normal fibrinogen. A simultaneous turnover study of autologous and homologous fibrinogen labeled with ^{125}I and ^{131}I, respectively, was performed in two affected members. Autologous ^{125}I-fibrinogen half-life was short in both members (1.91 and 1.58 days, respectively) and the fractional catabolic rate was also increased (48.9 and 91.37% of plasma pool per day). In contrast, the homologous ^{131}I-fibrinogen half-life of 3 and 3.08 days and the fractional catabolic rates of 27.4 and 27.89% fell within normal range. After both labeled fibrinogens were injected, a plasma sample was filtered through a Sepharose 4B column. Both labeled proteins eluted simultaneously in a single radioactivity peak. The low plasma fibrinogen concentration, the prolongation of thrombin and reptilase times, the normal immunoelectrophoretic characteristics, and the high catabolic rates of these patients' own fibrinogen differentiate this abnormality from dysfibrinogenemias previously described. We propose this abnormal fibrinogen to be designated as Fibrinogen Philadelphia.

205. Purification of Peptide Hormone Receptors from the Kidney. STEPHEN J. MARX* AND G. D. AURBACH, Bethesda, Md.

Renal receptors for parathyroid hormone (PTH), calcitonin (CT), and vasopressin (AVP) have been identified by determining the response of adenyl cyclase in homogenates of renal tissue. We have purified from rat kidney a membrane fraction containing these receptors by homogenization, sedimentation in 0.25 M sucrose, and centrifugation in a continuous gradient of 32–42% sucrose (w/w). This last step greatly diminished contamination with mitochondria. In the crude homogenates of whole rat kidney the maximal specific activities of the PTH- and CT-stimulated adenyl cyclases were 390 and 290 pmoles (3',5'-AMP)/mg protein per 30 min incubation. In the purified membrane fraction these specific activities were increased to 9300 and 10,600 pmoles per 30 min, respectively. The adenyl cyclase responses to PTH and CT were additive, indicating that the receptors for the two hormones are distinct. As has been shown previously with crude homogenates, PTH receptors were found

mainly in the renal cortex, and AVP receptors principally in the renal medulla. The receptors for calcitonin have been characterized further by studying the binding of ^{125}I-labeled calcitonin (salmon). Unlabeled calcitonin inhibited this uptake process. This "specific" binding was half maximal at 2×10^{-9} M salmon calcitonin, but half maximal for bovine calcitonin at 10^{-7} M. The high potency of salmon calcitonin relative to mammalian calcitonin in vivo must then be explained at least in part by the high affinity of salmon calcitonin for specific receptors.

206. Oxygen Toxicity: Influence of Hyperoxia on Pulmonary Protein Synthesis and Lung Mechanics. DONALD MASSARO, GERARDO GACAD,* AND GLORIA D. MASSARO,* Washington, D. C.

The mechanism by which hyperoxia adversely alters pulmonary mechanics is unclear, but a decreased synthesis of surfactant lipoprotein could play a role. Because of this possibility we sought to determine if the synthesis of protein in a surface-active lung fraction (SAF) is altered by hyperoxia, and, if so, whether this alteration preceeded changes in pulmonary mechanics. We exposed rats of 98% O_2 or compressed air at ambient pressure for 48 hr. Lung slices were incubated with leucine-U-^{14}C at 40°C. We measured radioactivity in acid-insoluble protein. The SAF was obtained by ultracentrifugation of homogenized lung, adjusted to density 1.21 g/ml, at 105,000 g for 16 hr at 4°C. The SAF floated as a pellicle. It lowered surface tension to < 10 dynes/cm at 37°C with 0.15 M NaCl as subphase. We measured free leucine, DNA, and protein and made pressure-volume (P-V) measurements on excised lungs. The specific activity of total protein in the air group was 621±39 cpm/mg protein (mean±SEM) and in the O_2 group 486±28 cpm/mg protein ($P < 0.05$). The specific activity in the SAF of the air group was 1051±60 cpm/mg protein and in the O_2 group 622±58 cpm/mg protein ($P < 0.001$). Differences between groups were also statistically significant when acid-insoluble radioactivity was expressed per milligram of DNA or per millimicromole of free leucine. At this time (48 hr) the P-V characteristics of the lungs from the O_2-exposed rats were virtually identical with those of air-exposed rats. However, after 72 hr of hyperoxia the P-V characteristics were markedly abnormal. We conclude that hyperoxia decreases the synthesis of protein in a lung SAF. This may play an important role in the altered mechanical properties of oxygen-poisoned lungs.

207. Studies on Mechanism of Hypocalcemia in Acute Renal Failure in Man. SHAUL G. MASSRY, ALLEN I. ARIEFF,* JACK W. COBURN,* GENARO M. PALMIERI,* AND CHARLES R. KLEEMAN,** Los Angeles, Calif. and Oklahoma City, Okla.

Skeletal resistance to parathyroid hormone (PTH) may be largely responsible for hypocalcemia in chronic renal failure. In acute renal failure (ARF), hypocalcemia has been attributed to the elevated serum phosphorus (P). It is not known whether resistance to PTH action may develop in ARF and underlie the hypocalcemia. Also data on blood levels of PTH in ARF are limited. In 10 patients with ARF, blood levels of Ca, P, and immunoreactive PTH were measured frequently. Infusions of parathyroid extract (PTE), IU/kg per hr × 10, were given during the oliguric and diuretic phases of ARF and 2–3 months later when renal function was normal. Hypocalcemia of 5.3 to 8.4 mg/100 ml developed as early as 2 days after the onset of ARF and persisted during the diuretic phase when serum P was normal or low. Blood levels of PTH were elevated 2 to 5 times above normal in 9 of 10 patients. The infusion of PTE produced a 15- to 40-fold increase in PTH levels but failed to cause a calcemic response in nine patients during the oliguric phase and in eight of nine restudied during the diuretic period. The change in serum Ca was 0–0.3 mg/100 ml (normal 1.0–2.2 mg/100 ml). The failure of response to PTE was not related to blood levels of P. Four patients restudied with PTE infusion when their renal function was normal had a normal increase in serum Ca of 1.2–2.2 mg/100 ml. The data show that (*a*) blood levels of PTH are elevated in ARF in man, (*b*) skeletal response to both endogenous and exogenous PTH is impaired in ARF and this abnormality is probably a major factor underlying hypocalcemia in ARF, and (*c*) skeletal responsiveness to PTH returns to normal after recovery of renal function. (Supported by USPHS Contract 43-68-1040.)

208. Effect of Thyroxin on Growth of Cardiac Ultrastructures: Quantitative Measurement of Tissue Electron Micrographs. L. P. MCCALLISTER* AND ERNEST PAGE, Chicago, Ill.

Thyroxin-stimulated growth of cardiac ultrastructures was measured by point counting and line integration on electron micrographs of rat left ventricles (Page et al. 1971. *Proc. Nat. Acad. Sci. U. S. A.* **68**: 1465 and Mobley and Page. 1972. *J. Physiol.* **220**.). 200-g female rats were thyroidectomized; 30 days postthyroidectomy, animals were weight-matched and one of each weight-matched pair of rats was injected with 36 μg thyroxin every 4th day (total of six injections). On the 54th day postthyroidectomy, electron micrographs of the ventricles from injected rats were compared with those from uninjected hypothyroid controls. Thyroxin caused a 40% increase in myocardial cell volume. The fractions of cell volume made up of mitochondria (Vmi), myofibrils (Vmf), T-systems (Vts), and sarcotubules (Vst), were (values from thyroxin-injected rats italicized): Vmi 0.32±0.01, *0.36±0.01,* Vmf 0.50±0.01, *0.49±0.01,* Vts 0.007±0.002, *0.011±0.002,* Vst 0.026±0.001, *0.029±0.001.* Membrane areas per cell volume (μ^2/μ^3) were: external sarcolemma 0.35±0.02, *0.30±0.02,* external sarcolemma + T-system 0.41±0.02, *0.41±0.02,* sarcotubular membrane 1.2±0.1, *1.2±0.1;* mitochondrial cristal membrane areas per unit mitochondrial volume (μ^2/μ^3) were *22±2, 34±2.* Conclusions: As myocardial cells grow in response to thyroxin (*a*) T-system membrane area increases, thereby maintaining constant the ratio (total plasma membrane area per cell volume); (*b*) myofibrils, sarcotubular membrane areas, and cell volume increase proportionately; (*c*) mitochondrial volume increases proportionately more than cell volume; (*d*) mitochondrial cristal membrane area per unit mitochondrial volume increases. (Supported by USPHS HE 10503 and by Chicago Heart Association.)

209. The Management of Chronic Myelocytic Leukemia (CML) by Leukapheresis. K. B. McCredie,* C. S. Vallejos,* and E. J. Freireich, Houston, Tex.

11 previously untreated patients with Ph chromosome positive CML were managed with leukapheresis for periods ranging from 1–24 months. For each procedure an average of 1.1×10^{11} (110 g) leukocytes were removed from 10 liters of blood processed on a continuous flow basis with the blood cell separator over an average of 3 hr. Six patients had intensive leukapheresis at the rate of 4–5 procedures each week for a total of 18 (7–23) in 28 days (8–48). This resulted in a median reduction in the leukocyte counts of 19%/wk of therapy and an overall leukocyte count decrease of 68% (26–91) of 106,000/cm (25–300). Four patients had over 50% reduction in spleen size. All patients had a subjective improvement. One patient had three periods of leukapheresis over a period of a year and another two periods in 6 months. Both had disappearance of splenomegaly with each period of leukapheresis. Five other patients had intermittent leukapheresis at the rate of five procedures each month for a median of 47 per patient (32–100) over an 8 month period. (5–23). Three patients showed reduction in spleen size and all had subjective improvement. Circulating leukocyte counts reduced by 50% (40–70). Two patients became refractory after 5 and 7 months. Three are still controlled at 9, 16, and 23 months. Leukapheresis has objective beneficial effects on clinical and hematologic abnormalities of CML and offers a potentially useful technique for management of the benign phase of CML. (Research supported by grants from NIH and American Cancer Society.)

210. Mammalian Keratinocytes and Cytochalasin B. Joseph McGuire, Stephanie Arnesen,* and Gisela Moellmann,* New Haven, Conn.

The influence of a drug, cytochalasin B, on the morphology and motility of guinea pig keratinocytes was investigated. Guinea pig keratinocytes were cultivated in Cruickshank chambers. Keratinocytes are motile and exhibit ruffling of the advanced cell margin. The keratinocytes form desmosomes in culture and also show evidence of synthesis and aggregation of keratin. Cytochalasin B, a drug obtained from culture filtrates of *Helminthosporium dematioideum,* disrupts microfilaments in a number of cell types. Recently we have shown that cytochalasin B prevents centrifugal translocation of pigment granules in epidermal melanocytes of *Rana pipiens.* Cytochalasin B added to cultivated keratinocytes produces profound morphologic changes. Ruffling stops, and the advancing cell border retracts leaving delicate strands in its place. The effect of cytochalasin B is reversible. Keratin filaments, microtubules, and desmosomes appear to be intact after exposure of the cell to cytochalasin B. The rapid alteration of cell morphology induced by cytochalasin B suggests that microfilaments are important structural elements in the keratinocyte. (Research supported by grants from NIH: AM 13929, AM 1003, and CA 04679.)

211. Quantitative Analysis of Estradiol-Binding Protein in Human Mammary Carcinoma. William L. McGuire,* San Antonio, Tex. (introduced by S. J. Friedberg).

We recently reported that experimental mammary carcinomas which regress after ovariectomy contain a specific cytoplasmic 17β estradiol-binding protein (EBP), whereas mammary carcinomas which continue to grow after ovariectomy lack this protein. These studies have been extended to human mammary carcinomas to see if the presence of EBP may be used to predict the tumor response to endocrine ablation therapy. We now report the quantitative analysis of EBP in the human mammary tumor specimens. EBP is measured by two specific methods: (*a*) the presence of a estradiol-^{3}H-EBP peak in the 8–10S region of 5–20% sucrose gradient centrifugation which can be displaced by preincubation of EBP with unlabeled estradiol, (*b*) Scatchard analysis of the estradiol-^{3}H-EBP binding curve (dextran-coated charcoal) which yields the number of EBP sites per milligram cytosol protein and the dissociation constant (*Kd*) of the interaction. We find that fibroandenomas (10) do not appreciably bind estradiol. Primary mammary carcinomas (30) contain 0–618 femtomoles EBP/mg cytosol protein. Metastatic mammary tumors to bone, liver, skin, and lymph nodes (18) contain 0–196 femtomoles EBP/mg cytosol protein. *Kd*'s from both primary and metastatic lesions are very low, 1×10^{-10} M, indicating very high affinity binding. Sucrose gradient centrifugation reveals specific 8–10S binding peaks in tumors containing more than 11 femtomoles EBP/mg cytosol protein. We anticipate that values above this level will indicate hormone dependence whereas values below this level will indicate autonomous mammary carcinomas. Conclusions: (*a*) EBP can be easily quantitated in primary and metastatic human breast carcinoma; (*b*) This analysis should identify hormone-dependent tumors and lead to a more physiological approach to breast cancer therapy.

212. Immunoglobulin Synthesis In Vitro by Human Lymphoid Tissues. Robert McMillan,* Robert L. Longmire,* Robert Yelenosky,* and Charles G. Craddock,** La Jolla, Calif.

The ability to quantitate IgG synthesis by human lymphoid tissues in vitro would greatly facilitate study of the immune response in normal and diseased subjects. Employing a sensitive Fab-anti-Fab assay we have determined the net IgG synthesis in vitro by cells from normal, human lymphoid tissues including bone marrow, spleen, blood, lymph node, and thymus. Leukocyte suspensions were cultured for 10 days at 37°C in 20% fetal calf serum in Dulbecco's medium. Mean Ig synthesis (nanograms N IgG/10^6 lymphocytes) by these tissues were: bone marrow, 1475±1136; spleen, 33.4±15.7; blood leukocytes, 62.3±28.5; blood lymphocytes (purified with brushed nylon fibers), 16.4±10.5; lymph nodes, 2.8±0.4, and thymus < 1.0. Synthesis could be blocked with mitomycin C or low temperatures. Accelerated Ig production in the presence of smallpox vaccine could be shown in splenic and peripheral blood cultures; no stimulation was noted in marrow, nodal, or thymic cultures. The following conclusions can be drawn: (*a*) the Fab-anti-Fab system is useful for evaluating IgG synthesis in vitro by human tissues; (*b*) the bone marrow is the major site of IgG production under normal circumstances (>90%); (*c*) increased Ig production by smallpox vaccine occurred only

in splenic and blood cultures. These data support the contention that the bone marrow is the most important site of IgG "bursa-dependent" cells and would offer a reasonable explanation for the tendency of most plasmacytic tumors to originate primarily in the marrow cavity. (Research was supported by NIH grant CA 11800.)

213. Histamine Receptors and Immunologic Function of Leukocytes. KENNETH L. MELMON, GENE M. SHEARER,* YACOB WEINSTEIN,* AND MICHAEL SELA,* San Francisco, Calif. and Rehovot, Israel.

Hormones (e.g., histamine and catecholamines) stimulate adenyl cyclase and have pharmacologically specific receptors on human leukocytes. We report physically identifying functional extracellular histamine receptors on various leukocytes with specific immunologic activity and determining that histamine modulates the immunologic function of mouse spleen cells (Balb/Bl immunized with sheep RBC). Histamine (H) was insolubilized by linking it to Sepharose (S) or by attaching it first to rabbit serum albumin (R) and then to S. After incubation human leukocytes (not red cells or platelets) bind to S-R-H, but not to S-R, activated S, or S-H. Binding was specifically prevented by three antihistamines but not antagonists of catecholamines; histamine (10^{-3} M) inhibited binding by 27%; only selected cells would bind to S-R-H, 80–90% of beads coated with human leukocytes; 40–50% coated with normal or immunized mouse spleen cells; 97% coated with mouse macrophages; 15% coated with thymus cells; and only 5–10% coated with mouse myeloma cells. Sepharose to which either basic polyamino acids (polylysine) or polyamines (polyethyleneimine) were attached bound all types of cells which were not displaced by antihistamines or pH changes that displaced leukoctyes from S-R-H. S-R-H or R-H activated human leukocyte adenyl cyclase. Such facts physically demonstrate specific histamine receptors. Histamine (10^{-5} M) inhibits 60± 7% of mouse spleen cell 19S plaques but has no effect on rosette formation. When the incubate of S-R-H with cells is poured into a column, the unbound cells make 54±12% less 19S plaques than controls. Rosette formation is unchanged. When unbound cells are transferred to radiated recipients there is a 4- to 6-fold increase in 19S and 7S plaque formation. We imply that: (*a*) selected leukocytes develop specific cell membrane histamine receptors; (*b*) stimulation of receptors inhibits antibody release from spleen cells; (*c*) presence of the receptor may herald some cells' immunologic competence. (Research supported by grants from NIH and Weizmann Institute.)

214. A New Assay for Anti-Penicillin Antibody Production in Man. ROBERT J. MEYER,* DON E. GRISWOLD,* AND PAUL CALABRESI,** Providence, R. I.

The immune response to penicillin in 24 human subjects was investigated using a hemolysin plaque-forming cell technique designed to measure anti-benzyl-penicilloyl (BPO) antibodies. Using specific developing antisera allows the quantification of IgM, IgG, and IgE antibody production. Human leukocytes were used as the source of immunocompetent cells, and mouse red blood cells (MRBC) conjugated with penicillin were used as target cells. The response to both MRBC and BPO-MRBC was assayed. The assay of leukocytes from 12 subjects with a positive history of penicillin allergy revealed IgM anti-BPO production in 4 out of 12 and IgE anti-BPO production in 6 out of 11. Among individuals with a negative history of penicillin allergy, IgM and IgE anti-BPO production was detected in only 1 out of 12 subjects. The production of IgM to mouse antigens was detected in 2 out of 12 individuals and IgE production to mouse antigens was observed in 1 out of 11 individuals with a positive history of penicillin allergy. None of the subjects with a negative history of penicillin allergy demonstrated IgM or IgE antibody response to MRBC. Two subjects with significant anti-BPO antibody production have been studied serially for a period of 42 days. During this time considerable fluctuations of IgM, IgG, and IgE anti-BPO antibody production were observed. These findings indicate that the Jerne plaque analysis is applicable to human studies. With the technique described it is feasible to measure specifically IgM, IgG, and IgE anti-BPO antibody production in clinical situations. These studies suggest that such an assay may be clinical value in predicting or determining the kinetics of penicillin and other drug allergies in man.

215. Pharmacologically Induced In Vivo Alterations of the Oxygen-Hemoglobin Equilibrium Curve in Man. LEONARD MILLER,* HARVEY SUGERMAN,* WILLIAM CROMIE,* DONALD TOMASELLO,* MARIA DELIVORIA-PAPADOPOULOS,* AND FRANK OSKI, Philadelphia, Pa.

The in vitro addition of inosine, pyruvate, and phosphate to human blood results in an increase in erythrocyte 2,3-diphosphoglycerate (DPG) and a decrease in the affinity of hemoglobin for oxygen. After demonstrating that the intravenous administration of this compound to rhesus monkeys produces similar alterations, studies were conducted in human volunteers. Seven subjects received intravenously 0.1 M inosine (5–15 ml/kg) and six subjects received a combination of 0.1 M inosine and 0.04 M pyruvate and phosphate (5–20 ml/kg) and serial measurements of DPG and P_{50} were performed. The combination of inosine, pyruvate, and phosphate proved more effective than inosine alone. With combination therapy, red cell DPG levels rose 0.9 to 1.8 μmoles within 4 hr and the P_{50} increased from 2 to 4 mm Hg. The infusions were associated with transient headaches, nausea, and drowsiness in some of the subjects while no alterations in blood pressure, renal or hepatic function were observed. The administration of inosine did result in a rise in serum uric acid levels that was prevented by the administration of 300 mg of allopurinol before infusion. It would appear that the administration of inosine, pyruvate, and phosphate to man produces no serious toxic reactions. The changes produced by this therapy may prove to be an effective means of increasing tissue oxygen delivery. (Supported by NIH Grant GM-15001 and The John A. Hartford Foundation, Inc.)

216. Mammalian and Bacterial Metabolism of Guanidinosuccinic Acid. SHELDON MILSTIEN* AND PETER GOLDMAN,* Bethesda, Md. (introduced by J. Wolff**).

The increased excretion of guanidinosuccinic acid (GSA) in the urine of uremic patients has suggested that this compound is a "uremic toxin." A new enzyme has been isolated

from a soil bacterium which hydrolyzes GSA to L-aspartate and urea. The enzyme, named *N*-amidino-L-aspartic acid amidinohydrolase, is not active with GSA derived from D-aspartate, nor with other guanidino compounds. Since GSA of both rat and human urine is completely hydrolyzed by this enzyme, its configuration can be considered as that of L-aspartate. Increased specificity of the GSA assay is achieved using this enzyme. The urinary excretion of GSA has been studied in five normal volunteers on normal and chemically defined (Vivonex-100) diets. On the 5th day of Vivonex, GSA excretion decreased to 46–61% of the control values; no further decrease was found after 10 days on Vivonex. Individual variation was less on Vivonex than on the normal diet. Since Vivonex had a lower nitrogen content than the normal diet, the effect of dietary protein on GSA excretion was examined. After a low protein diet (10–20 g daily) for 6 days, the urinary excretion of GSA was 43–47% of the value on a normal protein diet. On a high protein diet (150–200 g daily) for 6 days, the excretion of GSA rose to 130–220% of the normal value. Cultures of several strains of bacteria isolated from human feces are capable of degrading GSA. This observation and the finding of increased excretion of a compound provisionally identified as GSA in the urine of germ free rats suggests that GSA can be synthesized by the mammalian host and at least partially degraded by its intestinal microflora.

217. Maximum Expiratory Flow Volume Curves: Data Collection and Processing by Digital Computer. C. A. MITCHELL,* R. W. TUTTLE,* J. A. VIRGULTO,* AND A. BOUHUYS,** New Haven, Conn.

Maximum expiratory flow volume (MEFV) curves provide sensitive assessment of pathological airway obstruction in smokers, and in patients with bronchial asthma, cystic fibrosis, and obstructive industrial lung diseases. Clinical application of the method has been hampered by lack of standardized equipment and by the need to read instantaneous flow rates. We have developed an on-line data collection, processing, and reporting system that makes MEFV tests suitable for clinical and epidemiological studies. The computer first accepts the subject's personal data, checks its validity, and generates error messages if needed. Next, five successive forced expirations are performed through a pneumotachograph. Subjects and operator can monitor expiratory flow on an oscilloscope screen, so that technical errors can be immediately corrected. The flow signal is amplified, digitized by an A-D converter, and stored in computer core. 200 flow points are sampled per second for a maximum of 10 sec. The program only retains data on the two blows with the highest $FEV_{1.0}$; about 6K of memory is needed. The software includes semiautomatic calibration features, to compensate for base line drift. A special algorithm detects the start and stop of a forced expiration with adjustable thresholds distinguishing between signals and noise. The fully documented program is highly modular, allowing easy changes in subroutines controlling, e.g., the number and type of questions asked, the number of blows, flow sampling rate, and the equations used for prediction of normal values. The output data, including FVC, $FEV_{1.0}$, $FEV_{3.0}$, peak flow, and flows at 50 and 20% of FVC, in actual values and as per cent of predicted normal, are presented as a Polaroid print of a scope report and on paper type for statistical analysis.

218. Antibody to Fibrinopeptide A: Preparation and Use for the Rapid Detection of Fibrinogen Hydrolysis by Thrombin. J. L. MOAKE* AND D. R. SCHULTZ,* Miami, Fla. (introduced by Leon Schiff).

A rapid assay specifically reflecting fibrinogen hydrolysis by thrombin should be an important aid in the diagnosis of disseminated coagulation. During blood clotting, thrombin hydrolyzes two fibrinopeptide A (fpA) and two fpB molecules from each molecule of fibrinogen to generate fibrin monomer. Therefore, the presence of circulating fpA indicates thrombin-fibrinogen interaction, either at local sites or as a disseminated process. Since only fpA molecules are cleaved from fibrinogen by a purified enzyme from Malayan pit viper venom, this enzyme was used to hydrolyze human fibrinogen. FpA was separated by gel filtration on Sephadex G-25, linked to human albumin by a carbodiimide, and the conjugate was used to immunize goats. The γ-globulin fraction of goat antiserum did not visibly precipitate column-purified on synthetic fpA, as the mol wt of fpA is only 2000. The antibody does react with fibrinogen, but not with fibrin monomer, and absorption of this antibody with column-purified or synthetic fpA impairs subsequent interaction between the anti-fpA γ-globulin and fibrinogen as revealed by immunodiffusion in agar gel. A tanned red cell hemagglutination inhibition immunoassay (TRCHII) was devised for in vitro detection of fpA. Diluted anti-fpA γ-globulin was reacted with dilutions of column-purified or synthetic fpA in microtiter plates. Since unbound anti-fpA antibody agglutinated fibrinogen-tanned RBC, the last dilution of fpA to produce hemagglutination inhibition was the level of fpA in the test sample. The anti-fpA TRCHII will detect fpA concentrations in the microgram range. Determination of fpA levels in defibrinated plasma should be useful in the diagnosis of disseminated clotting and other hypercoagulable states and investigation of the role of coagulation in, for example, the genesis of atherosclerosis in low and high risk populations.

219. Restoration and Maintenance of Glomerular Filtration (GF) by Mannitol during Renal Hypoperfusion. C. RICHARD MORRIS,* FRANK J. BRUNS,* EDWARD A. ALEXANDER,* AND NORMAN G. LEVINSKY, Boston, Mass.

The effect of mannitol on GF was studied in rat during renal hypoperfusion (HP) by aortic clamping to BP = 40 mm Hg. Single nephron filtration rate (SNGFR) in superficial (S) and juxtamedullary (JM) nephrons was determined by a modified Hanssen technique. Minimum detectable SNGFR was < 0.05 nl/min. Five hydropenic rats were anuric during HP; SNGFR was not detected in 50 S or 50 JM nephrons. Urine persisted during HP in six rats infused with 5% mannitol in saline. C_{in} was 0.12±0.02 (SE) ml/min per kidney. In 61 S and 60 JM nephrons, SNGFR was 5.8±1.3 nl/min, without difference between S and JM. Seven rats receiving 1.7% NaCl were anuric. In three, GF was undetectable. In the other four, despite anuria, mean SNGFR was 9.5±4 nl/min, and was comparable in 45 S and 44 JM nephrons. In the above studies, infusions began before

HP. The following experiments were performed to determine whether GF could be reestablished after anuria for 15–30 min. Urine restarted promptly in five hydropenic rats given 5% mannitol in saline C_{In} was 0.22±0.04 ml/min per kidney. SNGFR averaged 10.3±4 nl/min without difference between S and JM. Eight rats received 0.85% saline (2), furosemide (3), or 1.7% saline (3) after HP. Anuria persisted in all. By observation of lissamine green appearance, there was no evidence of GF after 0.85% saline or furosemide. However, some nephrons filtered in each rat given 1.7% saline. We conclude that mannitol maintains or restarts filtration in nephrons which would otherwise not filter. The mechanism is uncertain; but mannitol need not be present in the tubular lumen. Hypertonic saline promotes GF in some cases, but anuria persists, implying complete reabsorption of filtrate. The effects of HP and mannitol on S and JM nephrons are equivalent. (This work has been supported by USPHS Grants AM 11793, AM 14004, and 5T01-AM5209.)

220. Bile Formation in Modified Acute Canine Viral Hepatitis. THOMAS Q. MORRIS,* New York (introduced by Stanley E. Bradley**).

Six adolescent purebred beagles, which had been raised in isolation, were immunized against leptospirosis and distemper before cholecystectomy and implantation of a Thomas duodenal cannula. After recovery from surgery studies of hepatobiliary function were made in each dog. Bile flow and electrolyte excretion were measured in response to infusion of both sodium taurocholate and secretin. Addition of tracer amounts of sodium taurocholate-24-^{14}C to the bile salt infusion permitted estimation of bile salt clearances. Fractional clearance of bromsulphalein (BSP) (k_{BSP}) was used to monitor the course of the disease in addition to the serum transaminase (SGOT) and alkaline phosphatase. After control studies each dog was infected with 300 $TCID_{50}$ of infectious canine hepatitis (ICH) virus. At the first change in k_{BSP} each dog received 5 cc of antiserum containing a high titer of anti-ICH antibody, a procedure previously developed to produce a prolonged acute hepatitis. The disease peaked from 7 to 10 days after infection. Two animals died with extensive hepatic necrosis at this time when BSP retention and elevation of the serum enzymes were marked. Infusion of sodium taurocholate (10–12 μEq/min) at the height of the disease always produced bile with normal flow and electrolyte characteristics. The transport maximum for bile salts, however, was reduced by 50% on the average as was the clearance of labeled sodium taurocholate at even the low rates of infusion. By contrast, secretion produced the usual choleresis and changes in bile composition. These results demonstrate impaired but viable secretory mechanisms at the canalicular level and intact ductular responses throughout the course of a severe experimental hepatitis. (Research supported by grant AI 08890 from NIH.)

221. Intrahepatic Biliary Atresia: a Defect in Hepatic Uptake of Bile Acid. KEVIN P. MORRISSEY,* ENGELINE KOK,* ELLIOT SIEGAL,* AND NORMAN B. JAVITT, New York.

We have studied bile acid metabolism and excretion in three children varying in age from 1 to 108 months who were explored during the neonatal period for the possibility of extrahepatic atresia. Subsequently, jaundice disappeared but conjugated hyperbilirubinemia (less than 2 mg/100 ml) persisted together with increased levels of cholesterol, bile acids, and 5′-nucleotidase. Urine, feces, and serum were analyzed qualitatively and quantitatively after solvolysis and/or hydrolysis by electrophoretic, thin-layer, and gas chromatographic techniques. Studies were done before and during cholestyramine therapy. In all instances, cholestyramine therapy caused a reduction in serum cholesterol and bile acid levels. One child had a reduction in serum cholesterol from 1300 to 300 mg/100 ml, disappearance of xanthoma, and marked clinical improvement. Daily excretion of bile salt on cholestyramine was 4 mg/kg (fecal) and 0.6 mg/kg (urine). Interruption of therapy caused a reduction in fecal bile salt excretion with a prompt rise in serum and urine (2.3 mg/kg per 24 hr) levels. Qualitative analysis of serum and urine in these children revealed predominantly chenodeoxycholic and cholic acids. Although recovery of added glycolithocholate sulfate was quantitative, we were unable to detect significant amounts of endogenous monohydroxy bile acids. The findings are consistent with the view that the liver cell fails to remove bile salts returning from the intestines. (Research supported by grants from the NIH.)

222. Fibrinogen Turnover in the Early Period after Experimental Coronary Thrombosis. C. B. MOSCHOS,* K. LAHIRI,* AND H. OLDEWURTEL,* Newark, N. J.

Although the initial platelet-aggregate in an artery is transformed to a permanent thrombus within 1–4 hr, only after deposition of fibrin, fibrinogen turnover in the early period after coronary thrombosis has not been clearly defined. Fibrinogen decay patterns during this period were assessed by means of ^{131}I-labeled fibrinogen (F) infused in eight dogs, immediately after formation of a platelet coronary thrombus by a catheter electrode. Total plasma and clottable plasma fraction radioactivity were determined in a well-type scintillation counter in samples taken at 15-min intervals for 4 hr. Using the 30 min post-F infusion sample as 100%, total plasma F-decay during the first hour was 11% in five controls vs. 32% in the dogs with thrombus. Cumulative 4 hr decay was 19.5% for controls vs. 62.3% for dogs with thrombus. Counts in thrombi and myocardial ischemic tissue specimens taken after inducing cardiac arrest exceeded those in nonischemic myocardium by ratios of 30:1 and 2:1. The findings suggest that during the early postthrombotic period there is a change in the pattern of F-decay coincident with the presence of a thrombotic process. Although the presence of a thrombus may be a primary factor, tissue injury appears to be, at least partly, responsible for the observed fibrinogen loss in the peripheral blood.

223. Evidence of Inappropriate Adrenergic Stimulation in Early Acute Myocardial Infarction in Man. HILTRUD MUELLER,* STEPHEN AYRES, AND STANLEY GIANNELLI,* New York.

Myocardial metabolic studies in 25 patients with acute myocardial infarction (AMI) demonstrated a direct correlation between arterial free fatty acid (FFA) content and myocardial extraction of FFA ($P < 0.001$) and an inverse correlation with glucose extraction ($P < 0.05$). 13 patients

with arterial FFA above 800 μEq/liter had increased extractions of FFA (244 μEq/liter, $P < 0.05$), lactate (208 mmoles/liter, $P = 0.30$), and oxygen (12.31 ml/100 ml, $P < 0.05$) but decreased glucose extraction (−7.3 mg/100 ml, $P < 0.10$) compared to the remainder which had extractions of 65 μEq/liter, 111 mmoles/liter, 10.6 ml/100 ml, and 7.7 mg/100 ml. Coronary blood flow (CBF) and stroke index were decreased in the high extraction group even though myocardial substrate uptake was increased. The relationship between these findings and possible sympathetic hyperactivity was studied by administering *l*-norepinephrine and propranolol to 10 patients. The difference between these two interventions was taken to represent the effect of adrenergic stimulation, which markedly increased arterial pressure ($P < 0.001$), CBF ($P < 0.001$), myocardial oxygen consumption ($P < 0.01$), and FFA extraction (NS) while decreasing lactate extraction ($P < 0.001$) and myocardial respiratory quotient ($P < 0.05$). The level of adrenergic activity appears to determine whether carbohydrate or FFA are the predominant myocardial substrate in AMI. Since increased FFA uptake was associated with increased oxygen extraction and decreased ventricular performance, catecholamine stimulation may not necessarily be a beneficial response. Exogenous *l*-norepinephrine produced transient metabolic deterioration in three patients, while propranolol uniformly improved lactate extraction. Therefore, sympathetic hyperactivity may magnify myocardial hypoxia. These studies suggest that beta adrenergic blockade may play an important therapeutic role in early AMI without pump failure, while our earlier work demonstrated that adrenergic stimulation improved myocardial metabolism in AMI with shock. (Research supported by grants from NIH and John A. Hartford Foundation, Inc.)

224. Cortisol (F) and Cortisone (E) in the Previable Human Fetus. BEVERLEY E. P. MURPHY AND ROBERT DIEZ D'AUX,* Montreal, Canada.

Despite extensive studies of steroid enzyme systems in pregnancy there are no published data of steroid levels in fetal blood or tissues. Fetal material was obtained immediately after hysterotomy performed at 12–18 wk gestation. The plasma steroids were extracted into ethyl acetate, while the tissues were homogenized in ethanol, centrifuged, and the ethanolic supernate used directly. After the addition of tracer amounts of tritiated steroids, the extracts were evaporated to dryness, redissolved in column solvent, and passed through a column of Sephadex LH-20. The eluate was split so that the pattern of the isotopes could be compared with that assayed by competitive protein binding to corticosteroid-binding globulin (CBG). Further identification and quantitation of the cortisol and cortisone peaks was made by comparing the binding with that of authentic standards in four different CBG systems (dog, chicken, human, monkey) and by chromatography in multiple Sephadex LH-20 systems. For $n = 3$ to 6, the levels found (mean±SE, nanograms/milliliter or nanograms/gram) were, for fetal serum: F 7.0±2.0; E 26.8 ±4.1; fetal adrenal: F 475±35, E 246±58; maternal serum: F 450±40, E 37±12; placenta: F < 10, E 118. In one 5 wk old whole-body homogenate the levels were F < 2, E 21 ng/g. These results suggest that (*a*) cortisone is derived from both the placenta and the fetal adrenal while cortisol is derived only from the fetal adrenal, (*b*) maternal cortisol crossing the placenta is largely converted to cortisone, (*c*) the fetal adrenal functions actively by the end of the first trimester. (Research supported by grant from the Medical Research Council of Canada.)

225. The Hemoglobin-Haptoglobin Reaction as a Probe for Hemoglobin Conformation. RONALD L. NAGEL AND QUENTIN H. GIBSON,* New York and Ithaca, N. Y.

The dissociation of hemoglobin to dimers is a prerequisite for the reaction of hemoglobin with haptoglobin and no binding has been observed with deoxyhemoglobin. Hence, we have used this reaction to investigate the conformational state of different hemoglobins. The reaction is followed by fluorescence quenching in a stopped flow apparatus. Mixed ligand hybrids ($\alpha_2\beta_2^*, \alpha_2^*\beta_2$) prepared by mixing one isolated chain in the cyanmet form and another chain in the oxy form, show normal reaction rates towards haptoglobin when in the fully liganded form; when the α- or β-chains become deoxygenated, the reaction rate slows considerably. This suggests that conformational changes affecting the $\alpha_1\beta_2$ interfact occur after two like-chain sites are deoxygenated, although the new conformation is not identical with fully deoxygenated hemoglobin. An interesting effect of phosphates is also uncovered by this reaction. In the presence of phosphate, deoxy Hb (hemoglobin) A does not bind Hp (haptoglobin); but when stripped of phosphate it is capable of binding at a slow rate. This suggests that deoxy Hb exhibits two conformational states, affecting the $\alpha_1\beta_2$ area of contact, related to the presence or absence of phosphate. Deoxy-Hb Hiroshima and deoxy-Hb Chesapeake are also capable of binding in the stripped form but this property decreases significantly in the presence of phosphates, especially in the case of Hb Hiroshima. This finding helps to understand the structural basis of the alter function of these mutants. These results demonstrate the usefulness of the hemoglobin-haptoglobin reaction in the study of conformational states of hemoglobin A and its variants. (Research supported by NIH and New York Heart Association.)

226. Genetic Analysis of Hemoglobin Proportions in Sickle Cell Trait. WALTER E. NANCE* AND JOHN GROVE,* Indianapolis, Ind. (introduced by Walter J. Daly).

Replicate determinations were made of the proportion of abnormal hemoglobin found in 272 subjects with sickle cell trait by the starch-gel electrophoretic method of Sunderman. The sample was a Brazilian population of mixed racial origin which included the affected parent and 184 children in 67 families. A single observer classified each individual into one of seven racial admixture groups based on skin color and other anthropologic characteristics. The distribution of the per cent of Hb (hemoglobin) S was negatively skewed with a mean of 42.6% and a range of 27–50%. Individual variation was significantly greater than the variation between replicates ($P < 0.001$) and the variation among sibships exceeded the variation within sibships ($P < 0.001$) suggesting a genetic influence on the trait. The mean proportion of Hb S in children was positively correlated with the proportion in their carrier parent ($P < 0.001$) and the regres-

sion accounted for almost 25% of the among family variance. Age and sex had no significant effects, but the degree of Negro ancestry, as judged by the ethnicity index, had a highly significant effect ($P < 0.001$) which persisted even when the per cent Hb S of the carrier parent was included in an among-family regression ($P < 0.02$) or when the effect was tested on a within-family basis ($P < 0.05$). The analysis indicates that two separate genetic factors influence the variation in per cent Hb S found in heterozygous carriers. One factor probably arises from genetic variation at the Hb β locus while the other effect is related to the racial background of the individual and may well be polygenic. These findings may be relevant to the known variation in clinical severity that is seen in patients with sickle cell anemia.

227. Two Step Control of Ammoniagenesis. ROBERT NARINS,* ALBERT TIZIANELLO,* AND ARNOLD RELMAN,** Philadelphia, Pa. (introduced by Isaac Starr**).

A recent suggestion that ammoniagenesis from glutamine ($GA\text{-}NH_3$) in the rat kidney may be independently regulated in cortex and medulla was based upon two observations: (*a*) chronic acidosis increased $GA\text{-}NH_3$ in cortex, but not in medulla; (*b*) in vitro, alkalosis enhanced $GA\text{-}NH_3$ in medulla, but not in cortex. Our studies confirm (*a*), but we find that both cortical and medullary $GA\text{-}NH_3$ are increased by alkalosis in vitro. This effect is relatively small in aerobic cortex, but is enhanced by anaerobiasis. In vitro stimulation of $GA\text{-}NH_3$ by alkali results from increased glutaminase-I activity because changes in $GA\text{-}NH_3$ parallel changes in glutamate production; furthermore, inhibition of all alternative pathways in the medulla did not alter its pH response. Ammoniagenesis from glutamate ($glut\text{-}NH_3$) in vitro is easily demonstrable in aerobic (but not anaerobic) cortex, but there is no $glut\text{-}NH_3$ in medulla under any conditions. In cortex $glut\text{-}NH_3$ is enhanced by acidosis whereas, as noted, acidosis inhibits $GA\text{-}NH_3$. We conclude that $GA\text{-}NH_3$ in cortex reflects two sequential, separately controlled processes: glutaminase-I, followed by $glut\text{-}NH_3$. Glutaminase-I is anaerobic and is directly stimulated by alkalosis, whereas the pathway for $glut\text{-}NH_3$ is aerobic and is directly activated by acidosis. In medulla glutaminase is solely responsible for ammoniagenesis, but in the aerobic cortex it is the balance between the two processes that determines the acute response to pH change. This balance is presumably modified in chronic acidosis by the consequences of enzyme induction in one or both pathways. (Research supported by NIH grant 5R1 AM-14207-03.)

228. Evidence for Triiodothyronine (T_3) Stimulation of Thyrotrophin-Releasing Hormone (TRH) Secretion in Man. JOHN T. NICOLOFF* AND PETER A. SINGER,* Los Angeles, Calif. (introduced by John E. Bethune).

Current evidence indicates that pituitary thyroid-stimulating hormone (TSH) release is controlled by the net result of the quantity of endogenous TRH secreted by the hypothalamus and the amount of TRH inactivated by a thyroid hormone-dependent pituitary enzymatic system. While the effect of thyroid hormone on the anterior pituitary is known, its action on hypothalamic TRH secretion is as yet unclear. Recent studies by Reichlin and coworkers in the rat indicate that thyroid hormone may stimulate TRH production. Employing a dual iodine isotopic method (1970. *J. Clin. Invest.* **49**: 269.) for the evaluation of thyroidal iodine release (TIR), the effects of exogenously administered T_3, TRH, and prednisolone on TIR and serum immunoassayable TSH (I-TSH) were studied in six euthyroid subjects. The stimulatory action of repetitive alternate day injections of TRH (100 μg) on TIR and TSH was not affected by the simultaneous administration of prednisolone (60 mg/day), thus verifying in man that prednisolone suppression of TIR is mediated by a decrease in endogenous TRH secretion. In similar studies, employing large doses of T_3, no rise in TIR or I-TSH was observed when TRH was administered. Incomplete suppression of TIR was observed with physiologic (75–100 μg/day) T_3 doses alone and no further suppression was seen with pharmacologic doses (150–300 μg/day). However, the addition of pharmacological doses of prednisolone to T_3 administration produced a prompt suppression of TIR. Conclusions: (*a*) T_3 administration does not completely suppress TIR. (*b*) Continued TIR during T_3 administration is mediated by endogenous TRH secretion. (*c*) Since exogenous TRH produced no change in TIR or I-TSH levels during T_3 administration, endogenous TRH secretion is probably augmented in order to overcome the inhibitory effects of T_3 on the pituitary.

229. Interaction of Cultured Human Fibroblasts with Fibrin and Its Modification by Aging and Drugs. STEFAN NIEWIAROWSKI* AND SAMUEL GOLDSTEIN,* Hamilton, Ontario (introduced by J. Fraser Mustard).

Since mouse L cells adhere to polymerizing fibrin (PF) and cause fibrin retraction, we have examined the interaction of human fibroblasts (HF) with PF. The HF were obtained by skin biopsy, grown by standard techniques, released from monolayer by trypsin, and resuspended after several washings in Tyrode-albumin solution. PF was prepared from ^{125}I-labeled human fibrinogen by incubation with small amounts of thrombin or reptilase. HF (10^6–5×10^6 cells/ml) adhered to polymerizing fibrin as indicated by increased light transmission through the cell suspension and increased radioactivity associated with the cell sediment. HF caused fibrin retraction within 1–2 hr followed by complete lysis of the fibrin within 2–24 hr. Intact cells were required for fibrin retraction. Aged (late-passage) cells produced significantly less retraction than young (early-passage) cells while fetal cells were more active in lysis than adult cells. 10^{-5} M prostaglandin E_1 significantly inhibited fibrin retraction. 3×10^{-4} M acetylsalycilic acid and 3×10^{-6} M phenylbutazone enhanced this process both in time and extent. Neither of these drugs affected fibrinolysis. Growth kinetics of cells were not altered in the presence of the oral contraceptives norethindrone (6×10^{-7} M) and mestranol (3×10^{-6} M), alone or in combination. However, each agent inhibited HF adherence to PF and retraction of fibrin by 50%; effects on lysis were not consistent. In conclusion, HF adhere to polymerizing fibrin, and cause fibrin retraction and lysis, all of which are probably important in tissue repair. This system should be useful for the study of the interaction of fibroblasts, blood coagulation, and fibrin in

tissue injury and repair, including the effects of aging and drugs. (Supported by an Ontario Health Grant, the Medical Research Council of Canada, the Canadian Diabetic Association Foundation Fund, and the Hoechst Pharmaceutical Company during the tenure of Dr. Goldstein's Medical Research Council Scholarship.)

230. Hemoglobin Olympia (β20 Val → Met): an Electrophoretically Silent Variant Associated with High Oxygen Affinity and Erythrocytosis. PETER E. NUTE,* GEORGE STAMATOYANNOPOULOS, AND DONALD FUNK,* Seattle, Wash.

In a family with erythrocytosis, routine hemoglobin studies failed to show the presence of a hemoglobin variant. Hemoglobin studies under various electrophoretic and chromatographic conditions, elution patterns of hemoglobin chains, and tryptic peptide maps of isolated chains were indistinguishable from normal. However, the oxygen dissociation curve of blood from affected individuals was shifted to the left of normal (P_{50} affected = 18.6 mm Hg, normal = 26.5 mm Hg) and this shift persisted when oxygen equilibria were studied on dialysed hemolysates (at pH 7.09, affected = 6.8 mm Hg, normal = 8.8 mm Hg). In spite of the normal findings of the hemoglobin study, a mutant hemoglobin was evidently present in the red cells of the affected persons and it was responsible for the increased oxygen affinity and the erythrocytosis. Specific staining of tryptic peptide maps from β-chains of the affected persons showed that peptic βT_3 was positive for a sulfur-containing amino acid. Amino acid analysis yielded a composition identical to that of normal βT_3, except that there were 2.6 residues of valine and 0.4 residues of methionine (normal composition, Val = 3.0, Met = 0.0). This suggested that the β-chains of affected individuals were a mixture of two kinds of chains, 40% of which had a methionyl residue in βT_3. Cleavage at methionine with cyanogen bromide permitted isolation, by gel filtration, of the CNBr fragments from the abnormal β-chain. Structural studies on isolated CNBr fragments demonstrated unequivocally that, in the abnormal β-chains, valine in position 20 is replaced by methionine. The new hemoglobin mutant is designated Hemoglobin Olympia (β20 Met). The stereochemical basis of its abnormal oxygenation is not apparent, since residue β20 is not located in any of the known functionally important areas of the hemoglobin molecule. (Research supported by NIH grants GM 15253 and RR-00166.)

231. A Binding Protein for Fatty Acids in Cytosol of Intestinal Mucosa, Liver, and Other Tissues. ROBERT K. OCKNER,* San Francisco, Calif. (introduced by R. Schmid).

Translocation of fatty acid (FA) from cell surface to endoplasmic reticulum and mitochondria is fundamental to intestinal absorption and to FA utilization by liver, muscle, and other tissues, but it is not known how these poorly soluble compounds traverse the cytosol. We showed previously that absorbed unsaturated FA are reesterified in jejunum more rapidly than saturated FA. As this was not explainable by differences in uptake or microsomal activation, a cytoplasmic carrier for FA seemed implicated. Gel filtration chromatography (GFC) of oleic acid-^{14}C with 105,000 *g* supernate from rat jejunal mucosa exhibited a peak of FA bound noncovalently to a protein of mol wt ~ 12,000. Polyacrylamide gel electrophoresis of partially purified jejunal fatty acid-binding protein (FABP) showed association of radioactivity with a single band. A similar FABP was isolated from human jejunal mucosa, and was identified in rat liver, myocardium, adipose tissue, and kidney, but not serum. On GFC of liver supernate with FA and sulfobromophthalein (BSP), FABP and the smaller ("Z") of two previously described cytoplasmic anion-binding proteins behaved identically. Identity of these proteins was further supported by competition for binding between FA and BSP. FA did not bind significantly to "Y" protein. Relative binding with jejunal FABP was: oleate 100, linoleate 72, stearate 7, palmitate 24, laurate 1.5, octanoate 0.1, methyl palmitate 3.8, hexadecanol 1.5, BSP 11. Thus, binding of unsaturated long-chain FA exceeded that of saturated and medium-chain FA, and BSP. Binding depended not on negative charge but rather on FA chain length and saturation, and possibly on hydrogen bonding to oxygen. In gut, FABP may explain observed differences in intestinal absorption of FA. Moreover, it may play an important role in uptake and utilization of FA and other lipid-soluble compounds by many mammalian tissues. (Supported by NIH grants AM-13328 and AM-14795.)

232. Identification of Apparently Specific Triiodothyronine (T_3)-Receptor Sites in Rat Adenohypophysis and Hepatic Nuclei. JACK H. OPPENHEIMER, ALAN R. SCHADLOW,* DIONA KOERNER,* HAROLD L. SCHWARTZ,* AND MARTIN I. SURKS,* Bronx, N. Y.

The capacity of rat adenohypophysis to bind T_3 and thyroxine (T_4) in competition with plasma proteins was calculated from the isotopic partition of tracer hormone injected intravenously and from the relative strength of plasma proteins binding as assessed by equilibrium dialysis. In normal animals, the anterior pituitary binds T_3 approximately 7–10 times as avidly as T_4. No major sex-related differences in pituitary binding of T_3 and T_4 were noted. In addition to the expected increase in pituitary weight, thyroidectomy resulted in a 58% increase in T_3-binding per gram of tissue without any change in T_4 binding. In normal rats, increasing intravenous doses of T_3 effected a progressive reduction in pituitary/plasma concentration ratio of T_3. Calculations indicated that the pituitary T_3 receptor sites were close to saturation at normal endogenous T_3 plasma concentrations and thus theoretically capable of regulating TSH release in an on-off fashion. No dose-related changes in pituitary/plasma T_4 concentration ratios were observed. Similarly, the tissue/plasma concentration ratios of liver, kidney, and brain either for T_3 or T_4 were not grossly influenced by the dose injected. When hepatic nuclei, however, were separated from other cellular constituents, a progressive reduction in the proportion of cellular T_3 bound to nuclei was observed as the dose of T_3, injected 3 hr earlier, was increased from 0.1 μg/100 g body weight (9.3% in nuclei) to 10.0 μg/100 g body weight (3.0% in nuclei). Only a minimal fall in the proportion of tracer T_4 associated with nuclei was observed when comparable molar doses of intraveneous T_4 were given. These findings thus raise

the possibility that nonspecific T_3-binding in other tissues may also obscure the existence of stereospecific nuclear receptor sites. The identification of specific T_3-binding sites in whole pituitary and hepatic nuclei is of special interest in connection with recent kinetic data suggesting that T_4 must be converted to T_3 before exerting hormonal action. (Supported by NIH, Department of the Army, and Health Research Council of New York City.)

233. Alcohol, Fasting, and Albumin Synthesis. MURRAY ORATZ,* SIDNEY SCHREIBER,* JOSEPH MONGELLI,* AND MARCUS A. ROTHSCHILD,** New York.

Albumin synthesis is rapidly inhibited by fasting, or by the administration of alcohol. These effects can be reversed by feeding or by adding tryptophan. The present studies were undertaken to determine if the liver derived from a fasted donor was more sensitive to this inhibitory effect of alcohol and to examine means of protecting against this inhibition. All male donor rabbits were fasted 48 hr before perfusion. ^{14}C-labeled CO_2 was used to label newly synthesized albumin. The perfusate consisted of whole blood or washed red cells with known amino acid content and 2.5 g/100 ml rabbit albumin. Albumin synthesis in fasted livers were 16±3 mg/100 g wet liver per 2.5 hr of perfusion compared to 35±2 mg in fed livers. Perfusion with 220 mg/100 ml alcohol, decreased albumin synthesis further to 9±2 mg. Tryptophan, 10 mM and/or high levels of amino acids failed to increase this low rate of albumin synthesis in the fasted-alcohol perfused liver. When cortisone was added to the perfusate containing alcohol, albumin synthesis rose to 22±2 mg. Fasting reduced hepatic RNA/DNA ratios from 2.30± 0.10 to 1.80±0.20, and the addition of alcohol lowered the ratio further to 1.52±0.07. Excess tryptophan did not change this ratio but cortisone resulted in an increase in hepatic RNA and the ratio rose to 1.90±0.09. Polysome profiles showed disaggregation in the fasting and fasting-alcohol studies, and perfusion with cortisone acetate and tryptophan resulted in partial reaggregation. These studies show that fasting and alcohol are additives in lowering albumin synthesis. Cortisone acetate does reverse the alcohol-induced inhibition of albumin production in the fasted liver, probably by maintaining hepatic ribosomal RNA. (Supported in part by the NIH and the Licensed Beverage Industries, Inc.)

234. ACTH and ACTH Fragments in Tumors Causing the Ectopic ACTH Syndrome. DAVID N. ORTH,* WENDELL E. NICHOLSON,* WILLIAM M. MITCHELL,* AND DONALD P. ISLAND,* Nashville, Tenn. (introduced by Eric Engel**).

The structure of the tumor product causing the ectopic ACTH syndrome has not been completely elucidated. Therefore, tumors of three patients with this syndrome were obtained at surgery, frozen immediately, extracted with cold glacial acetic acid, partially purified on Amberlite CG-50, and subjected to molecular sieve chromatography on Sephadex G-50 Fine. Original extracts and chromatographed fractions were characterized in ACTH and melanocyte-stimulating hormone (MSH) bioassays and in five radioimmunoassays: one specific for the N-terminal ACTH sequence, one for the central ACTH sequence, one for the C-terminal ACTH sequence, one for alpha-MSH, and one for beta-MSH. Each tumor contained a material having all of the properties of pituitary ACTH. Each tumor also contained in great abundance a smaller molecular moiety (approximately 25 amino acids) having only the properties of the biologically inactive C-terminal fragment of ACTH. Each tumor contained, again in great abundance, a still smaller molecular moiety (approximately 15 amino acids) having biologic MSH activity, little or no ACTH activity, and immunologic similarity only to the N-terminal fragment of ACTH. Similar characterization of human pituitary revealed a material having all the properties of ACTH, but no N-terminal or C-terminal ACTH fragments. Conclusion: Tumors causing ectopic ACTH syndrome contain, in addition to ACTH, ACTH fragments, some of which have biologic activities other than those of ACTH itself. (Supported by grants from NIH.)

235. Characterization of Bilirubin Photoderivatives in Gunn Rat Bile. J. DONALD OSTROW* AND COLIN S. BERRY,* Philadelphia, Pa. (introduced by Francis C. Wood**).

Phototherapy, widely used to decrease jaundice in neonates and Gunn rats, acts by accelerating the conversion of bilirubin to polar derivatives which can be excreted in bile without conjugation. The present work constitutes the first reported isolation and characterization of some of these photoderivatives. 10 hr after intravenous injection of bilirubin-^{14}C into Gunn rats, fistula bile was collected during successive periods of darkness and phototherapy. By solvent partition, each sample was divided into four fractions which were further fractionated by two-stage preparative thin-layer chromatography. Radioassay revealed that most of the labeled pigments excreted during phototherapy were also present in control bile. During phototherapy: (*a*) Excretion of some pigments was not augmented, including the brilliantly yellow-fluorescent compound described by Garay et al., which proved to be riboflavin; (*b*) Unconjugated bilirubin accounted for 47% of excreted radioactivity; (*c*) Of the nonbilirubin radioactivity, 27% partitioned into chloroform at pH = 7.0, suggesting that these products might diffuse into the brain and contribute to kernicterus in vivo; 21% was chloroform insoluble even at pH = 3.0. The principal bilirubin *photo*derivatives were yellow pigments with an absorption maximum at 400–445 nm and usually another peak at 270–285 nm also, typical of dipyrroles. The major photoproduct, accounting for 16% of the nonbilirubin radioactivity, was not apparent in chromatograms from bile excreted in the dark. It was yellow and nonfluorescent, possessed a single sharp maximum at 433 nm, was diazo-negative but pentdyopent-positive, and readily oxidized to brownish material resembling bilifuscins, but not to tetrapyrrole products. These properties indicate this derivative is a dipyrrole. (Supported by grant AM-14543 from NIH and the Veterans Administration.)

236. Hyperbilirubinemia of Fasting: Studies in Man during Parenteral Feedings. JOSEPH H. OYAMA, Chicago, Ill. (introduced by Robert M. Kark).

An inverse relationship between serum bilirubilin (SB) concentration and caloric intake has been observed in man when oral intake of food has been acutely restricted. The

mechanism of this effect is not well understood. The effect of supplying calories by other than the gastrointestinal route has not been previously described. Serum bilirubin was serially measured in five healthy male participants who took no food by mouth for 96 hr while receiving 1200 kcal daily as a constant infusion of dextrose or dextrose and amino acids in water. Free access to water orally was permitted. Mean base line SB of 0.74 mg/100 ml rose to 1.22 mg/100 ml within 24 hr of the study. The rise of SB was highly significant ($P < 0.001$). SB continued to rise for the next 72 hr to a peak of 1.90 mg/100 ml. Statistical analysis showed a linear relationship of SB and duration of study to 96 hr. Infusions were terminated at 96 hr and subjects allowed to eat ad lib. SB fell to 0.88 mg/100 ml within 24 hr of resuming oral feedings. The fall in SB was highly significant ($P < 0.001$). There was no significant difference between base line and post refeeding SB level. Other parameters of liver function and hematological indices did not change significantly during the study. These data suggest that the reduced hepatic ability to take up and store bilirubin during fasting may not depend on caloric deficiency per se but depends rather on factors related to gastrointestinal presence or absorption of food. (Supported in part by a grant from the USPHS No. 5 T01 AM 05505-06.)

237. Direct Identification in Human Leukocytes of Epstein-Barr Virus DNA by Complementary RNA-DNA Hybridization Tests. JOSEPH S. PAGANO AND MEIHAN NONOYAMA,* Chapel Hill, N. C.

There are suggestions but no proof that infection with the Epstein-Barr virus (EBV) is necessary before human leukocytes can be propagated in vitro. We now show unequivocally that EBV genome is present in established leukocyte lines but not in freshly isolated leukocytes by RNA-DNA hybridization tests. Tritiated complementary RNA (cRNA) specific for EBV DNA is synthesized in vitro with *Escherichia coli* DNA-dependent RNA polymerase and hybridized to denatured leukocyte DNA on membrane filters. Between 45 and 100 EBV genome equivalents per cell have been detected in the leukocyte lines so far tested, but less than 2 equivalents in fresh cells. The melting (*Tm*) profiles of cRNA-DNA hybrids from four leukocyte lines are identical, which indicates the similarity of the viral DNA in the cell lines. The viral DNA is neither freely replicating nor integrated by covalent bonding in the cellular DNA. By a refinement of the cRNA-DNA hybridization technique, in situ cytohybridization, we have localized by radioautography the EBV DNA in a portion of the cell population. We are now studying circulating lymphocytes in acute infectious mononucleosis with this powerful and specific probe for the presence of viral DNA sequence. (Supported by the John A. Hartford Foundation, Inc.)

238. Content and Intracellular Localization of Parathyroid Hormone in Human Tumors. GENARO M. A. PALMIERI,* ROBERT E. NORDQUIST,* AND GILBERT S. OMENN,* Oklahoma City, Okla. and Seattle, Wash. (introduced by Leonard P. Eliel).

This is a study of the content and localization of parathyroid hormone (PTH) in parathyroid adenomas and ectopic tumors by immunologic techniques. PTH was determined by radioimmunoassay in acetone-acetic acid extracts of parathyroid adenomas and ectopic tumors. Adjacent tissue blocks from the same series of tumors were studied by immunofluorescence using an anti-PTH guinea pig serum coupled to a rabbit anti-guinea pig serum and by radioautographic techniques using a guinea pig anti-PTH ^{131}I-IgG. PTH was readily detected by radioimmunoassay in four parathyroid adenomas and in nine ectopic tumors. The concentration of PTH in adenomas was 10–100 times higher than in ectopic tumors on the basis of protein content. Similarly, immunofluorescence demonstrated the presence of PTH in virtually all cells in parathyroid adenomas. However, the intensity of the fluorescence was somewhat dim when compared with the much brighter individual cell fluorescence observed in ectopic tumors. In the majority of these tumors less than 50% of the cells were fluorescein positive. Immunoradioautographic localization showed similar results to those observed with immunofluorescence. These results demonstrate the presence of PTH in the cells of parathyroid adenomas and in hormone-producing malignancies. Normal tissues and other tumors not associated with hypercalcemia gave negative results with all the techniques described. The disparity in the percentages of PTH-containing cells in ectopic tumors could indicate the coexistence of different cell clones with different biological properties in some of these tumors, or conversely, that PTH might be produced as a cyclic event. (Research supported by the Veterans Administration and the Oklahoma Medical Research Foundation.)

239. *Bordetella pertussis*-Induced Alterations in Lymphocyte Cyclic-AMP Metabolism. CHARLES W. PARKER AND STEPHEN I. MORSE, St. Louis, Mo. and Brooklyn, N. Y.

The intravenous injection of killed *B. pertussis* organisms in mice and rats produces a marked alteration in lymphocyte distribution with a striking lymphocytosis analogous to what is observed in human *B. pertussis* infections. Injected animals also exhibit increased sensitivity to histamine and a reduced physiologic and biochemical (blood glucose, lactate, and pyruvate) response to adrenergic drugs. Material which has lymphocytosis-producing activity is released into culture medium during a late growth phase and has been partially purified. In the present study the interaction of the pertussis fraction with nylon fiber-purified (> 98%) human peripheral blood lymphocytes in vitro has been studied. Suspensions of 5×10^6 lymphocytes were maintained at 37°C in the presence and absence of the bacterial fraction for various periods, and then stimulated with 10 and 1 mM concentrations of isoproterenol or 4 μM prostaglandin E_1 (PGE_1) for 10 min at 37°C. After centrifugation the cyclic-AMP content of the cells was determined by radioimmunoassay. In the absence of the *B. pertussis* fraction isoproterenol increased intracellular cyclic-AMP concentration 4- to 12-fold, whereas PGE_1 produced a 4-fold increase. Preincubation of cells with the bacterial fraction for 90 min or longer essentially abolished both the isoproterenol and the PGE_1 response. Results with broken cell preparations indicate that adenylate cyclase activity is altered by the *B. pertussis* fraction, indicating an early site of action of the bacterial fraction at the external

cell membrane. A change in membrane structure or function could also account for the altered lymphocyte distribution in vivo. (Supported by grants from the NIH.)

240. Microvillus Dense Bodies: Candidate Calcium-Binding Sites on Small Intestinal Brush Border Membrane. JOHN C. PARTIN AND WILLIAM K. SCHUBERT,* Cincinnati, Ohio (introduced by Edward L. Pratt**).

We have consistently observed electron scattering ("dense") plaques adherent to the inner lamina of the microvillus plasma membrane of human small intestinal epithelium when the tissues were fixed in phosphate-buffered (Millonig's) glutaraldehyde with $CaCl_2$ and osmium; similar plaques are present in rat duodenum. A possible physiologic significance of the microvillus dense bodies (MVDB) became apparent when serial proximal jejunal biopsies were obtained from a patient with idiopathic hypoparathyroidism and steatorrhea. When this 9 yr old boy was receiving 400,000 vitamin D_2 in corn oil and calcium lactate the MVDB were increased in size and number (compared to his pretreatment biopsies and to control tissue prepared in the same reagents). The MVDB, or very similar structures, could be found in terminal web cytoplasm, adherent to Golgi membrane and adherent to lateral intercellular space membrane. The MVDB were clearly visible in unstained sections of phosphate-buffered glutaraldehyde with $CaCl_2$ and osmium-fixed human microvilli. The MVDB were diminished in size and density when fixed in phosphate-buffered osmium alone for 1 hr. In rat microvilli, MVDB are clearly seen after phosphate-buffered glutaraldehyde with $CaCl_2$-osmium fixation. They are present but diminished in density in bicarbonate-buffered glutaraldehyde-osmium fixation and they are greatly diminished after either phosphate-osmium or bicarbonate-osmium fixation. In rat, electron-scattering plaques, probably MVDB, are visible in phosphate-buffered glutaraldehyde with $CaCl_2$-fixed unstained tissue. We propose MVDB are morphologic entities. They require glutaraldehyde (protein cross-linker) for maximum preservation. Since their density is enhanced by phosphate calcium-buffered fixation, and (in our patient) under conditions expected to result in increased calcium uptake, we propose these structures as candidate calcium-binding sites at the microvillus border of the small intestine. (Supported by NIH grant RR-123.)

241. Degradation of Microbial Phospholipids during Phagocytosis. PIERLUIGI PATRIARCA* AND PETER ELSBACH,** New York.

The fate of the fatty acid-^{14}C-labeled lipids of several microorganisms exposed to intact or disrupted rabbit granulocytes was studied. Whereas the phospholipids of intact *Escherichia coli* undergo degradation of no more than 25%, even when killing is virtually complete, spheroplasts prepared from *E. coli* are degraded by as much as 80% in 2 hr. This degradation probably was due in part to activation of *E. coli* phospholipases which remain associated with the bacterial cell membrane during formation of spheroplasts. When *E. coli* phospholipases were inactivated, however, either by autoclaving the microorganism, or by treatment with detergents, bacterial lipid degradation by intact or disrupted granulocytes was as great as that seen with untreated spheroplasts. In contrast to the extensive degradation of *E. coli* lipids after (partial) removal of the outer layers of the bacterial envelope, the lipids of *Mycoplasma hominis,* an organism naturally devoid of a cell wall, are not broken down under similar conditions. Further, the lipids of Gram-positive *Micrococcus lysodeikticus* are also incompletely broken down even though its cell is readily removed by (granulocyte) lysozyme. The extent of microbial lipid degradation by granulocytes appears to depend on accessibility of the lipids and not on their substrate properties with respect to granulocyte phospholipase(s). Accessibility of the lipids seems determined by other structural components of the bacterial coat that resist the action of the granulocyte's degradative enzymes. In different microorganisms such barriers occupy different locations within the envelopes. Thus, in *E. coli* the outer layers limit lipid degradation, while the limited lipid breakdown of *Micrococcus lysodeikticus* and *Mycoplasma hominis* may reflect properties of the cell membrane proper. The results also indicate that granulocyte phospholipases participate in the digestion that accompanies phagocytosis and that this lipid-splitting activity takes place within the phagocytic vacuole. The products of degradation are used for ganulocyte lipid synthesis. (Supported by USPHS Grant AM 05472.)

242. Rapid Effects of Parathyroid Hormone on Isolated Bone Cells: a New Sensitive In Vitro Bioassy. WILLIAM A. PECK,* KIRK MESSINGER,* AND JANET CARPENTER,* Rochester, New York (introduced by John R. Jaenike).

We have examined the sequential effects of bovine parathyroid hormone (2000 U/mg) on isolated bone cells dispersed from fetal rat calvaria and cultured in chemically defined medium. Upon treatment with 1 μg/ml PTH, bone cell cyclic 3′5′-adenosine monophosphate (cAMP), assayed by protein binding, rose from a control level of 10.5±0.4 pmoles/culture to 140±5.0 pmoles/culture in 30 sec, peaked at 315 ±8.5 pmoles/culture in 150 sec, then gradually diminished. cAMP increased in direct proportion to PTH concentration, and with as little as 5 ng/ml (+ 50%, $P < 0.01$). ACTH, thyrocalcitonin, and insulin were ineffective. Theophylline (1 mM) did not increase cAMP, but nearly doubled the effect of simultaneously added PTH. The PTH-mediated rise in cAMP was followed by increased incorporation of uridine-2-^{14}C into free nucleotide pools and RNA (+ 30% in 15 min at 1 μg/ml ($P < 0.01$), + 85% in 30 min [$P < 0.01$]). This stemmed in part from enhanced uridine phosphorylation, since PTH increased the incorporation of uridine-2-^{14}C into free nucleotide pools during simultaneous blockade of RNA synthesis with actinomycin D. Stimulation was directly proportional to PTH concentration (5 ng/ml–1 μg/ml), 5 ng/ml producing a small (+ 18%) but significant ($P < 0.05$) effect. Dibutyryl cAMP (DBC, 0.1–10 mM) also enhanced uridine incorporation, exhibiting the same time-course of action as PTH. PTH and DBC together at maximal stimulatory concentrations produced nonadditive stimulation. Other adenine nucleotides, thyrocalcitonin, basic polypeptides, and oxidized PTH were nonstimulatory. Hence, PTH appears to stimulate uridine phosphorylation by increasing bone cell cAMP levels. Isolated bone cells are sensitive to near physiological concentrations of PTH and re-

spond more rapidly than previously described systems. (Supported by NIH grant AM09865.)

243. Suppression of Experimental Inflammation by Pregnancy Serum. ROBERT H. PERSELLIN,* SHARON E. VANCE,* AND ASHTON PEERY,* San Antonio, Tex. (introduced by George W. Frimpter).

Inflammatory diseases frequently subside during pregnancy and recur several weeks after parturition. A nonsteroidal lysosome stabilizer developing in pregnancy could account for the diminution of inflammation. Experiments were performed to study the effect of pregnancy serum and its membrane-stabilizing fraction on an in vivo model of inflammation. Carrageenin, a sulfated mucopolysaccharide known to rupture lysosomal membranes, was used to induce an inflammatory reaction in the hind paw of the rat. Foot volume was measured plethysmographically before injection of 0.1 ml of 3% carrageenin and at 4 hr, the height of the reaction. The serums, their fractions, or the pharmacologic agents studied were injected intraperitoneally (i.p.) 1 hr before or at the time of carrageenin. Activity was calculated as the percentage inhibition of paw swelling compared with edema in saline-treated (i.p.) controls. A marked inhibitory effect was detected when pregnancy serum was given intraperitoneally and controls received saline; this was dose related (the difference induced by 10 ml serum being significant at $P < 0.001$). Partial suppression was detected with serum pools from nonpregnant females and males but no anti-inflammatory activity was found using cord serum. These results resembled in vitro lysosome stabilization studies; activity found in pregnancy serum was not in cord serum; it could be induced in normal females with synthetic estrogens. Corticosteroids suppressed inflammation but antihistaminic and antiserotonin compounds did not. Studies with the nonsteroid-containing serum fractions suggest that the lysosome stabilizer in pregnancy serum could be responsible for suppression of the carrageenin model of inflammation. (Research supported by grants from the Robert A. Welch Foundation, Houston.)

244. Differential Effect of Alloxan Diabetes on "Bound" and "Free" Hepatic Ribosomes. DANIEL T. PETERSON,* EVE P. REAVEN,* AND GERALD M. REAVEN, Palo Alto, Calif.

In an effort to understand why hepatic protein synthesis is reduced in experimental diabetes, changes in hepatic ultrastructure have been correlated with the capacity of isolated hepatic ribosomes to synthesize protein. Alloxan diabetes results in fragmentation of the rough endoplasmic reticulum (RER) of the hepatocyte, the severity of which is highly correlated with reduction in total hepatic protein synthesis ($r = 0.7$, $P < 0.001$). It is likely that this reduction in protein synthesis is due to the marked decrease in number of polyribosomes ("bound") which are normally associated with the RER. On the other hand, polyribosomes free ("free") in the cytosol seem unchanged or increased in number. To further study alloxan's effect on these two populations of hepatic ribosomes, "bound" and "free" ribosomes were isolated by ultracentrifugation from liver of normal and alloxanized rats. Alloxan diabetes results in a decrease in both amount (milligrams ribosomal RNA/gram liver) and protein synthetic capacity (amino acid incorporation/milligram ribosomal RNA) of "bound" ribosomes, whereas "free" ribosomes increased in amount and protein synthetic activity. These changes were statistically significant, and are consistent with the effects of alloxan diabetes on hepatic ultrastructure and overall protein synthesis. Apparently the increase in protein synthesis by "free" ribosomes does not compensate for the loss of the majority of hepatic polyribosomes that occurs with destruction of the RER and its population of "bound" polyribosomes. Finally, these observations indicate that insulin deficiency (or its physiological sequelae) has opposite effects on the two ribosomal populations present in the hepatocyte, and suggest that alloxan diabetes results in a decrease in activity of polyribosomes which manufacture protein primarily for export ("bound") and an increase in activity of polyribosomes which synthesize proteins primarily for intracellular use ("free"). (Supported by NIH grant HE 08506.)

245. The Influence of Immunoglobulin Concentration on Virus Antibody Levels in Human Sera. PAUL E. PHILLIPS* AND CHARLES CHRISTIAN, New York.

A significant direct correlation was previously shown between both measles and parainfluenza antibody levels and gamma globulin levels in random sera from individuals with connective tissue and other diseases. It therefore seemed likely that changes in immunoglobulin levels in individual subjects would be accompanied by corresponding changes in virus antibody levels. From 2 to 12 sequential serum specimens were obtained from each of 12 subjects, 7 of whom had systemic lupus erythematosus (SLE). The sera spanned periods of 1–6 yr. Measles antibody was measured by hemagglutination-inhibition (HI) and IgG by radial immunodiffusion. All of an individual's sera were done in duplicate in the same test. Reproducibility was checked by retesting, and mean values recorded for each specimen. In SLE patients, as much as an eightfold change was observed in both measles antibody and IgG. Lesser changes were found in other subjects. There was striking correspondence between changes in levels of the two variables in all subjects, with increases and decreases tending to occur in concert. In several subjects IgM and parainfluenza type 1 HI antibody were measured; changes here tended to parallel those in measles antibody and IgG. It was again noted that correlation between these variables was not close enough to allow, knowing one, quantitative prediction of another. However, the fact that individuals with changing immunoglobulin levels have similar changes in these virus antibody levels supports earlier data that populations with higher gamma globulin levels have higher levels of these virus antibodies. Thus variation in immunoglobulin concentration may influence the results of virus antibody testing in both individuals and groups. (Supported by grants from NIH and Hartford Foundation.)

246. In Vitro Studies on the Interrelationship of Immunologic Challenge and Mouse Leukemia Virus Activation. S. MICHAEL PHILLIPS,* MARTIN S. HIRSCH,* CHARLES B. CARPENTER, AND CATHERINE J. SOLNIK,* Boston, Mass.

Chronic in vivo and acute in vitro allogeneic stimulation was used to investigate the relationship between altered

cellular immunity, mouse leukemia virus (MLV) activation, and neoplasia. Chronic GvH was induced in F_1 recipients (GvH-F_1) by injection of parental (P) lymphocytes. GvH-F_1 lymphocytes, when compared to normal (N-F_1) animals' lymphocytes, showed increased initial metabolic and mitotic activity. Responses to phytohemagglutinin (PHA) and allogeneic lymphocytes (MLC) were reduced in proportion to the severity of GvH. In addition, GvH-F_1 cells, when mixed in vitro with N-F_1 cells, actively suppressed the expected response of the N-F_1 cells to PHA and in MLC. MLV was identified in the GvH-F_1 cultures by XC and S + L-tests. Previously standardized Gross and Rauscher virus suspensions suppressed PHA and MLC responses in vitro, an effect removed by specific viral neutralizing antibody. The origin and mechanism of release of MLV from normal cells was studied with MLC's or iododeoxyuridine IUDR treatment. Both manuevers were associated with blastogenesis and MLV release. Blastogenesis per se was not sufficient to release MLV since PHA and Concanavalin-A (CON-A) stimulated cultures did not release virus. MLV release by GvH-F_1 cells was also found using P and F_1 combinations which do *not* develop neoplasms in the course of their GvH. In addition, the supernatants of GvH-F_1 lymphocyte cultures suppressed in high, and stimulated in low, concentrations PHA and MLC responses of N-F_1, P, and indifferent mouse cells. These activities, although extremely labile at 4, 20, or 37°C and unstable to freezing and thawing, were recovered from ultracentrifuged supernates (105,000 *g* for 2 hr), which were free of particulate virus. It is concluded that allogeneic stimulation results in activation of latent MLV and associated suppression of cellular immunity. In susceptible hosts these factors may be responsible for the development of neoplasia. (Research supported by grants from NIH.)

247. Affinity Cytotoxicity for Tumor Cells with Antibody-Enzyme Conjugates. GORDON W. PHILPOTT,* RICHARD J. BOWER,* AND CHARLES W. PARKER, St. Louis, Mo.

Conjugation of cytotoxic molecules to antibodies specific for tumor antigens is one possible method of obtaining more selective tumor chemotherapy. To test possible toxins, we have developed a model system utilizing hapten-substituted tumor cells and anti-hapten antibody. Viable hapten-substituted cells were obtained by brief exposure of HeLa, L, and Hep 2 cells to 2,4,6-trinitrophenyl (TNP) sulfonic acid. High affinity anti-TNP antibody was purified from the serum of rabbits immunized with TNP-bovine gamma globulin. Selective cytotoxicity was obtained using a combination of antibody-conjugated glucose oxidase (which generates H_2O_2 from glucose) and lactoperoxidase (which iodinates cells in the presence of H_2O_2 and I^-). Highly purified glucose oxidase (*Aspergillus niger*) was conjugated to purified anti-TNP antibody by means of a bifunctional imidoester. TNP cells were exposed for 30 min to low concentrations (5–70 μg/ml) of the antibody-enzyme conjugate, washed, and cultured in complement-free media containing lactoperoxidase (50 μg/ml) and iodide (20μM). Using a modified plating efficiency technique in microtest plates 25–100% of the tumor cells were dead at 24 hr. There was little or no cytotoxicity if I^-, lactoperoxidase, or antibody-glucose oxidase was omitted, if the cells did not contain TNP, or if uptake of antibody onto TNP cells was blocked by free hapten. The cell kill obtainable with the antibody-enzyme system was considerably greater than the kill obtained with comparable amounts of unconjugated antibody and complement. This work supports the concept that antibody-mediated cytotoxicity can be improved by conjugating enzymes or toxins to antibodies. This approach is potentially applicable to the treatment of drug-resistant infections as well as tumors. (Supported by grants of the NIH.)

248. Fibrinogen Synthesis by Cultured Avian Hepatocytes: Effect of Various Steroids. JOHANNA PINDYCK,* MICHAEL W. MOSESSON,* AND RICHARD D. LEVERE,* Brooklyn, N. Y. (introduced by Paul Dreizen).

Modification of hepatic fibrinogen production by synthetic steroids may be related to the role these compounds play in the genesis of thromboembolic disease. These studies were undertaken, therefore, to develop a model system for the investigation of normal and steroid-influenced hepatic fibrinogen synthesis. Livers were obtained from 16 day old chick embryos, the hepatocytes dissociated and grown as monolayer primary cultures by a modification of the method of Granick. After 24 hr of incubation, the medium was changed and the test steroid, in propylene glycol vehicle, or propylene glycol alone (1 μ/ml medium) was added. Cultures were maintained for another 24 hr, after which the elaboration of fibrinogen into the medium was assayed by electroimmundiffusion, using monospecific antiserum against chicken fibrinogen. The fibrinogen measured by this technique was thrombin-clottable. Control cultures produced 1.0±0.5 μg of fibrinogen/100 μg cell protein. Stimulation of fibrinogen production was noted with three synthetic steroids, with maximum stimulation (137–160% of control levels) at the following concentrations; 17-ethinyl estradiol 3-methyl ether, 10^{-11} M ($P < 0.025$), 3-acetyl estrone, 10^{-12} M ($P < 0.05$), and medroxyprogesterone acetate, 10^{-7} M ($P < 0.001$). The natural steroid, hydrocortisone, also produced significant stimulation (121% of control at 10^{-11} M, $P < 0.03$). No stimulation of hepatocyte fibrinogen production was noted with the synthetic steroids 17-methyl estradiol 3-methyl ether, or norethynodrel, nor with the natural steroids estradiol, progesterone, and testosterone. These investigations establish that primary culture of chick hepatocytes provides a unique and reliable quantitative approach to the study of fibrinogen synthesis and its modification by natural and synthetic steroids. The results obtained indicate hepatic fibrinogen synthesis is significantly increased by certain synthetic estrogenic and progestational agents. (Research supported by NIH grant HE-13767.)

249. Neural Regulation of Pancreatic Glucagon Secretion. D. PORTE, JR., E. B. MARLISS,* L. GIRARDIER,* J. SEYDOUX,* Y. KANAZAWA,* A. RENOLD,** AND J. POSTERNAK,* Geneva, Switzerland and Seattle, Wash.

Despite the presence of numerous nerve endings within the endocrine pancreas and the known effects of neurotransmitters on the beta cell, a role for neural control of the alpha cell has never been demonstrated. To study this question, a new approach to defining pancreatic neuro-endocrine control

was devised. The mixed autonomic pancreatic nerve of the dog was isolated at its entrance to the pancreas, cut, and the distal end placed in a simulating electrode. Hormone output was determined by diverting all pancreaticoduodenal vein flow through an extracorporeal circuit for measurement of flow and immunoreactive glucagon (IRG) concentration before its return to the portal vein. IRG was inferred to be of pancreatic origin on the basis of the sampling site and verification of selected studies with a specific antibody. When the nerve was stimulated at 40/sec IRG output promptly increased from a basal value of 25 ng/min to 55 ng/min after a 10 min stimulation ($n = 19$, $P < 0.001$). Output increased as stimulation frequency was increased between 5/sec and 100/sec. Atropine pretreatment had no effect on this response (mean Δ output ±SEM: without atropine, 250 ng ±80/15 min, $n = 8$; with atropine, 280±100 ng/min, $n = 11$), suggesting that this stimulation was via sympathetic and not parasympathetic fibers. To determine the sensitivity of this response to circulating glucose, a 0.1 g glucose pulse was given intravenously. It promptly suppressed IRG output by 25% from basal ($P < 0.005$, $n = 11$) when given alone, and prevented the response to nerve-stimulated IRG output ($P < 0.001$, $n = 6$). This prevention may explain the difficulties in demonstrating hyperglucagonemia during systemic catechol infusions which elevate blood glucose. This is the first direct demonstration that the autonomic nervous system can stimulate glucagon secretion and suggests that exercise- and stress-related hyperglucagonemia may be due to increased sympathetic nervous system activity.

250. Resin Sponge-Induced Dissociation of Thyroxine from Serum Proteins in Man. B. N. PREMACHANDRA* AND I. I. IBRAHIM,* St. Louis, Mo. (introduced by Sven G. Eliasson).

In competitive protein binding T_4 assay, separation of free and bound hormone is effected by second antibody, resin sponge, charcoal, etc. In techniques utilizing resin sponge, duration of its contact with reactants in the medium is critical since, with time, dissociation of bound T_4 is induced by the sponge. This property of resin sponge was used to study dissociation of T_4 from thyroxine-binding proteins in human sera. For conferring additional specificity, ^{125}I-T_4-labeled antithyroglobulin serum (containing T_4 binding antibodies) was used; displacement of ^{125}I-T_4 from immune serum and its uptake by the sponge reflected changes in T_4 concentration in the medium effected by dissociation. 0.1 ml portions of ^{125}I-T_4-labeled antiserum were added to tubes followed by the addition of 0.5 ml phosphate buffer (pH 7.4) and 0.4 ml human serum. The reactants were equilibrated at 37°C for 30 min and sponges were added. The mean count of the few tubes represented initial radioactivity. Starting 1 min after insertion of sponges in the medium and up to 24 hr, ^{125}I-T_4 uptake was studied. Four plateaus in sponge ^{125}I-T_4 uptake were noted, two of short duration (lasting approximately 4 min) occurring at 1 and 6 min, and two of longer duration at 4½ and 6½ hr (lasting approximately 45 min). By correlation of sponge uptake of ^{125}I-T_4 in normal, hypo- and hyperthyroid, and pregnancy sera (supplemented by dialyzable free T_4 measurements), the first plateau was considered to represent free T_4 equilibrium, and the remaining plateaus to signify dissociation of T_4 from albumin, prealbumin, and thyroxine-binding globulin (TBG), respectively. The concept of free T_4 equilibrium as derived from these observations has been successfully used to measure free T_4 concentration in human sera.

251. A Rapid Radioimmunoassay for Serum "Free" Thyroxine. B. N. PREMACHANDRA* AND I. I. IBRAHIM,* St. Louis, Mo. (introduced by C. Kirk Osterland).

Principle: Nonradioactive thyroxine in unknown serum or standards is allowed to displace labeled T_4 from ^{125}I-T_4 antithyroglobulin serum (containing T_4-binding antibodies); the displaced ^{125}I-T_4 radioactivity and its abstraction by resin sponge is a function of "free" T_4 equilibrium established between various T_4-binding agents, provided sponge uptake is measured at appropriate intervals. 0.5 ml phosphate buffer (pH 7.4) was added to 0.1 ml diluted (1:10) ^{125}I-T_4 antithyroglobulin serum followed by addition of 0.4 ml thyroxine standards or unknown serum. After 30 min equilibration sponges were added. Three tubes were counted to represent initial radioactivity. 2 min after sponges were inserted they were washed three times and counted (sponge radioactivity). Sponge radioactivity, expressed as per cent of initial radioactivity and corrected for dilution, was multiplied by total T_4 and resultant values represented "free" T_4 concentration. Alternatively, sponge radioactivity could be related to free T_4 levels by constructing calibration curves with T_4 standards. Mean equilibrium free T_4 values of 17 serum samples obtained by immunoassay (0.039) were comparable to those obtained by dialysis technique (0.037). Furthermore, increases in equilibrium "free" T_4 values were noted with diluted human sera similar to that seen in dialysis technique. Also, separation of "free" T_4 values between various sera was obtained by immunoassay: normal 3.98, hypothyroid 108, hyperthyroid 7.84, pregnancy 3.67 ng/100 ml (mean). Immunoassay specificity was shown by lack of effect of various substances in the medium (L-T_3, phenformin, dilantin in pharmacological doses) on "free" T_4 concentration. This extraordinarily sensitive, simple, and reproducible immunoassay of "free" T_4 has attributes which might prove useful clinically, thereby enabling further understanding of the significance of "free" T_4 in thyroid regulation.

252. Spurred Red Cells and Plasma Mesophase Lipid in Patients with Alcoholic Hepatitis. S. QUARFORDT,* W. KREMER,* AND L. JAKOI,* Durham, N. C. (introduced by M. P. Tyor**).

Four patients with biopsy-documented alcoholic hepatitis were noted to have spurred red blood cells and plasma liquid crystals. These patients had shortened red cell survivals, elevated plasma hemoglobins, and increased plasma phospholipid and free cholesterols (C). Most of the plasma lipid was isolated in the very low and low density (S_f 0–400) fractions and some of this lipid was found to be in liquid crystalline form. The liquid crystals were isolated free of the conventional lipoproteins by combined sucrose density gradient ultracentrifugation and gel filtration chromatography. They were almost entirely lecithin (L) and free cholesterol (C), demonstrating a spectrum of L/C molar ratios ranging from 1/0.4 to 1/1.5. By polarized and elec-

tron microscopy this mesophase was of lamellar configuration. When incubated in vitro with normal red cells the liquid crystals isolated from these patients with L/C molar ratios of 1/0.5 were noted to induce spurring of the cells and those with molar ratios of 1/1.5 to hemolyze. In vitro prepared lecithin cholesterol liquid crystals with L/C molar ratios ranging from 1/0 to 1/1 were incubated with normal red cells. The red cell response to the liquid crystals differed depending on the relative cholesterol content. With increasing cholesterol in the liquid crystals spurring was first noted at L/C ratios from 1/0.5 to 1/0.7 and overt hemolysis at L/C ratios from 1/0.8 to 1/1. These data suggest that the plasma mesophase lipid found in these patients with alcoholic hepatitis may induce not only red cell spurring, but intravascular hemolysis. (Research supported by NIH grant No. 1R01-HE-14313-01.)

253. Endotoxin and Granulopoiesis. PETER QUESENBERRY,* ALEC MORLEY,* PATRICIA BEALMEAR,* EERO NISKANEN,* MARILYN MILLER,* DONALD HOWARD,* AND FREDERICK STOHLMAN, JR., Boston, Mass.

The role of endotoxemia in the regulation of myelopoiesis was studied in irradiated conventional and germfree mice and in normal mice treated with 5 μg of *Salmonella typhosa* endotoxin. In vitro myeloid colony-forming cells (CFC), serum colony stimulating factor (CSF), and bone marrow myelopoiesis were evaluated. Sephadex G-150 gel filtration of normal and endotoxin serums separated serum inhibitors from CSF and indicated the appearance of a stimulator after endotoxin. Control animals had low to absent CSF. Conventional animals developed significant increases in CSF activity 6–8 days after 850 R. Germfree animals studied up to 10 days after irradiation failed to show elevations of CSF. Endotoxin, when given to normal mice, increased CSF within 10 min and peak values were seen between 2 and 8 hr. Tibial CFC decreased to 72% within 20 min, remained depressed for 24 hr, and returned to normal by 48 hr. During this period there was a wave of granulocytic differentiation manifested by sequential increases in the myeloblast-promyelocyte, myelocyte, and mature granulocyte compartments. Administration of endotoxin to conventional irradiated mice with myeloid aplasia, and low to no serum CSF activity, significantly increased serum CSF. Incubation of endotoxin with lung in vitro generated CSF but incubation with bone marrow or blood did not. The data indicate that CSF may be a long range humoral regulator of granulopoiesis and that endotoxemia (and, by inference, host flora) is one determinant of CSF levels. Lung appears to be one site of production. Mice with myeloid aplasia may have no demonstrable CSF and yet can generate increases in CSF after endotoxin suggesting that the number of granulocytes are not responsible for the CSF elevations seen after irradiation or endotoxin. (Research supported by grants from National Heart and Lung Institute and USPHS.)

254. Multiple Organ Disease from Light-Chain Deposition. R. E. RANDALL, JR.,* W. J. S. STILL,* M. Y. TUNG,* U. JAIN,* S. LOMVARDIAS,* E. S. BEAR,* AND C. W. MONCURE,* Richmond, Va. (introduced by G. W. James).

Two patients with marrow plasmacytosis were sustained by hemodialysis. Progressive deposition of kappa light-chains (KLC) occurred in multiple organs associated with significant functional and pathological abnormalities: (*a*) Kidney: tubular degeneration, membrano-proliferative (lobular) glomerulonephritis, and renal failure; (*b*) Liver: hepatomegaly and decompensation; (*c*) Neurological: choroid plexus and peripheral nerve deposits with neuropathy, diffuse cerebral dysfunction, abnormal EEG, papilledema, and increased KLC, gamma globulin, and protein in CSF; (*d*) Spleen: splenomegaly; (*e*) Pancreas: fibrosis and atrophy; (*f*) Endocrine: massive deposits and possible hypofunction of adrenal, pituitary, thyroid, parathyroid, and pancreatic islets; (*g*) Heart: conduction defect and arrhythmias; (*h*) Gastrointestinal: degeneration, dysfunction, and malabsorption; (*i*) Lung: oxygen diffusion defect; (*j*) Skin: itching and dermatitis. Deposits appeared in the basement membranes and in perivascular areas, similar to amyloid, but were nonfibrillar by electron microscopy and lacked the specific tinctorial characteristics of amyloid. They stained as KLC by immunofluorescent techniques and accepted conventional stains in a manner similar to basement membrane glycoprotein. The clinical and pathological manifestations of light-chain deposition in numerous organs represents a heretofore unrecognized risk of light-chain dysproteinemia, particularly in patients with renal failure who are supported by dialysis.

255. Suppression of Thyrotropin (TSH) Release by L-Dopa in Long-Standing Hypothyroidism. B. RAPOPORT,* S. REFETOFF,* V. S. FANG,* AND H. G. FRIESEN,* Chicago, Ill. and Montreal, Canada (introduced by A. T. Kenyon**).

Synthetic thyrotropin-releasing hormone (TRH) stimulates human TSH and prolactin (hPr) release. L-Dopa inhibits hPr release but in one study had no effect on TSH in euthyroid men. To reexamine the possibility for a common pathway of hypothalamic control for TSH and hPr, 0.5 to 1.0 g of L-Dopa was given orally to seven hypothyroid patients and serum obtained hourly for 6 hr. Hypothyroidism in four (group A) was at least of 1 yr duration and in three (group B) of less than 2 months. Mean serum TSH±SD in group A was 66.5±11.6 and in group B 27.5±25.8 μU/ml. Results are reported as per cent change ±SEM. Basal variation of TSH was 4.7% without distinct pattern. In group A, L-Dopa lowered TSH in all, with a nadir of −37±3.9% at 2–4 hr. A rebound of +17±9.5% occurred at 6 hr. No significant effect on serum TSH was observed in group B. 400 μg TRH intravenously produced a mean ±SD TSH rise of 174±18 in group A and 48±22 μU/ml in group B. hPr response to TRH and L-Dopa paralleled that of TSH as previously described. Since L-Dopa probably inhibits hPr secretion by stimulation of hypothalamic prolactin inhibitory factor (PIF) and TRH directly stimulates hPr and TSH release, the response of TSH to L-Dopa in this study suggests that the control of both hPr and TSH may be mediated by a common pathway: stimulation via TRH and inhibition via PIF. In man, however, the main control of TSH is via TRH and hPr via PIF. In long-standing hypothyroidism, with expected low levels of PIF, L-Dopa may decrease TSH by stimulation of PIF secretion, though a possible inhibition of endogenous TRH cannot be excluded. Thus,

TRH may well be also a prolactin-stimulating hormone (PRH) and PIF a TSH inhibitory factor (TIF). (Supported by grants NIH -F55 and AM 15,070.)

256. Central Neural Mechanisms Governing Orthostatic Reflexes. DONALD J. REIS AND NOBUTAKA DOBA,* New York.

To evaluate the role of the brain in regulating orthostatic reflexes, sites were sought in the brain of anesthetized paralyzed cats from which a comparable circulatory pattern could be evoked by electrical stimulation. Since stimulation of the fastigial nucleus (FN) of cerebellum produces elevated blood pressure (BP) and tachycardia (the fastigial pressor response [FPR]) and vestibulo-fastigial mechanisms control postural motor reflexes, the cardiodynamic changes associated with the FPR were investigated. The FPR is characterized by an α-adrenergically mediated decrease in blood flow and increase in resistance in femoral, axillary, renal, and mesenteric arteries. Carotid arterial flow increases passively suggesting suspended autoregulation. Total peripheral resistance increases 400%. Despite increased heart rate and myocardial contractility, stroke volume falls due to increased afterload thereby resulting in unchanged cardiac output. Venous pressure and pupillary size are unchanged. On the basis of the pattern of cardiovascular activity the FPR therefore appears to simulate the orthostatic reflex. Further support for this contention was established by studying the effects of FN or VIIIth nerve lesions on cardiovascular responses to head up tilting. In controls, tilting to 30° or 60° for 1 min results in a maintained BP, tachycardia decreased flow in femoral and renal arteries. Bilateral lesions of FN impair the reflex resulting in a fall of BP during tilt and decreased vasoconstriction. Dennervation of systemic baroreceptors or VIIIth nerves reduced the reflexes to the same extent. Maximal impairment occurred with combined FN and baroreceptor lesions. We conclude (*a*) that the FN can differentially excite sympathetic networks subserving orthostatic reflexes and (*b*) that the FN, triggered by vestibular mechanisms, participates in initiating and sustaining orthostatic reflexes in concert with systemic baroreceptors. (Supported by NIH and the Harris Foundation.)

257. Are There Anatomic Arteriovenous Shunts in Paget's Disease of Bone? BUCK A. RHODES,* N. DAVID GREYSON,* CARLOS R. HAMILTON, JR.,* AND H. N. WAGNER, JR.,** Baltimore, Md.

The circulatory abnormalities associated with Paget's disease of bone have been thought to result from anatomic arteriovenous shunting of blood through the diseased bone. To test this assumption, shunting was measured in patients with clear-cut radiographic and biochemical evidence of Paget's disease. ^{99m}Tc-labeled albumin microspheres, 15–30 μ in diameter, were injected into the artery supplying the diseased bone. Normally this size particle becomes trapped in the capillary bed. However, when arteriovenous (A-V) anastomoses are shunting blood, a proportionate fraction of the microspheres are carried through the extremity to become trapped in the pulmonary capillary bed. Shunting was quantified by measuring the fraction of the injected radioactivity which passed through the extremity and was detected in the lungs. Two measurements were made in each extremity. The regional distribution of capillary blood was determined by obtaining a rectilinear scan of the distribution of the radioactivity in the diseased extremity. In every case the perfusion scan showed increased blood flow in the involved bone in the areas corresponding to radiographic changes. In the eight involved regions of the six patients in the series no significant A-V shunting was detected. The overall mean was 0.18±1.04% (SD). The normal range for patients without shunting was 0±3.0%. An additional patient with concurrent pulmonary osteoarthropathy with clubbing of the fingers, a condition previously shown to be associated with A-V shunting, had a 4.8% shunt. We conclude that the increased blood flow found in Paget's disease of the bone is due to hyperperfusion of the diseased bone through vessels less than 15 μ in diameter rather than through larger arteriovenous communications. (Supported by USPHS grant GM 10548.)

258. Serum β_2-μGlobulin Concentrations (S_{β_2G}): a Measure of Renal Viability during Anuric Phase of Cadaveric Transplant. EDMOND S. RICANATI,* VEENA SAHNI,* SATWANT SINGH,* AND PHILIP W. HALL,* Cleveland, Ohio (introduced by George P. Gabuzda**).

Serial measurements of urine β_2-μglobulin excretion (U_{β_2G}) and S_{β_2G} were made before and after cadaveric renal transplantation in six nonnephrectomized chronic uremic patients, using the single immunodiffusion technique. Before transplant, U_{β_2G} excretion averaged 8 mg/24 hr (range 0–26) and S_{β_2G} averaged 167 μg/ml (range 80–190). S_{β_2G} decreased from 167 to 36 μg/ml (range 19–44) within 11 days after the transplant in four of the six patients, while U_{β_2G} remained at pretransplant levels. Assuming S_{β_2G} to be distributed in extracellular fluid, this decrease represents the disappearance of more than 1500 mg of S_{β_2G}. Renal excretion accounts for only 5% of this amount. Urine volume was less than 100 ml/day during this 11 day posttransplant period. Thereafter rapid improvement in glomerular filtration rate and excretory function occurred. These individuals have transplants which continue to function 4 or more months after transplant. The other two patients showed no change in S_{β_2G} after transplantation. Clinical course, laboratory, isotope, and radiologic studies for the first 11 days posttransplant in all six patients were otherwise identical. Within 25 days the two patients without initial posttransplant decreases in S_{β_2G} had the homografts removed. One showed evidence of hyperacute rejection, the other had renal arterial thrombosis. β_2-μglobulin, like gamma-G L-chains, is normally metabolized by the renal tubule. Our data suggest that this metabolism is occurring in the absence of detectable excretory function. A decreasing S_{β_2G} during the anuric phase after cadaver transplantation provides an indication that the graft is viable.

259. Pathogenetic and Therapeutic Correlations in Chronic Mucocutaneous Candidiasis. ROBERT R. RICH,* CHARLES H. KIRPATRICK,* AND TERRILL K. SMITH,* Bethesda, Md. (introduced by Sheldon M. Wolff).

On the basis of cellular immune functions, patients with chronic mucocutaneous candidiasis may be classed into three general categories: (*a*) those without demonstrable defects in cellular immunity; (b) those with an isolated defect in

cellular immune responses to candida antigens; and (*c*) those with inability to express delayed cutaneous hypersensitivity to any antigen tested. Studies of three patients in categories *a* and *b* suggested that therapy directed at complete elimination of the antigen burden with intravenous amphotericin B and avulsion of infected nails may be curative. Previously, neither treatment alone had produced sustained remissions. After combination therapy, these patients remain disease-free for periods of 6–12 months Moreover, the two patients in category *b* with isolated cellular immune defects to candida antigens developed normal delayed cutaneous hypersensitivity to these antigens after therapy. This was not associated with changes in serum anti-candida antibody titers. The data suggest that the immune defect in these patients was induced and maintained by excessive antigen. Three patients from category *c*, anergic to all antigens tested, have been similarly treated after reconstitution of cellular immune functions with soluble transfer factor. Transfer of delayed cutaneous hypersensitivity to several antigens, including candida, and synthesis of the lymphocyte mediator, macrophage migration inhibitory factor, was effected in all three patients with transfer factor alone, although clinical improvement was not observed. The patients were subsequently cleared of fungal infection with intravenous amphotericin B and nail avulsion. Both clinical remissions and intact cellular immunity have since been maintained by periodic readministration of transfer factor.

260. Feedback Control of Pituitary and Hypothalamus in Patients with Various Thyroid Disorders. E. CHESTER RIDGWAY,* JOSE L. CEVALLOS,* BRUCE D. WEINTRAUB,* C. THOMAS GRAEBER,* MARCO DANON,* HANS H. BODE,* AND FARAHE MALOOF,** Boston, Mass.

Synthetic thyrotropin-releasing hormone (TRH), 200–1000 μg, was given intravenously to patients with various thyroid disorders. Serial measurements were made of serum T_4, T_3, and thyroid-stimulating hormone (TSH) (radioimmunoassay), normal 3.6±0.6 μU/ml. In 12 normals, base line TSH rose to peak level of 15 μU/ml at 20 min, T_4 from 5.9 to 6.8 μg/100 ml in 120 min. In 10 patients with primary hypothyroidism, TSH (20–80 μU/ml) rose 2- to 10-fold and peaked at 30–60 min. In 21 prehypothyroid patients (TSH, 10–30 μU/ml; T_4, 4.0–8.0 μg/100 ml) TSH rose 3- to 10-fold and peaked at 20–45 min, resulting in a variable and nonparallel increase in T_4 (by 0–2 μg/100 ml) and in T_3 (by 0–100 μg/100 ml). Similar data were observed by the administration of bovine TSH. Complete suppression of the TRH response was achieved by doses of L-T_4 ranging from 150 to 300 μg/day. In seven patients with Graves' disease (TSH < 0.25 μU/ml; $T_4 = 14.0$ μg/100 ml; $T_3 = 350$ ng/100 ml) there was no change in TSH or usually in T_4 or T_3. In eight patients with hyperfunctioning thyroid nodules and complete suppression of paranodular tissue (TSH < 0.25 μU/ml; $T_4 = 8.4$, $T_3 = 250$) there was no change in TSH, T_4 or T_3. In one patient with incomplete suppression of paranodular tissue TSH increased from 1.7 to 7.8 μU/ml, T_4 from 7.0 to 9.5, and T^3 from 150 to 250. In three siblings with thyroxine-binding globulin (TBG) deficiency (4 μg/100 ml) TSH rose from 3.0 to 30 μU/ml at 20 min and T_4 rose from 2.5 to 6.0 μg/100 ml. In one patient with partial resistance to thyroid hormone (T_4, 21 μg/100 ml, F-T_4, 4.2 ng/100 ml; TBG, 15 μg/100 ml) TSH increased from 3.5 to peak of 21 μU/ml at 20 min, T_4 from 21 to 27 μg/100 ml, and T_3 from 475 to 730 ng/100 ml. Prednisone altered the TRH response; TSH rose from 0.75 to 10 μU/ml; there was no change in T_4, but T_3 rose from 505 to 745 ng/100 ml. Summary: TRH is effective in releasing biologically active TSH in a variety of metabolic states as evidenced by an early rise in T_4 and T_3 and its effect is inhibited by various serum levels of thyroid hormone. (Research supported by grants from USPHS: R01 HD 05195-01, AM 13916-02, AM-04501, AM 5205-12.)

261. The Importance of Plasma Osmolality in Regulating Antidiuretic Hormone Secretion in Man. GARY ROBERTSON,* AND ERMELINDA MAHR,* Indianapolis, Ind. and Chicago, Ill. (introduced by Jacob Robbins**).

The relative importance of blood volume and osmolality in regulating the secretion of antidiuretic hormone, arginine-vasopressin (AVP), in normal man has not been established. To evaluate this, we produced small changes in blood osmolality and/or volume of three types—increased osmolality and decreased volume by fluid deprivation; increased osmolality and volume by hypertonic saline infusion; and decreased volume alone by phlebotomy—and compared the effects on plasma AVP concentration (P_{avp}) as determined by radioimmunoassay (1971. *Clin. Res.* **19**: 562). In recumbent adults, basal P_{avp} averaged 3.8±1.6 pg/ml and plasma osmolality (P_{osm}) 288±3 mOsm/kg (mean±SD). Fluid deprivation sufficient to reduce body water by at least 4% caused 2- to 4-fold increases in P_{avp} that correlated closely with the increases in P_{osm} as described by the regression equation $P_{avp} = 0.52\ [P_{osm} - 278]$ ($r = 0.7$, $P < 0.001$). Hypertonic saline, in amounts sufficient to increase both plasma volume and P_{osm} by about 4%, produced equally precise but slightly smaller rises in P_{avp} as described by $P_{avp} = 0.36\ [P_{osm} - 275]$ ($r = 0.87$, $P < 0.001$). Phlebotomy, which reduced blood volume by about 5% without altering blood pressure or P_{osm}, did not increase P_{avp}. Thus, increases in P_{osm} as small as 1% consistently produced readily detectable increases in P_{avp}. Hypovolemia of severalfold greater magnitude did not effect P_{avp} in the absence of osmotic changes, but appeared to potentiate slightly the response to hypertonicity. We conclude that in healthy adults the secretion of AVP is regulated primarily by small changes in P_{osm} and is relatively insensitive to comparable changes in blood volume.

262. Mechanism of Cholesterol Gallstone Formation in the Essential Fatty Acid-Deficient Hamster. SANDER J. ROBINS* AND JOAN FASULO,* Boston, Mass. (introduced by Lauran D. Harris).

Essential fatty acid-deficient (EFAD) hamsters develop lithogenic bile and cholesterol gallstones. To determine whether overproduction of cholesterol or bile salt (BS) deficiency is responsible for stone production, we studied BS kinetics and hepatic cholesterol secretion. Age-matched, young hamsters, fed either an EFAD or laboratory chow diet, were studied at intervals up to 60 days. Hepatic bile for cholesterol and BS measurements was obtained from non-fasted animals during the first ½ hr after common duct

cannulation. Fractional daily excretion of BS was determined after the intraduodenal administration of cholate-^{14}C, and pool size estimated by isotope dilution. All values were calculated in relation to body weight. 70% of EFAD and none of the chow-fed hamsters developed gallstones. There was no evidence of BS deficiency in EFAD animals; (*a*) cholate pool size (12.7±4.9 mg/100 g) was not significantly different from controls (9.8±2.5 mg/100 g); (*b*) fractional excretion of the pool was less ($P < 0.01$) and reabsorption of cholate greater ($P < 0.001$); and (*c*) total BS secretion rates were, in fact, 60% higher ($P < 0.05$). However, there was biliary cholesterol overproduction in EFAD animals: (*a*) while BS secretion remained constant, cholesterol secretion rates increased progressively from 2.2 to 4.5 times the amounts in controls; (*b*) when cholesterol precipitation as stones was prevented by cystic duct ligation, EFAD animals had 7.7-fold greater cholesterol secretion than chow-fed, cystic duct-ligated controls and 2.5-fold greater secretion than EFAD animals with stones; and (*c*) although serum cholesterol was not increased, total hepatic cholesterol levels were 67% greater than controls ($P < 0.05$). These results demonstrated that lithogenic bile found in the EFAD hamster is not secondary to BS deficiency but rather results from markedly enhanced biliary secretion of cholesterol.

263. Excretion of DNA by Phytohemagglutinin-Stimulated Lymphocytes. JOHN C. ROGERS,* DAVID BOLDT,* STUART KORNFELD, AND C. ROBERT VALERI,* Chelsea, Mass. and St. Louis, Mo. (introduced by C. V. Moore).

Although phytohemagglutinin (PHA) induces lymphocytes to undergo blast transformation and to synthesize DNA, no consistent increase in DNA content or cell number has been found in PHA-stimulated cultures. Therefore we examined the fate of newly synthesized DNA in such cultures. Human lymphocytes were cultured for 3 days with PHA, then pulsed for 4 hr with thymidine-^{3}H. The cells were washed and resuspended in culture medium with PHA for an additional 3 days. The cells remained viable as judged by supravital dye exclusion and 70–90% underwent blast transformation. Chromosome preparations on days 3, 4, and 5 indicated that a minimum of 10–20% of cells underwent mitosis. Cell number and total cellular DNA content (measured colorimetrically) remained constant from day 3 to day 6. However 60–90% of the thymidine-^{3}H incorporated into cellular DNA left the cells and appeared in the medium during this time. The radioactivity in the medium was acid precipitable and could be solubilized with DNase but not with RNase or alkali. This DNA had a mol-wt $> 4 \times 10^6$ as determined by gel filtration on Sepharose 6B. Experiments using uridine-^{14}C indicated that RNA was not lost into the culture medium. DNA excretion occurred under many culture conditions. From our studies we conclude: DNA is selectively excreted by lymphocytes stimulated in vitro by plant mitogens. The reason for this phenomenon is unclear, but our evidence suggests that it may be the result of abortive cell division since cell number and DNA content remain constant in the presence of a significant mitotic rate. Alternatively the excreted DNA may represent a specialized species of DNA released for a specific purpose as yet undetermined.

264. An Enzyme in the Alternate Pathway to C3 Activation (the Properdin System) and Its Inhibition by a Protein in Normal Serum. FRED S. ROSEN AND CHESTER A. ALPER, Boston, Mass.

Factor B of the properdin system has recently been shown to be identical with glycine-rich β-glycoprotein (GBG). This protein is cleaved into two fragments, one of γ and one of α mobility, by the action of a hydrazine-sensitive enzyme in serum during complement activation. The serum of a patient, previously reported, with type I essential hypercatabolism of C3 is deficient in GBC and contains this enzymatic activity in activated form. Highly purified GBG, on addition to his serum, is completely cleaved in less than 15 min at 37°C into the α and γ fragments and factor B activity is lost. This reaction is temperature- and Mg^{++}-dependent. The purified enzyme from the patient's serum (GBGase) is an α_2-euglobulin of about 4S which is not inhibited in its activated state by hydrazine. Normal human serum inhibits the action of GBGase on GBG and on purification this GBGase inhibitor is a 5–6S heat-labile β-pseudoglobulin. The inhibitor is uniquely absent from the serum of the patient with C3 hypercatabolism but is present in the serum of patients with hereditary angioneurotic edema and of other patients with various deficiencies. It appears likely that GBGase occurs normally in serum in a zymogen form (proGBGase) and the latter is probably factor A in the properdin system.

265. Regulation of Transcription in the Human Peripheral Lymphocyte by Dibutyryl Cyclic Adenosine Monophosphate (DcAMP), Phytohemagglutinin (PHA), and Cortisol. MICHAEL G. ROSENFELD,* ITAMAR B. ABRASS,* JOHN MENDELSOHN,* BERNARD A. ROOS,* ROBERT F. BOONE,* AND LEONARD D. GARREN, La Jolla, Calif. (introduced by Henry O. Wheeler).

The effect of DcAMP, PHA, and cortisol on transcription of messenger RNA (mRNA) has been studied in human lymphocytes to gain insight into the early molecular events of hormone action and the control of lymphocyte transformation. To characterize mRNA synthesis in cultures of peripheral blood lymphocytes, rapidly labeled polyadenylate-rich nuclear and polyribosomal RNA (A-RNA) was isolated. mRNA function for this A-RNA is suggested by its ability to direct ^{3}H-met-tRNA binding to ribosomes and incorporation of amino acids into protein in a cell-free system. PHA and low concentrations of DcAMP increase A-RNA synthesis 40% within 2 hr, with 100–500% stimulation at later time points. Low concentrations of prostaglandin (PGE_1), which increases intracellular cAMP levels, also increase A-RNA synthesis. In contrast, high concentrations of DcAMP and PGE_1 inhibit synthesis of A-RNA by unstimulated and PHA-treated lymphocytes without damaging cells. This inhibitory effect of DcAMP depends upon its early and continued presence since removal of DcAMP at several time points allows full expression of the PHA effect. Cortisol, a known inhibitor of lymphocyte transformation, also inhibits A-RNA synthesis 80%. Cycloheximide at concentrations which inhibit protein synthesis inhibits A-RNA synthesis 80% in PHA-stimulated cells. These studies demonstrate that as part of the early molecular events of their action PHA and low concentrations of

DcAMP stimulate transcription of A-RNA in human lymphocytes, and that this effect is dependent upon protein synthesis. Cortisol, as well as high doses of DcAMP and PGE_1 suppress this stimulation. (Research supported by NIH American Cancer Society, and University of California, San Diego.)

266. Identification of a New Plasma Hypotensive Factor. JULIAN ROSENTHAL* AND WILLIAM HOLLANDER,** Boston, Mass.

In a previous study this laboratory reported the identification of a factor in aortic tissue of dogs that decreased arterial blood pressure (1971. *Clin. Res.* **19**: 713.). We now report that a similar substance is present in plasma of normal dogs and also of humans. Extraction and isolation from the plasma was achieved by selective membrane ultrafiltration, gel filtration, and polyacrylamide gel electrophoresis. The purified substance, when injected intravenously into the rat in a dosage of 5–10 μg, lowered rapidly the arterial blood pressure by 40–50 mm Hg. The blood pressure returned to base line values within 1 min. In these dosages the hypotensive factor also caused relaxation of the isolated, spirally cut rabbit aortic strip, but did not depress the myocardial contractility of the isolated rabbit papillary muscle. Biochemical and pharmacological characterization demonstrated the hypotensive factor to be a polypeptide with a mol wt of below 2000. The polypeptide analysis revealed the major constituents to be glutamic acid, proline, and glycine. In dogs with hemorrhagic shock induced by bleeding from the femoral artery, the hypotensive factor increased 5- to 6-fold as compared to the values in sham-shocked animals. Parallel increases of the hypotensive factor occurred in the arterial tissue during shock. Initial studies in normal humans and patients in shock (gastro-intestinal hemorrhage, myocardial infarction) showed a similar pattern. The hypotensive factor was present in the plasma in higher concentrations in shock patients than in normals. In conclusion: It appears from these data that the polypeptide isolated from arteries and now also from plasma may play a role in the regulation of blood pressure and peripheral vascular resistance. This hypotensive factor also appears to be involved in the pathogenesis of shock. (Supported by HE 13262.)

267. Mechanisms of Immune Hemolysis in Hereditary Erythroblastic Multinuclearity with Positive Acidified Serum Test (HEMPAS) and Paroxysmal Nocturnal Hemoglobinuria (PNH). WENDELL ROSSE, JOHN CROOKSTON,* MARIE CROOKSTON,* GERALD LOGUE,* AND JUDITH ADAMS,* Durham, N. C. and Toronto, Canada.

In HEMPAS (a recently-described hereditary dyserythropoietic anemia) the red cells resemble PNH cells in being lysed in the acidified serum (Ham) test. However, in this test, lysis of HEMPAS cells is initiated by a unique naturally occurring alloantibody, whereas lysis of PNH cells is initiated by activation of the third complement component (C3) without mediation of antibody. When exposed to anti-I and complement, HEMPAS cells are more readily lysed than normal cells. HEMPAS cells adsorb the same amount or somewhat more ^{125}I-labeled anti-I compared to normal cells but only one-tenth as much adsorbed antibody is needed for a given degree of lysis. When membrane-bound C3 was measured (using anti-C3 and the fixation and transfer test) 10 times as much C3 was found on HEMPAS cells for a given quantity of adsorbed antibody. Thus, normal and HEMPAS cells require the same amount of membrane-bound C3 for the same degree of lysis. In PNH, the complement-sensitive cells require little adsorbed antibody for lysis. The amount of C3 bound per molecule of adsorbed anti-I was the same for PNH as for normal cells but the amount of cell-bound C3 necessary to lyse PNH cells was much less than needed for the same degree of lysis of normal or HEMPAS cells. Thus, when complement is bound by anti-I, HEMPAS cells are readily lysed because more complement is bound per molecule of adsorbed antibody. By contrast, PNH cells are readily lysed because much less cell-bound C3 is needed for lysis of these cells than of normal or HEMPAS cells. (Research supported by NIH (National Cancer Institute) and the Medical Research Council of Canada.)

268. How Accurate Are Patients' Statements and Their Doctors' Estimates in Assessing Antacid Intake? HAROLD P. ROTH* AND HERBERT S. CARON,* Cleveland, Ohio (introduced by Reginald A. Shipley**).

Patients vary widely in the amount of prescribed medicine that they actually take. Yet the physician must decide how much medicine is taken to properly evaluate a given patient's progress. This study analyzes the accuracy of a series of estimates by three physicians and statements by 97 patients about antacid intake during a 2 yr outpatient followup study of peptic ulcer. The subjective estimates were compared with objective measures: (*a*) counts of empty bottles made at each regular delivery of free medication to the patient's home, and (*b*) blood levels of a trace element present in the medication. Utilizing objective measurements, the mean intake of antacid was found to be 51% of the amount prescribed, but the mean intake reported by patients was 91%. All three physicians overestimated patients' intake. Their mean estimates were respectively 61, 83, and 64% although their patients did not differ significantly from the grand mean of 51%. Inaccuracy of subjective judgements was also indicated by low correlation coefficients between the objective measures and either patients' statements ($r = 0.42$) or the three physicians' estimates ($r = 0.56$, 0.36, and 0.30). Examination of the data revealed differences according to race of patient and physician. Black patients reported taking about as much antacid, 90%, as did the white patients, 92%. However, the mean antacid intake was lower for black patients, 46% of prescription, than for white patients, 63% ($P < 0.005$). In turn, white doctors may be more accurate ($P = 0.06$) in assessing intake of their white patients ($r = 0.58$) than of their black patients ($r = 0.29$). (Research supported by grant from NIH.)

269. Humoral Stimulators of Granulocyte Production. GERALD ROTHSTEIN,* ERICH HÜGL,* PAUL CHERVENICK,* AND JOHN ATHENS,** Salt Lake City, Utah and Pittsburgh, Pa.

These studies were designed to determine whether diffusible granulocytopoietic stimulator (DGS) and colony stimulating factor (CSF) are the same. DGS stimulates granulocytopoiesis in vivo in Millipore chambers; CSF promotes granulocyte colony formation in vitro. Mice were given 40 μg endotoxin intravenously (i.v.) and CSF and DGS were assayed. Serum CSF increased 4–20 hr post endotoxin (mean, 181 colonies in 21 endotoxin mice vs. 18 colonies in 11 controls, $P < 0.05$). 72 hr after endotoxin CSF was normal. DGS was not detectable 4–20 hr after endotoxin even though CSF was increased. However, in Millipore chamber marrow cultures DGS was detected 72–96 hr after endotoxin (mean blast + promyelocytes [BP] 46,780 in 20 endotoxin hosts vs. 33,025 BP in 21 controls, $P < 0.05$) while CSF was normal. Thus, DGS and CSF were detected at different times. In other studies, host mice were given 25,000 U CSF (courtesy E. R. Stanley) in one intravenous dose, but no stimulation of BP growth occurred in the chambers during the succeeding 24 hr (30,500 BP in 11 CSF injected hosts; 26,000 BP in 11 controls, $P > 0.3$). Therefore CSF did not stimulate granulocyte production in chambers. These data suggest DGS and CSF are different factors. Also, DGS appears to act on identifiable granulocyte precursors (blasts and promyelocytes) while CSF does not; CSF may act to promote cloning of less mature cells. (Grant support: NIH, American Cancer Society, and LSA.)

270. Two Types of β-Thalassemia Distinguished by Inducibility of Cell-Free Synthesis of β-Chains by Nonthalassemic Reticulocyte Soluble Fraction. PETER T. ROWLEY,* FRANCESCO CONCONI,* LAURA DEL SENNO,* SANDRO PONTREMOLI,* SILVIO VOLPATO,* AND BARBARA KOSCIOLEK,* Rochester, N. Y. and Ferrara, Italy (introduced by Lawrence E. Young**).

β-Thalassemia is a hereditary anemia in which the β-chains of hemoglobin, though structurally normal, are synthesized abnormally slowly. Synthetic studies in intact cells in homozygotes distinguish two types, the usual type with reduced β-chain synthesis and the Ferrara type with no β-chain synthesis. An improved human reticulocyte cell-free system, which synthesizes globin at a linear rate for more than 2 hr, has permitted subcellular localization of the β-chain synthetic lesion. In the cell-free system, ribosomes from Ferrara subjects did not make β-chains in the presence of their own soluble fraction, but did when nonthalassemic soluble fraction was substituted. This effect was due to a factor other than messenger RNA because soluble fraction from subjects with sickle cell anemia, which lacks messenger RNA for normal β-chains, was equally effective. In contrast, ribosomes from non-Ferrara subjects did not increase β-chain synthesis when the subject's own soluble fraction was replaced by nonthalassemic soluble fraction. Recently Nienhuis and Anderson have presented direct evidence that in non-Ferrara β-thalassemics the defect is in β-chain messenger RNA. Our results are consistent with this finding, since reticulocyte messenger RNA is a component of the ribosomal fraction, and substitution of soluble fractions would not be expected to be corrective. Thus it appears likely that the defect in the non-Ferrara type is in messenger RNA and the defect in the Ferrara type is in a soluble component yet to be identified, a factor specifically determining human β-chain mRNA translation, possibly an initiation factor. (Supported by National Science Foundation and World Health Organization.)

271. Cyclic AMP-Dependent Protein Kinase Activity in Human Erythrocytes. CHARLES S. RUBIN,* JACK ERLICHMAN,* AND ORA M. ROSEN, Bronx, N. Y.

A membrane-associated cyclic AMP-dependent protein kinase has been found in preparations of human erythrocyte ghosts and has been characterized. The membrane-bound enzyme was stimulated 7-fold by the addition of 2×10^{-6} M cyclic AMP, catalyzed the transfer of 690 pmoles ^{32}P from $AT^{32}P$ to protamine/min per mg membrane protein, and accounted for greater than 70% of the total protein kinase activity present in the erythrocyte. Exogenous proteins, e.g., protamine, histone, and casein, as well as components of the erythrocyte membrane, are substrates in the phosphotransferase reaction, but stimulation by cyclic AMP was observed only when histones or protamine were serving as substrates for phosphorylation. The membrane-associated protein kinase resembles many soluble cyclic AMP-dependent protein kinases; it requires 20 mM Mg^{++} for maximal activity and possesses a high affinity for cyclic AMP ($K_m = 4 \times 10^{-8}$ moles/liter). Preparations of erythrocyte ghosts bind cyclic AMP avidly and specifically and the bound nucleotide is resistant to hydrolysis by cyclic nucleotide phosphodiesterase. The cyclic AMP-independent catalytic component of the cyclic AMP-dependent protein kinase could be solubilized by treating the membranes with NH_4Cl at high ionic strength. The cyclic AMP-binding component, however, remained firmly anchored in the membrane residue after such treatment. Since human erythrocytes possess very little adenyl cyclase activity (≤ 6 pmoles cyclic AMP formed per min per mg membrane protein), the membrane-associated cyclic AMP-dependent protein kinase may be primarily responsive to fluctuations in the plasma level of cyclic AMP. The accumulation of high local concentrations of cyclic AMP at the erythrocyte cell surface may influence the physiological state of a variety of tissues as well as the function of the erythrocyte itself. (Supported by grants from the NIH and ACS.)

272. Abnormality of the Alternate Complement Pathway in Systemic Lupus Erythematosus (SLE) and Hypocomplementemic Chronic Glomerulonephritis (HCGN). SHAUN RUDDY,* LAWRENCE G. HUNSICKER,* PETER H. SCHUR, AND K. FRANK AUSTEN, Boston, Mass.

Factor B of the properdin system is required for alternate pathway activation of the terminal components of complement (C3–C9). This protein was purified and monospecific antibody was used in radial immunodiffusion to measure serum levels. Serum complement components were measured by effective molecule titrations and/or radial immunodiffusion, and metabolism of third (C3) and fifth (C5) components was assessed with radiolabeled proteins. In 12 of 15 patients with hypocomplementemic systemic lupus erythematosus (SLE), factor B levels were below the normal range (mean$\pm$2 SD) in 25 healthy adults. In serial studies, reductions in factor B correlated with increased disease

severity, were often preceded by depressions of C4, invariably accompanied by decreased C3, and sometimes associated with reduced levels of C9. In the four patients studied, fractional catabolic rates (FCR) for C3 were increased: 2.9, 3.9, 5.1, and 5.7% of plasma pool/hr (mean±2 SD in 17 controls: 0.8–2.2%/hr). In a single study, the FCR for C5 was 3.8% as compared to 1.6, 2.1, and 2.3%/hr in three normals. In 9 of 18 patients with hypocomplementemic chronic glomerulonephritis (HCGN), levels of factor B and C3 were subnormal, and C3 catabolic rates were increased in the four patients studied: 2.8, 3.2, 5.9, 6.9%/hr. Serum C4 levels in HCGN were uniformly normal. Normocomplementemic patients with SLE or chronic nephritis had normal levels of factor B. In hypocomplementemic chronic glomerulonephritis, hypercatabolism of C3 is associated with decreased levels of factor B, implying activation of the alternate pathway. In SLE, depressions of serum C4 indicate classical pathway activation; with exacerbations of disease, depressions of C3 and factor B occur, indicating recruitment of the alternate pathway for activation of the terminal complement components.

273. Renal Osteodystrophy: a Defect in Bone Maturation? JEAN E. RUSSELL* AND LOUIS V. AVIOLI, St. Louis, Mo.

Although alterations in bone mineral metabolism which attend the uremic state have been exhaustively defined, the effect of chronic renal failure on the maturation of skeletal tissue is virtually unknown. The chronic uremic state was induced experimentally in adult rats which were sacrificed along with their pair-fed controls at 2, 5, and 11 wk thereafter. Skeletal tisue was "powdered" and fractionated into individual components of varying degrees of density (1.7–2.3 g/cm^3) on toluene-bromoform gradients wherein the less dense fractions correspond to newly synthesized collagen and surface labile mineral and most dense fractions, mature collagen and hydroxyapatite crystals. Despite normal levels of *total* bone collagen and mineral content for the first 5 weeks of the chronic uremic state, a 60% reduction in the concentration of the mature collagen-mineral complexes was observed at 2 wk; 5 and 11 wk of uremia resulted in a 70 and 80% decrease in the mature collagen-mineral complexes, respectively. Whereas the incorporation of single pulse labels of ^{45}Ca- and ^{3}H-labeled proline into mature bone fractions normally increased progressively with time, the uremic state resulted in an accumulation of both isotopes in immature collagen-mineral complexes. This defect in bone metabolism was unaffected by prior therapy with either vitamin D_3 or its active 25-hydroxycholecalciferol (25-HCC) metabolite. The accumulated data reveal that the chronic uremic state imposes significant alterations in bone collagen maturation as well as in the sequence of events which terminate in the mineralization of organic matrix. Since the metabolic error is resistant to vitamin D_3 and 25-HCC therapy, the lesion may result from an abnormality in 25-HCC metabolism, secondary hyperparathyroidism, an accumulation of uremic toxins, or combinations thereof. (Research supported by NIH Contract 70-2219.)

274. The Role of Metals in Resistance of *Staphylococcus aureus* to Methicillin and Other Penicillins. L. D. SABATH* AND S. J. WALLACE,* Boston, Mass. (introduced by M. Finland**).

A possible functional role for metals in methicillin resistance of *Staphylococcus aureus* was evaluated because methicillin-resistant (M-R) organisms had more elutable zinc on their surfaces than did methicillin-susceptible (M-S) organisms. Susceptibility tests in medium that had been depleted of metals with the resin Chelex-100, and also medium (brain heart infusion broth) to which chelating agents (ethylenediaminetetraacetic acid [EDTA]) of orthophenanthroline had been added, showed striking (up to 256-fold) suppression of methicillin resistance in M-R organisms but negligible (0 to 2-fold) changes in the susceptibility of M-S strains. The effect of EDTA could be completely reversed by the addition of equivalent amounts of Ca^{++}, Fe^{++}, Ni^{++}, and Mn^{++} and nearly completely by Cu^{++} and Mg^{++}; the ortho-phenanthroline effect was most effectively reversed by Co^{++}, Fe^{++}, and Ni^{++}; 8-hydroxyquinoline could not suppress methicillin resistance. This suggested that the expression of methicillin resistance in *S. aureus* (which is not due to penicillinase) requires certain divalent cations. The binding kinetics of benzylpenicillin-^{14}C are different in M-R cells (higher K_m) than in M-S cells, even in the presence of EDTA. This suggests that the role of the metals in penicillin resistance is to counteract the inhibitory effect of antibiotic that is attached to the cell but not to decrease the rate or amount of drug bound. (Supported by NIH and AHA.)

275. Prostaglandins: Release from the Lung during Mechanical Ventilation at Large Tidal Volumes. SAMI I. SAID, SATOSHI KITAMURA,* AND CAROL VREIM,* Richmond, Va. and Dallas, Tex.

The lung is an important site of synthesis and metabolism of prostaglandins (PG's), biologically potent lipids which influence blood pressure, respiration, and smooth muscle. We sought to determine whether mechanical distortion of the lung, as by hyperinflation, could lead to discharge of PG's. The left lower lobe in 16 dogs was perfused with Krebs solution at constant flow (15 ml/min, perfusion pressure [PP] = 8–17 mm Hg), and ventilated mechanically with 5% CO_2 in O_2. To detect active substances in the perfusate, it was made to drip onto strips of rat stomach and rat colon which had been rendered insensitive to catecholamines, histamine, serotonin, and acetylcholine. These tissues permitted the assay of PG's and some differentiation between PGE and PGF. At control tidals (V_T), little or no PG was detectable. On increasing V_T by 50–500%, while maintaining the same minute ventilation and effluent pH, both muscle strips contracted. The contractions began within 2 min of increasing V_T, correlated with breath size, were accompanied by a decrease in PP, and corresponded to the liberation of up to 150 ng/min of PG's, predominantly PGE. After infusing 1 mg aspirin into the lung to inhibit PG synthesis, hyperinflation no longer affected the smooth muscles or PP. We conclude that stretching of the lung probably triggers increased synthesis of PG's, and their discharge into the circulation. Release of PGE's, bronchodilators and vasodilators, might be an adaptive response to deeper breathing, but could contribute to systemic hypotension during pro-

longed assisted respiration. (Research supported by grants from NIH, American Heart Association, and NTRDA.)

276. Dissociation of Effects of Adiposity and Diet on Glucose, Insulin, and Adipose Tissue Metabolism in Experimental Human Obesity. L. SALANS,* E. DANFORTH,* E. HORTON,* AND E. SIMS,* Hanover, N. H. and Burlington, Vt. (introduced by Gilbert Mudge**).

Four normal volunteers were fed diets low (100 g/m²) and high (300 g/m²) in CHO both before and after 13–25% weight gain through overeating a balanced diet. Oral glucose tolerance (OGT), plasma immunoreactive insulin (IRI), and in vitro adipose tissue (AT) metabolism were measured after 3 wk on each diet at each weight and comparisons made as functions of CHO intake and adiposity. At normal body fat content and fat cell size (0.27 μg TG per cell), high CHO intake increased basal glucose metabolism and insulin effectiveness as demonstrated by increased OGT, decreased IRI response and, in AT, by both enhanced basal (CO_2, 131%↑; TG, 148%↑) and insulin-stimulated (158%↑; 203%↑) glucose metabolism. After weight gain, when body fat was increased 33–140% and cell size enlarged (0.51 μg TG per cell), glucose metabolism and insulin effectiveness were significantly decreased on either diet ($P < 0.01$). During low CHO intake, OGT was unchanged but IRI response was increased by the excess adiposity. At both levels of CHO intake, increased adiposity diminished the effect of insulin on AT metabolism (61–360%↓). In contrast to its effect at normal weight, high CHO intake at peak weight impaired OGT, further increased hyperinsulinemia, and only minimally increased basal (5%) and insulin-stimulated (8%) glucose metabolism by AT. These data indicate that the metabolic effects of antecedent diet and adiposity can be dissociated. Both may influence the metabolic alterations associated with this form of obesity, but hyperinsulinemia and insulin resistance are demonstrable after weight gain irrespective of dietary CHO intake. (Supported by NIM Grants AM 13321 and 10254.)

277. Sedimentation Behavior of Platelets in Plasma from Patients with Hyperlipoproteinemia. JEAN W. SALEH* AND SAMI A. HASHIM, New York.

The influence of lipoproteins on sedimentation behavior of platelets appears not to have been studied. EDTA plasma was obtained from 11 normal subjects, 6 patients with type II, and 12 with type IV hyperlipoproteinemia, all fasting, and from 11 normal subjects before and at intervals up to 6 hr after ingestion of 65 g fat. All blood samples were centrifuged at 1500 rpm for 5 min (platelet-rich plasma [PRP]), at 10,000 rpm for 10 min, and at 42,000 rpm for 30 min. Platelet counts were made on mixed PRP and, after high-speed centrifugation, on top and middle plasma layers and on the sediment, resuspended in imidazole buffer. Lipoprotein electrophoresis was performed on all fasting plasmas and their top layers after centrifugation. Also, cholesterol and triglyceride concentrations were determined on all plasma samples. The mean±SE number of platelets per mm³ in top layer among normal, type II, and type IV plasmas were 4000±405, 9000 ±70, and 21,000±195, respectively. The corresponding values expressed as per cent of platelets of PRP were 1.2±0.1, 3.0 ±0.2, and 7.5±0.6. In the top layer, PRP platelet ratio correlated well ($r = 0.75$) with plasma triglycerides but not with cholesterol ($r = 0.4$). The curve representing serum triglyceride changes in response to fat ingestion in normal subjects paralleled that of platelets in top layer. Addition of artificial triglyceride emulsion to normal plasma resulted in threefold increase in platelets "floating" after centrifugation. Thus, the sedimentation behavior of platelets is altered significantly in hyperlipidemic plasmas, particularly those with hypertriglyceridemia. The pathophysiologic consequences of this phenomenon in patients with hyperlipoproteinemia remain to be determined. (Research supported by Grant AM-08107 from NIH.)

278. Mechanism of Action of Thyroid Hormones: Studies in Cell Culture. HERBERT H. SAMUELS,* JIR TSAI,* AND RAQUEL CINTRON, New York (introduced by Saul J. Farber**).

In spite of extensive studies in vivo, the mechanism of action of thyroid hormones remains to be established. We have therefore developed a cell culture system which is unique in that it responds to physiologic concentrations of triiodothyronine (T3) and thyroxine (T4). T3 at 1.5×10^{-9} mole/liter or T4 at 0.8×10^{-7} mole/liter or greater induce an increase in the rate of glucose utilization, oxygen consumption, and cell growth. The increase in glucose oxidation is detected within 12 hr and attains a constant rate after 24 hr. Dose-response studies indicate that a maximal 3-fold increase in glucose utilization to 3 μmoles/24 hr per 10^6 cells is induced by T3 at concentrations of 3×10^{-8} mole/liter or greater. Half-maximal induction occurs at 4×10^{-9} mole/liter. Similar dose-response studies with T4 demonstrate that half-maximal induction occurs at 1.8×10^{-7} mole/liter. Iodothyronines inactive in vivo are also inactive in this system. After 12–15 hr of incubation with 3×10^{-8} M T3, the rate of uridine-^{3}H incorporation into RNA increased 2- to 4-fold. This is rapidly followed by an increase in the rates of incorporation of thymidine-^{3}H into DNA and of amino acid-^{14}C into cell protein. The population doubling time is 40–50 hr for cells incubated with 3×10^{-8} M T3 compared to 110–130 hr for cells incubated with hypothyroid media. At each comparative stage of growth (lag, exponential, and stationary phase), the rate of glucose utilization expressed as nanomoles per hour per microgram DNA was always greater in cells incubated with T3. These results indicate that thyroid hormones can control the rate of energy production in cell culture. These changes in energy control appear, in turn, to regulate the rate of cell replication. (Research supported by Grant 1 K04 AM 46546-01 from NIH and P-595 from ACS.)

279. Bone and Intestinal Response to Vitamin D in Anticonvulsant-Induced Osteomalacia. R. P. SANTANGELO,* S. M. FIDLER,* J. F. MACKIN,* AND J. J. CANARY,** Washington, D. C. and Bethesda, Md.

Recent observations have demonstrated an increased incidence of osteomalacia in patients on long-term anticonvulsant therapy. Drug-mediated liver enzyme induction leading to an increased inactivation of vitamin D metabolites has been suggested as the operant mechanism. We evaluated the effect of vitamin D_2 therapy on the chemical composition of

11th rib cortical bone, ^{47}Ca radionuclide retention, and appropriate serum and urinary variables in a 55-yr-old woman with severe bone pain on 450 mg diphenylhydantoin per day and 240 mg of phenobarbital per day for a period of 19 yr. Pre-D bone biopsy, roentgenograms, response to ^{40}Ca infusion, and appropriate serum and urinary chemical variables were consistent with a combined osteomalacicoteoporotic state. During the study period, the patient was maintained on her anticonvulsants and a known calcium-hydroxyproline-free diet. Post-therapy, bone ash weight increased from 43.2 to 60.1%, ^{47}Ca radionuclide retention increased from 37% (low Ca intake) to 95% (high Ca intake), histologic evidence of osteomalacia disappeared in biopsy of contralateral rib, phosphate clearance decreased, and urinary hydroxyproline decreased after transient rise. Serum chemistries reverted to the normal range. The increase of bone ash weight, high intestinal retention of Ca, and remission of the clinical, chemical, and histologic abnormalities indicate that vitamin D therapy is effective in individuals with osteomalacia associated with long-term anticonvulsant therapy. High-dose anticonvulsants, when required, need not be decreased provided vitamin D supplementation is maintained.

280. The Role of the Islets of Langerhans in Potassium Homeostasis. F. SANTEUSANIO,* G. R. FALOONA,* J. P. KNOCHEL,* AND ROGER H. UNGER,** Dallas, Tex.

K^+ stimulates insulin release in vitro and insulin lowers extracellular K^+ in vivo, suggesting a feedback relationship. Studies were designed to determine if increased serum K^+ stimulates insulin and/or glucagon secretion in vivo, and, if so, their possible roles in K^+ homeostasis. KCl (4 mEq/kg per hr) was infused intravenously for 60 min in 12 conscious dogs. Within 20 min, as serum K^+ reached 5.9 mEq/liter (SEM ±0.2), both hormones increased in all dogs; at 60 min K^+ averaged 7.7 (±0.2), insulin had risen 30 μU/ml (±7), glucagon 80 pg/ml (±14), and glucose 7 mg/ml (±2). Serum K^+ increment (KI) per mEq infused averaged 0.84 (±0.07) and postinfusion K^+ disappearance (KD) averaged 2.5 mEq/liter per hr (±0.2). The role of each hormone was individually examined by preventing secretion of the other. To prevent kaliogenic glucagon secretion, hyperglycemia averaging 150 mg/100 ml was induced by infusing glucose (15 mg/kg per min), before and throughout the KCl infusion. Now KCl increased insulin 20 μU/ml (±9) and, with hyperglucagonemia prevented, glucose declined 41 mg/100 ml (±6) in 60 min; KR and KD were unchanged. To prevent kaliogenic insulin secretion, 11 dogs were alloxanized and controlled with insulin before the study. In these dogs KCl dose was necessarily reduced to < 3 mEq/kg to avoid EKG changes; KI was 1.41 (±0.2) and KD 1.63 (±0.2), both significantly changed ($P < 0.001$; $P < 0.05$). Glucagon rose 292 pg/ml (±91) and, with hyperinsulinemia prevented, glucose rose 60 mg/100 ml (±34). In conclusion: (*a*) K^+ stimulates insulin and glucagon; (*b*) without glucagon, kaliogenic hyperinsulinemia causes hypoglycemia but K^+ is handled normally; and (c) without insulin, K^+ tolerance and disappearance fall markedly and exaggerated kaliogenic hyperglucagonemia causes hyperglycemia. This suggests that normally hyperkalemia elicits its own "self-treatment" with endogenous glucose and insulin, insulin increasing K^+ tolerance and glucagon providing enough glucose to prevent hypoglycemia. (Supported by NIH grant.)

281. Disorders of Bile Formation in Essential Fatty Acid Deficiency. I. J. SARFEH* AND J. A. BALINT,** Albany, N. Y.

Essential fatty acid-deficient (EFAD) hamsters develop cholesterol gallstones and have diminished biliary excretion of lecithin and taurocholate (TC). These defects of biliary excretion were investigated by studies of canalicular flow (erythritol-^{14}C clearance) and of hepatic excretory capacity for TC. for 12 wk 10 EFAD hamsters received fat-free diet containing 4% tripalmitin; 10 controls received similar diets with 4% safflower oil, or regular chow. Bile duct and inferior vena cava (IVC) were cannulated, and cystic duct and renal pedicles ligated. When awake 30 min later, a bolus of erythritol-^{14}C (2 μCi in 10 μ moles) was injected via the IVC, and then each animal was infused (IVC) with either 10 or 1.67 mM TC-^{3}H at 0.3 ml/hr for 4–5 hr. At the high infusion rate 64±3% of total infused TC-^{3}H radioactivity was recovered in bile of controls and 40±5% in EFAD hamsters ($P < 0.001$). All TC-^{3}H radioactivity not found in bile was present in the livers. Colorimetrically determined biliary TC excretion rates were 2.2±0.4 and 1.5±0.2 μmoles/hr, respectively ($P < 0.001$). At the low infusion rate biliary recovery of infused TC-^{3}H radioactivity approached 90% in both groups at 3, 4, and 5 hr, but was significantly reduced in EFAD hamsters at 1 and 2 hr. At both TC infusion rates bile: plasma ratios of erythritol-^{14}C were 0.98±0.04 in controls and 1.0±0.05 in EFAD animals, while canalicular flow was 0.04±0.009 and 0.024±0.005 ml/hr per g liver, respectively ($P = 0.008$). In EFAD animals the bile salt-independent fraction of canalicular flow was 1/2 to 1/3 that of controls. These data indicate that there is reduction of both bile salt-dependent and -independent fractions of canalicular flow, as well as impaired bile salt (TC) excretion in EFAD hamsters. These changes may contribute to gallstone formation. (Supported by grant from NIH.)

282. Exaggerated Natriuresis in Glomerulonephritis. ROBERT SCHACHT,* JOHN M. STEELE,* AND DAVID S. BALDWIN,** New York.

We have described a defect in sodium excretion in essential hypertension and have proposed that the phenomenon of exaggerated natriuresis (EN) may reflect the mechanism by which hypertensives nevertheless maintain sodium homeostasis. In the course of glomerulonephritis, sclerosis of glomeruli occurs and filtration is reduced in some nephrons even before overall filtration rate is affected, yet sodium balance is achieved. To examine the mechanism for sodium handling in glomerulonephritics we have studied their response to acute saline loading. After a liter infusion of 2.5% saline (20 ml/min), 11 of 13 normotensive poststreptococcal glomerulonephritics demonstrated EN with a mean sodium excretion of 2126 mEq/min. Control filtration rate ranged from 26 to 122 ml/min and increased an average of 15%. Neither the occurrence nor the magnitude of natriuresis was related to the extent of reduction in filtration rate. In five nephritics measurements were made of systemic and intrarenal hemodynamics during saline loading. Cardiac output increased 43%; no increase in arterial pressure or wedged

renal vein pressure occurred. Having observed little change in cardiac output or filtration rate and a marked increase in wedged renal vein pressure in hypertension, we have attributed EN to decreased tubular reabsorption of sodium resulting from transmission of systemic pressure to peritubular capillaries. In contrast, EN in glomerulonephritis appears to be related to increased cardiac output, decreased renal resistance, and increased filtration rate. The occurrence of EN in the glomerulonephritic, even when filtration rate is not reduced, suggests adaptation to a defect in sodium excretion. The two different physiologic responses accompanying EN in essential hypertensives and normotensive glomerulonephritics may reflect distinctive mechanisms for day-by-day sodium balance. (Research supported by grants from NIH and NYHA.)

283. Vitamin D and the Intestinal Transport of Zinc. DAVID SCHACHTER AND SZLOMA KOWARSKI,* New York.

Vitamin D is required for optimal transport of calcium via an active cation pump maximal in the proximal duodenum of the rat. Experiments with everted intestinal sacs in vitro demonstrate a similar influence of the vitamin on zinc transfer via an intestinal mechanism distinct from that for calcium. Rat intestinal sacs were incubated in an isotonic medium containing carrier Zn plus ^{65}Zn in the mucosal ambient medium, and mucosal uptake and transfer to the serosal surface were estimated. With initial concentrations below 1 mM Zn much of the cation taken up at the mucosal surface remained within the sac tissue after 1–1½ hr of incubation. With initial concentrations from 1 to 3 mM Zn an increasing fraction was transferred to the serosal surface. Serosal transfer was inhibited by iodoacetate, 2,4-dinitrophenol, cyanide, and incubation under N_2. Optimal transfer required a metabolizable hexose in the medium; the transfer was reduced by hexoses which increase the positive electrical potential of the serosal surface relative to the mucosal surface. Zn transfer dependent on cellular metabolism was rate limited and maximal in the jejunum, in contrast to the localization of Ca transport in the duodenum. Sacs prepared from vitamin D-depleted rats transferred less Zn than sacs from repleted animals. The effect of the vitamin was observed maximally with jejunal sacs. Whole particulate preparations of intestinal mucosa were assayed for ATPase and alkaline phosphatase dependent on divalent cations. Vitamin D increased both enzymatic activities dependent on Ca, Zn, Mg. Co, and Mn. The results suggest that vitamin D participates in the regulation of a number of cation transport mechanisms which may involve ATPase or alkaline phosphatase activities. (Research supported by Grant AM-01483 from NIH.)

284. On the Mechanism of Isolated Hypoaldosteronism. M. SCHAMBELAN,* J. R. STOCKIGT,* R. D. COLLINS,* AND E. G. BIGLIERI,** San Francisco, Calif.

Isolated hypoaldosteronism is a rare disorder characterized by hyperkalemia in association with subnormal aldosterone but normal cortisol production. While some infants with this syndrome have a defect in the final steps of aldosterone synthesis, the mechanism in adults remains uncertain; a primary defect in renin secretion could be present. Six patients (41–82 yr) were studied. All were normotensive and had hyperkalemia (6.2–6.8 mEq/liter) that was corrected by mineralocorticoid replacement. Creatinine clearance ranged from 32 to 72 ml/min. Urinary steroids were determined by double-isotope dilution techniques. Plasma renin activity (PRA), concentration (PRC), and renin substrate (RS) were measured by radioimmunoassay of angiotensin I. On 120 mEq sodium intake aldosterone excretion was reduced (3.1 ± 0.7 [SE] $\mu g/24$ hr, $n=4$–17) and showed a subnormal response to salt restriction (8.2 ± 1.9, $n=20$–67). Urinary excretion of tetrahydro-deoxycorticosterone, -corticosterone, and 17-hydroxycorticoids was normal and showed a prompt and sustained increase in response to 3 days of ACTH, whereas subnormal aldosterone levels increased only transiently. Supine PRA was reduced (1.3 ± 0.3 ng/ml per 3 hr, $n=5.5\pm0.9$) and failed to increase normally with upright posture (2.0 ± 0.4, $n=13.9\pm3.2$) or salt restriction (4.0 ± 0.6, $n=12.1\pm1.2$). PRA did not increase further after intravenous furosemide, 20 mg, during salt restriction in five of six and after correction of hyperkalemia with exchange resins in two of two. Supine PRC was subnormal (1.17 ± 0.08 ng/ml per hr, $n=8.35\pm1.48$) and remained so during the maneuvers, whereas RS was not decreased. Intuitively, primary mineralocorticoid deficiency should result in hyper-reninemia. Two patients with Addison's disease on glucocorticoid replacement had elevated supine PRA (78 and 156) that increased further with upright posture and salt restriction. We conclude that isolated hypoaldosteronism may result from a primary defect in renin secretion. (Work supported by NIH AM-06415 and HL-11046.)

285. Persistence of Hapten-Antibody Complexes in the Circulation of Immunized Animals after a Single Intravenous Injection of Hapten. DONALD H. SCHMIDT,* BETTE M. KAUFMAN,* AND VINCENT P. BUTLER, JR., New York.

To study the fate of a low molecular weight antigen (hapten) in the circulation of animals whose sera contain antibodies specific for that low molecular weight antigen, a single injection of digoxin-^3H (0.4 mg/kg) was administered intravenously to 12 rabbits. Seven animals (six nonimmunized and one immunized with bovine serum albumin) served as control animals. In five rabbits which had been immunized with a digoxin-bovine serum albumin conjugate and whose sera contained digoxin-specific antibodies, the mean 24-hr serum digoxin concentration was 8440 ng/ml (control: 84 ng/ml) and the mean serum concentration 10 months after the single injection of digoxin-^3H was 203 ng/ml. In digoxin-immunized rabbits, less than 10% of the digoxin-^3H was excreted in the first 10 days (control: 76% recovered in urine and feces) and the biological half-life of digoxin, as calculated from serum digoxin-^3H disappearance curves, varied from 1 to 3 months (control: 3.5 days). In sera of digoxin-immunized rabbits, more than 90% of the circulating digoxin-^3H was immunoglobulin bound, as determined by the double-antibody method. It is concluded that the biological half-life of a hapten may be markedly prolonged when the hapten is bound to specific antibody. The persistence of antibody-hapten complexes in the circulation suggests that these complexes may not be deposited in tissues and raises the possibility that low molecular weight determinants may be capable of preventing or reversing deposition of immune com-

plexes, containing macromolecular antigens, in the tissues of experimental animals and man. (Research supported by grants from NIH, New York Heart Association, American Heart Association.)

286. The Common Activation and Regulation of the Coagulation, Fibrinolytic, and Kinin-Generating Pathways in Human Plasma. ALAN D. SCHREIBER,* ALLEN P. KAPLAN,* AND K. FRANK AUSTEN, Boston, Mass.

Activated Hageman factor acts upon three unique plasma substrates to initiate three effector pathways of tissue injury: coagulation by converting pre-PTA to PTA; fibrinolysis by activating the precursor of the plasminogen proactivator (pro-PA) to a plasminogen activator (PA); and kinin generation by converting prekallikrein to kallikrein. Both intact activated Hageman factor (mol wt 110,000) and the Hageman factor fragments (mol wt 33,000) produced by plasmin digestion are active upon each of the above proenzymes. Three circulating protein inhibitors, C1INH, α_1-antitrypsin, and α_2-macroglobulin (α_2M), were isolated in highly purified form from Hageman-deficient plasma. The ability of the C1INH to inhibit the bradykinin-generating activity of kallikrein, the fibrinolytic activity of plasmin, and the clotting activity of PTA was confirmed. A time course analysis of the interaction of the C1INH with purified Hageman factor fragments demonstrated progressive inhibition of the activation of prekallikrein, pre-PTA, and pro-PA at a concentration which gave minimal inhibition of plasmin and no inhibition of kallikrein. The C1INH failed to inhibit the activation of plasminogen by PA. α_2M and α_1-antitrypsin did not inhibit the activity of the Hageman factor fragments upon any of its three proenzymes. In addition to the known inhibitory activity of α_2M on plasmin and kallikrein and the inhibitory activity of α_1-antitrypsin on plasmin, α_1-antitrypsin was also found to inhibit PA. Although regulation of coagulation, fibrinolysis, and kinin generation occurs at multiple points, the ability of the C1INH to inactivate the Hageman factor fragments provides a critical control mechanism at the common initiating enzyme of each pathway.

287. Abnormal Vacuole Formation in Erythrocytes from Patients with Hereditary Spherocytosis (HS). STANLEY L. SCHRIER, ISAAC BEN-BASSAT,* AND KLAUS BENSCH,* Stanford, Calif. (introduced by David A. Rytand).

The process of membrane internalization leading to in vitro vacuole formation in erythrocytes and in resealed ghosts provides an opportunity to study the interrelationship between Ca^{++}, Mg^{++}, and ATP and membrane events. Since these interactions are at the focus of the presumed genetically determined defect in hereditary spherocytosis (HS) we measured vacuole formation in erythrocytes and resealed ghosts in 15 patients with HS representing nine families, using a quantitative radioisotopic assay based on the membrane binding of vitamin B_{12}-^{57}Co. Vacuole formation in erythrocytes requires ATP plus addition of primaquine, hydrocortisone, vinblastine, or chlorpromazine. Membrane internalization in resealed ghosts is induced by addition of 2–10 mM Mg^{++} ATP and is blocked by 10–20 mM Ca^{++}. Normal and HS erythrocytes were incubated with primaquine and hydrocortisone and the extent of vacuole formation in HS erythrocytes was expressed as per cent of normal. Resealed ghosts from normal and HS patients containing from 2.5 to 10 mM Mg^{++} ATP were incubated with 0–20 mM Ca^{++}. Mean vacuole formation in HS erythrocytes was 25% of normal with 10 mM hydrocortisone, and 44% of normal with 1.5 mM primaquine. Vacuole formation within HS families was consistent, families showing either half-normal or essentially no vacuole formation. Surprisingly, vacuole formation in HS resealed ghosts was normal, as was the titered Ca^{++} inhibition of vacuole formation. Three patients with spherocytosis associated with autoimmune hemolytic anemia formed erythrocytic vacuoles poorly with 10 mM hydrocortisone (0, 7, and 10%) and their resealed ghosts formed almost no vacuoles. We propose that the spherocytic shape alone partly accounts for decreased erythrocytic vacuole formation, that HS encompasses several diseases with defects involving a block in association of Mg^{++} ATP with the inner membrane, and that resealing ghosts with Mg^{++} ATP circumvents this block. (Work supported by Grant No. R1 AM 13682 from NIH.)

288. Visualization of Regional Ventilation. ROGER SECKER-WALKER,* REXFORD HILL,* JOANNE MARKHAM,* AND E. JAMES POTCHEN, St. Louis, Mo. (introduced by E. James Potchen).

Regional ventilation has been measured during the wash-in and wash-out of Xenon-133, using a Pho-Gamma III Scintillation camera interfaced to a PDP 12/A computer. Xenon-133 was delivered from a dual bag-box system, and tidal volume and respiratory frequency were recorded by a wedge spirometer. The subject, seated with his back to the gamma camera, breathed air containing ^{133}Xe (1 mCi/liter) for the wash-in and air alone for the wash-out. Images were collected and stored on magnetic tape under program control, using short time frames while the count rate was changing rapidly and longer ones when the count rate was more stable, or low. The data were corrected for background activity, nonuniform response of the gamma camera and dead-time loss. Ventilation was calculated as the fractional exchange of air per second for each of the elements in the lung image, using the height/area approach. As Xenon-133 entered the blood and tissues during the study, a tissue background correction was incorporated into the calculation program, so that advantage could be taken of the sensitivity of the wash-out phase to regions of impaired ventilation. The validity of the tissue background correction had been determined by direct measurement in patients who had undergone pneumonectomy, and by comparison with figures for ventilation calculated from the wash-in, and from prolonged wash-out curves. The results are presented as: (*a*) Grey-scale "functional images" of the fractional exchange of air, of the distribution of tidal breathing, and of lung volume; and (*b*) figures for each of these values for selected regions in each lung. Examples will be presented from more than 120 studies and the clinical implications discussed. (Research supported by grant from AEC.)

289. Secondary Phosphoglycerate Kinase Blockade: the Cause of Deficient Na^+ Pumping in Pyruvate Kinase (PK)-Deficient RBC. GEORGE B. SEGEL,* STEPHEN A. FEIG,*

AND DAVID G. NATHAN, Boston, Mass. (introduced by Charles A. Janeway**).

PK-deficient RBC, with markedly reduced lactate production, demonstrate a severe impairment of ouabin-inhibitable (active) Na^+ transport, whereas active K^+ transport is increased. The metabolic basis of this dissociation and the role of phosphoglycerate kinase in Na^+ pumping (*Blood.* 1971. **38:** 832) were investigated in these cells. Pk-deficient RBC were incubated overnight at 4°C to increase the internal Na^+ concentration and permit examination of the response of the Na^+ and K^+ pumps to that physiologic stimulus. A twofold increase in internal Na^+ concentration, a threefold increase in total triose, and a 75% decrease in ATP occurred. When these stored cells were incubated at 37°C for 1 hr, lactate production immediately increased threefold, and Na^+ pumping increased sevenfold, while K^+ pumping declined slightly. In sharp contrast, fresh PGK-deficient RBC incubated at 37°C exhibited increased lactate production and total triose concentration. However, Na^+ pumping was reduced 50%, whereas K^+ pumping was normal. After overnight storage at 4°C to increase internal Na^+ and subsequent incubation at 37°C, PKG cells maintained lactate production, but had a paradoxical further decline of Na^+ pumping to 25% of normal. K^+ pumping was appropriately increased. These studies indicate that K^+ pumping in PK and PGK-deficient RBC is independent of the rate of flow of metabolites through PGK. In contrast the reduced Na^+ pump in PK cells is due to a secondary blockade of PGK. This blockade can be temporarily overcome by cold storage, which induces a change in the relative concentrations of phosphorylated intermediates leading to increased flow of metabolites through and hence greater production of ATP by PGK. This in turn permits activation of the Na^+ pump. However, this maneuver fails to alter the Na^+ pump when PGK is itself deficient.

290. Extra Adrenal Effect of Adrenocorticotropic Hormone (ACTH) upon Fibrinogen Synthesis. URI SELIGSOHN,* SAMUEL I. RAPAPORT,** AND PAUL R. KUEFLER,* Los Angeles, Calif.

Large doses of adrenocorticotropic hormone (ACTH) reportedly stimulate fibrinogen synthesis in rabbits. We studied this to clarify its mechanism and possible physiological implications. In 42 male rabbits mean fibrinogen level rose from 349±12 mg/100 ml to 470±12 mg/100 ml 24 hr after 12.5–18 U/kg of ACTH ($P < 0.01$). The level did not rise significantly in 30 controls. The ACTH effect varied inversely with the rabbit's initial fibrinogen level ($r = -0.4$, $P < 0.01$) and was attenuated in 6 rabbits given ACTH for a second time 2 or 5 days later. It was also dose dependent; 8 rabbits given up to 0.8 U/kg had no rise and 17 rabbits given 0.8–12.0 U/kg had intermediate rises. Fibrinogen rises could not be related to changes in plasma corticosterone levels (9 animals). When ^{75}Se-methionine was given 4–6 hr after 12.5–18 U/kg ACTH (16 animals) its incorporation rate into fibrinogen was 2.4 times greater than in 17 control animals. Adrenalectomy (4 animals) or sham-operation (4 animals) did not alter significantly the effect of ACTH upon fibrinogen level or ^{75}Se-methionine incorporation. Incorporation of ^{75}Se-methionine into other plasma proteins, separated by cellulose-acetate electrophoresis, was also studied in rabbits given ACTH (11 animals), 25 mg of hydrocortisone, cortisone or corticosterone (13 animals), or saline (7 animals). When compared with the saline controls, the ACTH-treated animals showed diminished radioactivity in α_2- and β_1-globulins whereas the glucocorticoid-treated animals showed greatly increased radioactivity in these fractions. These data establish that the ACTH effect upon fibrinogen synthesis is independent of the adrenal gland and partially dependent upon the animal's initial fibrinogen level. (Research supported by Grant HE-06128-11, NIH.)

291. Triiodothyronine: the Modulator of Pituitary Responsiveness to Thyrotropin-Releasing Hormone. LOUIS SHENKMAN,* TERUNORI MITSUMA,* ARAYA SUPHAVAI,* AND CHARLES S. HOLLANDER,* New York (introduced by H. Sherwood Lawrence**).

Thyroid hormone excess, caused by hyperthyroidism or administration of exogenous thyroid hormone, inhibits pituitary response to thyrotropin-releasing hormone (TRH). To assess the relative roles of triiodothyronine (T3) and thyroxine (T4) in modulating this response, we have determined TRH responsiveness in naturally occurring and pharmacologically induced situations in which either T3 or T4 is elevated. T3 and TSH were measured by radioimmunoassay: T4 by radioimmunoassay and competitive protein-binding analysis. 15 untreated hyperthyroid patients with elevated T3 and T4 levels showed no increase in thyrotropin (TSH) after 400 μg of TRH intravenously. After 10 days of therapy with propylthiouracil, 10 had normal T3 and T4 levels; 5 had persistent T4 elevations but normal T3 concentrations. Normal TRH responsiveness was restored in all 15 and the magnitude of the TSH rise was similar in both groups. Mean TSH rose from 0.8±0.4 to 11.2±1.6 μU/ml in patients with normal T3 and T4 and from 1.1±0.3 to 11.6±1.2 μU/ml in those with persistent T4 elevations and normal T3 levels. In five patients with T3 toxicosis who had high T3 levels (240, 260, 300, 360, and 400 ng/100 ml, normal range 96–172 ng/100 ml) and normal T4 and free T4 concentrations, TSH failed to rise after TRH. Two received propylthiouracil and, concomitant with the fall of T3 to normal, TRH responsiveness returned. In conclusion, the data suggest that T3 may well be the major modulator of pituitary responsiveness to TRH. (Research supported by Grants FR-96, 2R0IAM14314-02, and 1F03AM-5156-01 from NIH.)

292. Adrenergic Receptors and the Release of Parathyroid Hormone (PTH). LOUIS M. SHERWOOD* AND MINORU ABE,* Boston, Mass. (introduced by Howard H. Hiatt).

Our recent studies suggest that the release of parathyroid hormone (PTH) is stimulated by cyclic 3',5'-AMP (cAMP) and theophylline, and that adenyl cyclase is an intermediate in the effects of calcium on hormone secretion. In order to determine whether stimulation of hormone release might also involve alpha- or beta-adrenergic receptors, the effects of catecholamines on PTH secretion were investigated. After being preincubated for 2 hr in Krebs-Ringer bicarbonate buffer, normal bovine parathyroid glands were transferred

to fresh buffer containing low (0.5 mM) or high (2.0 mM) calcium concentrations and the agent to be tested. PTH was measured by radioimmunoassay of sequential samples during incubation. At 1 hr, the release of hormone in 0.5 mM calcium buffer was stimulated significantly by 10^{-4} M epinephrine (284±17 ng PTH/mg tissue protein vs. 215±10 ng/mg for control tissue) and 10^{-4} M isoproterenol (298±6 ng/mg) but not by 10^{-4} M norepinephrine (218±10 ng/mg). Addition of 10^{-4} M propranolol blocked the beta-stimulated effect. Thyrocalcitonin at a concentration of 100 mU/ml also stimulated hormone secretion (326±30 ng/mg), while 2.0 mM calcium had a suppressive effect (127±20 ng/mg). Measurements of cAMP in the incubation medium showed parallel results: 0.5 mM calcium, 25±2 pmoles cAMP per mg; 2.0 mM calcium, 16±2 pmoles/mg; isoproterenol, 65±11 pmoles/mg; and thyrocalcitonin, 45±3 pmoles/mg. In high-calcium experiments, release of PTH was also stimulated by epinephrine, isoproterenol, and thyrocalcitonin. These observations suggest that stimulation of beta-adrenergic receptors in parathyroid tissue causes increased hormone secretion, and that adenyl cyclase might mediate this effect. The close anatomical location of the thyroid and parathyroid glands and the production by parafollicular cells of both catecholamines and thyrocalcitonin suggest the possibility of a local noncalcium-mediated mechanism for the regulation of parathyroid secretion. (Research supported by grants from the NIH and John A. Hartford Foundation.)

293. Lipid Changes and Bacterial Killing in Phagocytosis of Peroxide and Nonperoxide-Producing Pneumococci. S. Shohet, J. Pitt,* R. Baehner, and D. Poplack,* San Francisco, Calif.

Chronic granulomatous disease (CGD) leukocytes are defective in both generating hydrogen peroxide and in killing ingested bacteria. Bacteria which themselves produce H_2O_2 are killed by CGD cells, whereas bacteria deficient in H_2O_2 secondary to catalase activity are not. This suggests a causal relationship between H_2O_2 production and killing. Mutant pneumococci deficient in H_2O_2 enabled us to test this hypothesis directly. Additionally, labeling bacterial lipids enabled us to follow lipid peroxidation in the phagocytic complex. Both peroxide ⊕ and ⊖ pneumococci were grown with arachidonic acid-^{14}C and palmitic acid-^{3}H to label their lipids with unsaturated and saturated fatty acids. CGD and normal granulocytes were incubated with each bacteria; ingestion, killing, and fatty acids of the cell-bacteria complexes were followed. $^{14}CO_2$ release from glucose-1-^{14}C was independently followed to measure glucose oxidation during phagocytosis. Ingestion was similar in all cell-bacteria combinations. CGD cells killed the H_2O_2 ⊕ pneumococci much more effectively than the H_2O_2 ⊖ mutant (96% vs. 47% reduction in viable cells). Normal cells killed both peroxide ⊕ and ⊖ pneumococci effectively (98% and 94% reduction in viable cells). Loss of arachidonic acid-^{14}C (28–46%) consistent with lipid peroxidation was observed in all normal cells and in CGD cells with peroxide ⊕ pneumococci. However, no loss of arachidonic acid-^{14}C occurred in CGD cells with peroxide ⊖ pneumococci. No loss of palmitic acid-^{3}H occurred in any cell-bacteria combination. Glucose oxidation was impaired 65–85% for CGD cells ingesting peroxide ⊖ compared to peroxide ⊕ pneumococci. These data directly support the hypothesis that bacterial killing is dependent upon an intact H_2O_2-generating system in the leukocyte-bacterial complex. They also suggest that bacterial peroxidation occurs during phagocytic bacterial killing. (Supported by NIH grants.)

294. Heterophile and Virus-Specific Antibody Responses in Squirrel Monkeys after Inoculation of Autologous Epstein-Barr Virus-Transformed Lymphoblasts. Thomas Shope* and George Miller,* New Haven, Conn. (introduced by Dorothy M. Horstmann**).

Squirrel monkey blood leukocytes exposed in vitro to a cell-free extract of Epstein-Barr virus (EBV) transform into continuous lymphoblastoid cell lines which contain EBV antigens. In order to evaluate the potential of these transformed cells to cause disease and stimulate immune responses in autologous animals, three EBV antibody-negative adult squirrel monkeys were given intravenous inoculations of approximately 10^9 autologous transformed cells. An EBV-negative uninoculated control monkey was observed in parallel. Differential white blood cell counts, heterophile agglutinins, and EBV antibodies were measured weekly. No apparent illness or hematologic abnormality consistent with infectious mononucleosis was observed; however, all inoculated monkeys developed heterophile responses and EBV-specific antibody. The heterophile response appeared between 1 and 2 wk after inoculation, peaked within a week, and fell to low levels or disappeared by 7 to 10 wk. Sera absorbed with guinea pig kidney antigen retained the agglutinin but sera absorbed with beef erythrocyte antigen did not. Immunofluorescent (IF) and complement-fixing (CF) antibodies appeared between 2 and 4 wk after inoculation, peaked within 2 wk of appearance, then fell to low or undetectable levels by 5–8 wk. Heterophile, IF, and CF antibody did not appear in the uninoculated animal. Attempts to recover EB virus from washed leukocytes of the test animals are in progress. The production of a heterophile response and EBV-specific antibodies by inoculation of EBV-transformed lymphoblasts is an encouraging first step in the study of EBV-induced infection of animals. (Supported by a grant from the Jane Coffin Childs Memorial Fund for Medical Research [JCC-257].)

295. Radioimmunoassay (RIA) for Australia Antigen (Au) in Blood Donors and Liver Disease Patients. James Shorey* and Burton Combes, Dallas, Tex.

Australia antigen (Au) was measured by a radioimmunoassay (RIA) method in which test serum (2 μl) was incubated with hemophiliac anti-Au serum for 20 hr; ^{125}I-labeled Au was then added and 4 hr later antibody-bound Au was precipitated by rabbit anti-human globulin plus polyethylene glycol. In the standard curve prepared from an Au(+) serum, (arbitrary titer 2000 Au U/2 μl), Au was clearly detectable in serum diluted 2000 × (1 U/2 μl). RIA was 120 times more sensitive than a standard counter-immunoelectrophoresis method (CIEP) which detected Au in the same serum at maximal dilution of 1:16. However, the apparent RIA titer of Au in CIEP (+) sera varied

considerably, and some CIEP (−) sera contained appreciable titers of Au by RIA. Thus the RIA/CIEP sensitivity ratio varied widely. The possibility that this is the consequence of different Au-antigenic subtypes, antibody specificities, or Au antigen-antibody complexes in test sera is currently being investigated. Establishment of a precise cut-off point clearly distinguishing Au (−) sera from sera containing very small amounts of antigen has been difficult. Because 20 of 21 CIEP (+) sera gave RIA titers of > 2 U, and because CIEP (−) sera with RIA titers > 2 U were clearly distinguishable from either normal controls or most patients with various liver diseases, we considered sera containing the equivalent of 2 U/2 μl or greater as being Au positive. Sera from 200 consecutive blood donors were all CIEP negative and containing < "1 U" by RIA. 3 of 40 CIEP (—) uncomplicated acute hepatitis patients were RIA (+). Only 1 of 16 patients with fulminant "viral" hepatitis was CIEP (+) while 5 were RIA (+). Sera from 13 primary biliary cirrhosis patients and 20 halothane hepatitis patients were negative by both tests, providing strong evidence that neither disease is due to Au hepatitis virus. The liver disease sera are being tested by RIA modified for detection of antibody, and by which 15% of 200 donor sera have been found to contain antibody. (Research supported by grants from NIH and VA.)

296. Evidence for X-Linked Inheritance of Ornithine Transcarbamylase Deficiency. ELIZABETH M. SHORT,* HAROLD O. CONN,* PHILIP J. SNODGRASS,* AND LEON E. ROSENBERG, New Haven, Conn. and Boston, Mass.

The major cause of ammonia intoxication in children is an inherited defect of any one of the five urea cycle enzymes. Hyperammonemia due to *partial deficiency* (< 25% of normal) of one of these enzymes, ornithine transcarbamylase (OTC), has been reported almost exclusively in females (9 of 10 patients). The present study of four families was undertaken to explain this unusual sex ratio and the mode of inheritance of OTC deficiency. In two pedigrees, male infants, who died of ammonia intoxication within the 1st week of life, had *complete absence* of hepatic OTC activity. Significantly, a brother of one proband and three maternal uncles of the second had died inexplicably in the neonatal period. Whereas the father of each proband had normal OTC activity, as determined by hepatic assay and/or a modified ammonia tolerance test, both mothers had partial OTC deficiency. The second proband also had a sister with partial OTC deficiency whose symptomatic hyperammonemia appeared at the age of 1 yr and was controlled by dietary protein restriction. In two other pedigrees, we found 1-yr-old girls with partial OTC deficiency and symptomatic hyperammonemia whose fathers *and* mothers had normal OTC activity. The striking clinical and biochemical differences noted between males and females with OTC deficiency, and the maternal inheritance noted in two pedigrees lead us to conclude that OTC is coded for by a gene on the X chromosome and that a mutation at this locus causes partial OTC deficiency in heterozygous females with unfavorable Lyonisation and complete absence of OTC activity in hemizygous, affected males. The failure to demonstrate maternal inheritance in the other two pedigrees is compatible with the high incidence of new mutations for X-linked traits or with favorable Lyonisation in carrier mothers.

297. Identification of Inorganic Pyrophosphate (PPi) in Human Platelets. DONALD C. SILCOX,* SERGIO JACOBELLI,* AND DANIEL J. MCCARTY, Chicago, Ill.

An isotope dilution method for quantification of inorganic pyrophosphate (PPi) in biological fluids and tissues has been developed by removal of inorganic orthophosphate (Pi) and protein, incubation with yeast inorganic PPiase, and measurement of reduced phosphomolybdate. PPi levels in normal plasma were ($\bar{X}\pm SD$) 2.80±1.1 μmoles/liter. Precision in duplicate analysis of normal plasma samples was ±10%. PPi levels in serum were 2- to 3-fold higher than those in plasma prepared from identical blood (e.g., 9.1 vs. 2.9 and 3.9 vs. 1.7). PPi levels were 2.33±0.68 mμmoles/10^8 platelets (range 1.45–3.2), sufficient to account for the plasma-serum difference. The presence of platelet PPi was confirmed by two additional methods, both using isotope dilution and washed platelets sonicated in the presence of PCA and ^{32}PPi tracer. (*a*) UDPG pyrophosphorylase to convert PPi to glucose-1-PO_4, phosphoglucomutase to convert this to glucose 6PO_4 and glucose-6-phosphatase to convert NADP to NADPH which was then read spectrophotometrically. Here PPi = 1.4 mμmoles/10^8 platelets. (*b*) Anion-exchange chromatographic separation of PPi after twice coprecipitating with calcium phosphate and passage over a cation exchange column to remove calcium and nucleotides; PPi = 2.8 and 4.5 mμmoles/10^8 platelets. Thrombin added to a suspension of platelets released PPi. Platelet neutral inorganic pyrophosphatase was also measured. PPi (mμmoles/10^8 cells) control pellet (CP) 1.39, control supernatant (CS) 0.00, thrombin pellet (TP) 0.57, thrombin supernatant (TS) 0.61; PPiase (μmole Pi/min per g protein) CP 3.1, CS 0.0, TP 3.5, TS 0.61; β-glucuronidase (units) CP 2.12, CS 0.09, TP 1.59, TS 0.96; protein (μg/10^8 cells CP 148, CS 46, TP 142, TS 69. These data indicate that platelets contain PPi in concentrations of 1–5 mμmoles/10^8 cells, about ½ the ATP concentrations reported by others. Its release on thrombin stimulation and its presence in a cell containing PPiase suggest that it is sequestered in a secretory granule. (Research supported by grants from NIH and The Arthritis Foundation.)

298. Effects of Dibutyryl Cyclic Adenosine 3′,5′-Monophosphate, Glucago, Isoproterenol, and *l*-Dihydroxyphenylaline on Renal Concentration in Man. TUSHAR K. SINHA,* CHARLES M. CLARK, JR.,* GARY L. ROBERTSON,* SUSAN AVERY,* AND NORMAN H. BELL, Indianapolis, Ind.

Studies in this laboratory have shown that porcine neurohypophyseal adenyl cyclase can be augmented by glucagon, isoproterenol, dopamine, and other neurohormones. To investigate the possibility that the adenyl cyclase system may be involved in vasopressin release, the effects of dibutyryl cyclic adenosine 3′,5′-monophosphate (DB-CAMP) and other agents on clearances of inulin (C_{In}) and free water (C_{H_2O}) were examined in hydrated normal subjects by standard clearance techniques. Subjects received 5% dextrose and water, 5 ml/min, throughout each study and for 1½ hr

before treatment. DB-CAMP (0.1 mg/kg per min for 1 hr), glucagon (10 μg/min for 1 hr) and *l*-dihydroxyphenylalanine (1 g by mouth) each consistently produced a negative C_{H_2O}. Isoproterenol (1 μg/min for 1 hr) was less effective in this regard and its effects were diminished by beta adrenergic blockade with propranolol. In none of the studies could the observed changes in urine volume (V) or C_{H_2O} be accounted for by variations in C_{In} or osmolar clearance. In contrast to the effects in normal subjects, DB-CAMP consistently increased V and C_{H_2O} in patients with diabetes insipidus. These changes were accompanied by increases in urine sodium and indicated an inhibition of proximal renal tubular sodium reabsorption. That DB-CAMP produced vasopressin release in the normal subjects was confirmed by radioimmunoassay of plasma vasopressin. It is suggested that vasopressin release may be influenced by the adenyl cyclase system and that secretion of the hormone is increased by beta adrenergic stimulation.

299. Heavy Chain Subclass of Human Anti-Platelet Autoantibodies. GREGORY W. SISKIND, PETER H. SCHUR, AND SIMON KARPATKIN, New York and Boston, Mass.

We have previously reported that by use of the platelet factor 3 (PF-3) immuno-injury test an anti-platelet activity could be demonstrated in the serum of approximately 65% of patients with idiopathic thrombocytopenic purpura (ITP) and 78% of patients with systemic lupus erythematosus (SLE). It was shown that the anti-platelet activity demonstrated in these conditions was an IgG autoantibody with platelet specificity. We have now further characterized these autoantibodies with respect to their distribution among the four known human IgG subclasses. This was determined by assaying the ability of antibodies specific for the individual subclasses to deplete the patient's serum of the anti-platelet activity demonstrated by the PF-3 test. It was found that the anti-platelet antibodies present in ITP are, within the limits of sensitivity of the method, restricted to the γG3 subclass in every one of 15 patients studied. In contrast, 9 patients with SLE who had anti-platelet antibodies were all found to have these antibodies present in three out of four or in all four γG subclasses. Five patients with drug-induced immunologic thrombocytopenia were studied. The anti-platelet antibodies which could be detected in the presence of the drug were of the γG1, γG2, and γG3 subclasses in four patients and of the γG2 and γG3 subclasses in one patient. Thus different distributions of anti-platelet autoantibodies occur in different clinical syndromes. The restriction of the anti-platelet antibody in ITP to the γG3 subclass which is normally present in relatively low concentration (4–7%) is particularly striking. (Research supported by grants from NIH, N. Y. Heart Association, John A. Hartford Foundation, N. Y. C. H.R.C.).

300. Vasopressin (VP) Kinetics in Man and the Rhesus Monkey Fetus Using Radioimmunoassay (RIA) Measurements. W. RONALD SKOWSKY* AND DELBERT A. FISHER, Torrance, Calif. (introduced by Joseph St. Geme, Jr.).

We have developed a radioimmunoassay (RIA) for measurement of arginine vasopressin (AVP) in human serum. Antibodies were produced by conjugating synthetic lysine vasopressin (LVP) to thyroglobulin, allowing the "tail" of LVP to be exposed. LVP was labeled with ^{125}I at two distinct sites, tyrosine of the "ring" and by conjugating iodotyrosine to the tail. Binding affinities of the two iodinated LVP's suggested that the early antibodies were directed largely toward the tail. With continued immunizations, cross-reactivity to AVP increased so that the antiserum currently is used in a dilution of 1:200,000 to RIA AVP with a sensitivity of 1.25 μIU/ml. Thyroglobulin or thyronines and oxytocin in 1000-fold excess do not cross-react. An effective serum extraction and concentration method employing Bentonite also was devised yielding 80% recovery from 4 ml of serum, increasing the RIA sensitivity to < 0.4 μIU/ml. The mean AVP level in normally hydrated ambulatory subjects is 1.42±0.09 (SEM) μIU/ml which increases to 10.0±1.8 μIU/ml after overnight dehydration. The mean AVP concentration in patients with nephrogenic diabetes insipidus is 4.4±1.2 μIU/ml, while in patients with inappropriate vasopressin (VP) secretion the level is greater than 35 μIU/ml. Kinetic studies were undertaken in water-loaded subjects using a pulse dose of unlabeled VP; the $t_{1/2}$ of vasopressin in serum was 6–10 min and the apparent volume of distribution (VD) 20–25 liters. Similar studies were conducted in the term rhesus monkey fetus; the results indicate a $t_{1/2}$ of 9–10 min and a VD of 1 liter. Thus, we have developed a sensitive RIA capable of measuring AVP in whole serum. Preliminary results in normals and in patients with abnormalities of AVP metabolism are in agreement with earlier bioassay data. (Supported by Grants HD 04270-04 and F03-AM43172 from NIH.)

301. Transfer of Rubella Virus Resistance from Rheumatoid to Rabbit Synovial Cells by Cell Fusion. CAROL SMITH* AND DAVID HAMERMAN,** Bronx, N. Y.

A number of differences have been observed between synovial cells in culture derived from rheumatoid and nonrheumatoid joints. In particular, the rheumatoid cells are resistant to lytic infection with rubella virus (RV), while nonrheumatoid controls show cytopathic effects and lysis within 14 days. A possible basis for this difference is the persistence of a microorganism in a noninfectious state within the rheumatoid cells. One might pursue this possibility experimentally by attempting to introduce the "agent" into susceptible cells by cell fusion. Inactivated Sendai virus was used as the factor to fuse cultured rabbit synovial cells with rheumatoid or control cells. Rabbit cell nuclei were labeled with thymidine-^3H for radioautographic identification. Heterokaryon formation in the fused cultures was found to be 2–4% between the rabbit and human cells. After 3–6 wk of growth and subdivision, the cultures contained only rabbit cells, as shown by growth characteristics and chromosomal analyses. When challenged with RV, four of five such cultures resulting from fusion of rabbit with rheumatoid cells showed resistance with little or no cytopathic effect up to 6 wk of observation and subculture; all four control cultures showed cell death and lysis within 14 days. Other control studies showed no transfer of RV resistance when rabbit and rheumatoid cells were merely cocultivated. No infectious organism has yet been isolated from the fused cultures, but the transfer of RV resistance by cell fusion indicates that addi-

tional methods should be applied in an attempt to demonstrate an agent in the rheumatoid synovial cells. (Research supported by grants from the NIH and the Arthritis Foundation.)

302. Inhibition of Thyrotropin (TSH) Response to Thyrotropin-Releasing Hormone (TRH) by Small Quantities of Thyroid Hormones. PETER J. SNYDER* AND ROBERT D. UTIGER, Philadelphia, Pa.

Inhibition of thyrotropin (TSH) release by chronic treatment with small quantities of triiodothyronine (T_3) + thyroxine (T_4) was evaluated by determining the serum TSH response to thyrotropin-releasing hormone (TRH) in normal and hypothyroid subjects. Response to TRH was determined before treatment and after each dosage of $T_3 + T_4$ had been given for 3–4 wk. Treatment of eight normal subjects with 15 μg T_3 + 60 μg T_4 reduced the maximum increase in serum TSH above base line (maximum ΔTSH) after 400 μg TRH from 15.2±2.7 (mean±SEM) to 3.7±1.5 μU/ml ($P < 0.01$) and after 25 μg TRH from 9.4±4.0 to 1.2±0.5 μU/ml ($P < 0.05$). Treatment of these subjects with 30 μg T_3 + 120 μg T_4 reduced the maximum ΔTSH after 400 μg TRH to 0.8±0.3 μU/ml ($P < 0.01$). To determine the effect of these dosages of $T_3 + T_4$ on serum T_3 and T_4 levels, these levels were measured repeatedly during a 24-hr period. The averaged 24-hr serum T_3 and T_4 levels before and during treatment with 15 μg T_3 + 60 μg T_4 and 30 μg T_3 + 120 μg T_4 were (T_3) 98±7, 129±10, and 181±7 ng/100 ml, and (T_4) 6.5±0.5, 6.5±0.4, and 7.9±0.3 μg/100 ml. Ranges of normal for serum T_3 and T_4 are 70–150 ng/100 ml and 5–12 μg/100 ml. Six primary hypothyroid patients were treated, sequentially, with 15 + 60, 22.5 + 90, and 30 μg T_3 + 120 μg T_4. For each patient there was one 7.5 μg T_3 + 30 μg T_4 increase in dosage which abruptly converted a maximum ΔTSH that was greater than, or at the upper limit of, normal (mean maximum ΔTSH, 33.0±8.6 μU/ml) to one that was subnormal (mean maximum ΔTSH, 1.8±0.5 μU/ml). This abrupt change occurred after the dosage was increased from 15 + 60 to 22.5 μg T_3 + 90 μg T_4 in three patients and from 22.5 + 90 to 30 μg T_3 + 120 μg T_4 in the other three patients. Concurrent with these six abrupt changes in TSH response, the mean serum T_3 level increased from 105±5 to 129±9 ng/100 ml and the mean serum T_4 level increased from 4.9±0.8 to 6.3±0.5 μg/100 ml. These data demonstrate the extreme sensitivity of TRH-induced TSH release to inhibition by the chronic administration of quantities of $T_3 + T_4$ which do not raise serum T_3 or T_4 levels above the normal range.

303. Lymphocyte Responses to Tumor-Specific Antigens in Patients with Malignant Melanoma and Results of Transfer Factor Therapy. LYNN E. SPITLER,* ALAN S. LEVIN,* M. SCOTT BLOIS,* WILLIAM EPSTEIN,* H. HUGH FUDENBERG, INGEGARD HELLSTROM,* AND KARL E. HELLSTROM,* San Francisco, Calif. and Seattle, Wash.

Lymphocyte responses to melanoma antigens were determined in patients with disseminated melanoma, localized melanoma, family members of patients with melanoma, and normal subjects. The ability of lymphocytes derived from these patients to undergo increased DNA synthesis and to produce migration inhibitory factor (MIF) in response to soluble melanoma antigens and their ability to kill plated melanoma cells in vitro was determined. Lymphocytes from seven patients with disseminated melanoma did not show increased DNA synthesis and only one showed MIF production; in contrast, lymphocytes from four patients with localized disease showed both responses. On the other hand, a cytotoxic effect of lymphocytes from both groups of patients was seen on cultivated melanoma cells. Seven of nine family members had immunity as demonstrated by one or more of the in vitro tests. Four patients with melanoma were treated with transfer factor from selected donors with immunity demonstrated by the in vitro tests. Three patients with widely disseminated disease showed no response. One patient who was regularly developing new skin metastases showed a spontaneous regression or response to transfer factor with regression of skin lesions, decrease in previously elevated urinary melanogens to normal levels, and conversion of lymphocyte transformation and has remained free of new lesions for 7 months under continuing therapy.

304. Randomized Trials of Physician Substitutes in Primary Care. WALTER O. SPITZER,* ROBIN S. ROBERTS,* MICHAEL GENT,* DAVID L. SACKETT,* JOHN C. SIBLEY,* DOROTHY J. KERGIN,* AND MAY A. YOSHIDA,* Hamilton, Canada (introduced by Alvan R. Feinstein**).

Two complementary randomized trials of physician substitutes are in progress in southern Ontario. A group of nurses performing traditional office functions in family medical practice have received innovative university-based training to assume most of primary care management as nurse practitioners. The associated physicians shift their role to general supervision and attention to difficult clinical problems referred by the nurse. The effects of this change are being tested in 16 family medicine practices during Spring, 1971–Summer, 1972. The first trial focuses upon effects on patients. In each of two practices, 270 families were randomly assigned to primary care from the nurse practitioner. The remaining 500 families in each practice continue with conventional care. The second trial concerns effects on physicians and nurses. In 14 practices, with physicians' support and commitment to participation, the nurses had applied for the new training. Seven applicants were randomly selected to receive the training and their corresponding practices became the experimental group, with the remaining nurses and practices retained as controls. The variates under assessment include the following in the patients, functional capacity, medical services utilization, acceptance of the nurse, and general satisfaction; in the nurses and doctors, alterations in clinical/nonclinical activities and professional attitudes; in quality of care, managerial strategy for "tracer conditions," medication, and referral decisions; and in the practice itself, growth and profitability. Only 5 of 540 families in the first trial refused assignment to a nurse practitioner and the augmented professional productivity has allowed the number of families in the two practices to be increased by over 20%. In both trials, the preliminary indicators of function, satisfaction, and acceptance have been favorable. In one practice of the second trial, the new technique was abandoned for reasons ascribed to financial and professional disadvantages.

305. Selective Deficiency of Granules Associated with Lysozyme and Lactoferrin in Human Polymorphs (PMN) with Reduced Microbicidal Capacity. J. K. SPITZNAGEL,* M. R. COOPER,* A. E. MCCALL,* L. R. DECHATELET,* AND I. R. H. WELSH,* Chapel Hill and Winston-Salem, N. C. (introduced by Carl W. Gottschalk**).

Defects in intraleukocytic killing have been associated with abnormal oxidative metabolism in human polymorphs (PMN), myeloperoxidase deficiency, and structurally abnormal PMN lysosomes (Chediak-Higashi syndrome). We now report a bactericidal defect associated with a selective granule abnormality. Defective intraleukocytic killing of *P. rettgeri, E. coli,* and α-streptococcus was observed in PMN from a 43-yr-old man with recurrent bacterial infections. In the defective PMN, lysozyme measured by lysis of *M. lysodeikticus* was less than half normal; lactoferrin, an antibacterial protein, was virtually undetectable by immunochemical assay. When homogenates of normal PMN are subjected to sucrose density centrifugation ($_0\int^t \omega^2 dt = 1.48 \times 10^{10}$ sec^{-1} with $R_{min} = 6.4$ cm and $R_{max} = 15.3$ cm, gradient volume = 50 ml, sucrose concentration linear 30–53%) a discrete band of granules with both lysozyme and lactoferrin is found at the level of 36–38% sucrose. This band was absent from homogenates of the patient's cells. Peroxidase-rich azurophil granules were present and recovered in the gradient at 46–48% sucrose. Normal values were obtained for alkaline phosphatase, O_2 utilization, hexose monophosphate shunt activity, iodination, and Nitroblue tetrazolium reduction. Electron microscopy demonstrated normal phagocytosis and normal degranulation of the azurophil's myeloperoxidase. Electron microscopy showed a reduction in total granules. (Research supported by AEC Grant AT-(40-1)-3628, NIAID Grant AI02430 and NIAID Grant AI09169.)

306. Human Peripheral Nerve Myelin: Qualitative and Quantitative Studies with Aging. NORTON SPRITZ, BARBARA GEYER,* AND HARBHAJAN SINGH,* New York.

The entire intraabdominal portion of femoral nerves were obtained from 14 people who died suddenly. After removal of collagen following treatment with glycine buffer (*Nature [London]*. 1968. **220**: 171) myelin (purity established by chloroform-methanol solubility and electron microscopy) was isolated quantitatively and purified in a cesium chloride continuous gradient. In eight people aged 23–42 yr, myelin content averaged 7.3±2.0 mg/g of nerve compared to six aged 60–75 yr in whom the average was 3.2±1.6 ($P < 0.001$). This striking difference was evident whether myelin content was related to whole nerve segment or unit nerve length, rather than to nerve weight, indicating that the lower values in the older group reflected an absolute rather than a relative decrease. Total nerve protein, cholesterol, and glycolipid did not appear to vary significantly with age. The density (ultracentrifugal flotation) and composition of myelin was essentially the same in both groups and resembled that found by O'Brien (*J. Neurochem.* 1967. **14**: 357) in ox spinal roots. Molar ratios of cholesterol : phospholipid : glycolipid were 3 : 3 : 1; and among the phospholipids, molar ratios of phosphatidyl choline : phosphatidyl ethanolamine : phosphatidal ethanolamine : phosphatidyl serine : sphingomyelin approached 2 : 1 : 4 : 2 : 5 in both age groups. Protein content was 35.3 and 34.5% respectively in the younger and older groups ($P > 0.5$), and Eng-Smith ratios 1.2 and 1.4. Decrease of myelin with aging as revealed by direct measurement may be the counterpart of altered neurophysiology with aging that is expressed by decreased nerve conduction velocity and loss of vibration sense. Whether the observed decrease reflects a loss of neuronal units or a fall in average myelin content per unit is not established, although the constancy with age of nonmyelin composition supports the latter. (Supported by VA and NIH Grant AM 13525.)

307. On the Action of Uricosuric Agents. THOMAS H. STEELE* AND GEOFFREY BONER,* Madison, Wis. (introduced by Edwin C. Albright).

The uricosuric actions of intravenous probenecid (BEN) and chlorothiazide (CTZ) were investigated by comparing increases in uric acid excretion (per unit inulin clearance) produced by these agents ($\Delta\ UV_{ur}/C_{in}$), before and after the suppression of urate secretion by pretreatment with pyrazinamide (PZA). In 11 normal men, base line urate-to-inulin clearance ratios (C_{ur}/C_{in}) averaged 0.078±0.005 (mean ±SE); C_{ur}/C_{in} averaged only 0.012±0.001 in paired studies performed after PZA pretreatment. BEN produced a $\Delta\ UV_{ur}/C_{in}$ of 1327±114 μg/100 ml. However, in paired experiments after PZA pretreatment, $\Delta\ UV_{ur}/C_{in}$ after BEN was only 199±30 μg/100 ml. The BEN-induced $\Delta\ UV_{ur}/C_{in}$ after PZA pretreatment was < 20% of the values without PZA in all five persons studied. CTZ produced a $\Delta\ UV_{ur}/C_{in}$ of 648±65 μg/100 ml in eight persons; in paired experiments after PZA pretreatment, $\Delta\ UV_{ur}/C_{in}$ after CTZ was only 340±83 μg/100 ml. Uricosuric responses to CTZ were most markedly attenuated after PZA in five persons with the greatest uricosuric responses originally. Interference by PZA with the actions of CTZ and BEN could explain these results, but peak natriuretic responses to CTZ were similar with and without PZA. In addition, in separate experiments, PZA pretreatment did not change PAH secretion (T_{PAH}). BEN decreased T_{PAH} equivalently, either with or without PZA pretreatment. Moreover, it seems unlikely that BEN and CTZ act primarily through stimulation of urate secretion. The results suggest that a portion of urate reabsorption might occur distal to secretory sites in nephrons. Presumably, urate reabsorption distally would be diminished after PZA inhibition of secretion. The administration of a uricosuric agent, inhibiting urate reabsorption at a proximal locus, would increase distal urate delivery. This could explain an amelioration of the effect of uricosuric agents during secretory blockade by PZA; the "distal" site then could reabsorb any increment in intratubular urate more avidly.

308. Cytosol-Binding Protein (CBP) of Thyroxine in Human and Rat Kidney Tissues. KENNETH STERLING,** MILTON A. BRENNER,* VICTOR F. SALDANHA,* AND PETER O. MILCH,* New York.

Earlier work, particularly that of Ingbar, has indicated the existence of intracellular thyroxine (T4)-binding sites in various tissues including liver, kidney, muscle, and others. The present investigation comprises studies of rat and human kidney tissues to elucidate whether one or more binding proteins exist in the cytosol. To date, studies have been

carried out on rat kidneys perfused in situ, as well as on the supernatant fluid of sonicated and centrifuged human kidney cells which had been grown in monolayer cell cultures (Microbiological Associates). In the rat studies, the aorta was cannulated and perfused with ice-cold isotonic saline for 30 min until the renal venous effluent fluid was crystal clear and virtually devoid of serum proteins. The homogenized tissue was subjected to centrifugation at 105,000 *g* to obtain clear cytosol, which was then mixed with tracer thyroxine-^{125}I, and subjected to various procedures including dialysis, gel filtration (Sephadex G-200), and electrophoresis on paper as well as Pevikon thin layer (Hamada and Ingbar). In all these studies the most conspicuous protein binder of thyroxine had an anodal mobility slightly greater than thyroxine-binding alpha-globulin (TBG) and apparent molecular weight slightly greater than albumin (approximately 70,000). This cytosol-binding protein (CBP) appeared similar in kidneys of rat and human origin. Its properties and biological role are under intensive study. (Research supported by grant from NIH.)

309. Computer Simulation of an Osmotic Gradient without Active Transport in the Renal Inner Medulla. JOHN STEWART* AND HEINZ VALTIN, Hanover, N. H.

It has been generally supposed that the osmotic gradient observed in the inner medulla of mammalian kidneys during antidiuresis results from countercurrent multiplication of active sodium reabsorption from the thin ascending loop of Henle. However, disagreement persists on whether the thin ascending loop of Henle is capable of such active transport. Recently an alternative model has been proposed by Kokko and Rector. In this model water reabsorption from the descending loop of Henle creates a high tubular concentration of sodium at the bend of the loop, so that sodium can move passively out of the ascending thin loop. Computer simulation of this model has shown that it can indeed result in an osmotic gradient in the inner medulla, on condition that sodium movement out of the thin ascending loop results in the tubular fluid becoming hyposmotic to adjacent interstitium. This can be achieved if the descending loop is permeable to water but relatively impermeable to urea and electrolytes, and the ascending limb is less permeable to water and urea than to electrolytes. In this model, a key role is played by urea which constitutes a substantial proportion of the interstitial osmolality, and hence provides a driving force for water reabsorption from the descending limb. The importance of urea is illustrated in the model, firstly by simulation of a low-protein diet (i.e., low urea excretion), and secondly by simulation of water diuresis. In both situations, reduced reabsorption of urea from the late collecting ducts causes a reduced interstitial urea concentration, which in turn causes a severe reduction in interstitial electrolyte concentration. (Supported by NIH Research Grant AM 08469-GM; Career Program Award 6-K3-GM 21,786; Traveling Fellowship from the British MRC.)

310. A Model System Simulating Bacterial Phagocytosis and a Screening Test for Phagocytic Dysfunction. THOMAS P. STOSSEL,* Boston, Mass. (introduced by David H. Smith).

Neutrophils engulfed droplets of paraffin oil containing oil red O and stabilized with *E. coli* or *Salmonella* lipopolysaccharide only if the emulsion was preincubated with normal fresh serum. The initial rate of phagocytosis of opsonized lipopolysaccharide-coated droplets was quantitated from the optical density of dioxane extracts of washed neutrophils after incubations of cells with the particles. The absorption maximum of Nitroblue tetrazolium (NBT)-formazan in dioxane (580 mμ) differs from that of oil red O (525 mμ) permitting simultaneous measurement of oil red O ingestion and NBT reduction. Oxidative activity was corrected for the phagocytic rate, since NBT reduction occurs within the phagocytic vacuole. The opsonic effect of patients' sera, the ingestion rate of their phagocytes, and the oxidative activity of the cells were rapidly analyzed using 8-ml blood samples. The assay clearly differentiated hereditary and acquired C3-deficient, agamma- and hypogammaglobulinemic, and newborn sera (subnormal oil red O uptake, normal formazan/oil red O ratio) and chronic granulomatous disease phagocytes (normal oil red O uptake, diminished formazan/oil red O ratio) from normals ($n = 60$) without overlap. Sera from individuals with certain bacterial infections promoted supernormal mean rates of oil red O ingestion and NBT reduction by normal leukocytes. Opsonins in this system were labile to heat, hydrazine, EDTA, and zymosan. Droplets coated with lipopolysaccharide modified by alkaline hydrolysis or acetylation had opsonic requirements identical with particles prepared with native lipopolysaccharide. However, paraffin oil emulsions made with deacylated lipopolysaccharide were ingested in the absence of serum. This model facilitates quantitative study of serum opsonins and of the molecular basis of bacterial resistance to phagocytosis.

311. Thiazide Diuretics: Differential Effect on Distal Tubule Calcium and Sodium Transport. R. A. L. SUTTON,* B. R. EDWARDS,* N. L. M. WONG,* P. G. BAER,* AND J. H. DIRKS, Montreal, Canada.

The transport of Ca and Na in the distal tubule of the dog was studied by micropuncture in hydropenia and after thiazide diuretics, a natriuretic agent that reduces hypercalcuria. Tubule fluid Ca and Na were measured with the helium-glow photometer. In hydropenia mean distal tubule fluid to ultrafilterable plasma (TF/UF) Ca was 0.31 while mean TF/P Na was 0.34 (38 tubules from 23 dogs). A highly significant correlation between TF/P Na and Ca was observed indicating parallel distal Na and Ca reabsorption in hydropenia. In further experiments three phase recollection micropuncture studies of distal Ca-Na transport were carried out in hydropenia, 3% body weight saline infusion, and after thiazide administration (chlorthiazide 20 mg/kg per hr) in 10 dogs. Urine flow was carefully matched with Ringer's infusion after thiazide. Saline alone did not significantly change distal TF/P Na and TF/UF Ca. After thiazide, fractional Na excretion increased from 1.7 to 6.2% while fractional Ca rose only from 1.1 to 1.7%. After thiazides, mean distal TF/P Na increased from 0.26 to 0.54 ($P < 0.01$) but mean TF/UF Ca remained unchanged (0.22 vs. 0.21). Mean fractional rejection of Na increased by 6% in the distal tubule while that of Ca was not significantly altered. Thiazide diuretics clearly dissociated the normal proportional distal

transport of Ca and Na by selectively inhibiting Na reabsorption. This differential transport effect forms the basis of the therapeutic usefulness of thiazides in hypercalcuria. (Research supported by grant from MRC(C).)

312. Distribution of Cholesterol Feedback Control in the Guinea Pig. ALAN SWANN* AND MARVIN SIPERSTEIN,** Dallas, Tex.

While it is widely assumed that cholesterol-mediated feedback control of cholesterol synthesis is limited to liver, this assumption is based almost exclusively on data obtained in a single species, the rat. This study was undertaken to determine whether this pattern of cholesterogenesis is representative of other species. Cholesterol synthesis and its feedback control were therefore determined in seven tissues of a second widely used laboratory animal, the guinea pig. Guinea pigs were fed either 0 or 5% cholesterol diets for 7 days or injected with Triton 1339, tissue slices incubated with acetate-2-^{14}C, and the apparent K_m and V_{max} for cholesterol synthesis determined. In this species intestine has the highest cholesterogenic capacity (130 nmoles/g per 2 hr); however, unexpectedly, lung synthesizes cholesterol at a rate (22) exceeding liver (7.4). The apparent K_m's for cholesterol synthesis in these tissues were similar. Most surprising, cholesterol feeding causes marked feedback inhibition of cholesterogenesis not only in liver, 93%, but in lung, 78%, intestine, 71%, spleen, 95%, lymph node 81%, adrenal 72%, and brain, 80%. Accumulation of cholesterol was apparent in all tissues studied; and except for brain and liver, inhibition of cholesterol synthesis was proportional to tissue cholesterol accumulation. Triton WR-1339 caused stimulation of cholesterol in all tissues but brain and lymph node, again demonstrating susceptability to exogenous cholesterol. These studies demonstrate that, in contrast to the limited localization of the cholesterol feedback system in the rat, cholesterol feedback control is operative in a wide range of guinea pig tissues. The most unexpected finding of this study is that in this species lung represents a major site of cholesterol synthesis and, as in liver, cholesterogenesis in lung is under sensitive feedback control. (Research supported by grants from NIH and DRMFCS.)

313. Molecular Organization of Sickled Hemoglobin. PAUL H. SWERDLOW,* BEATRICE MAGDOFF-FAIRCHILD,* AND JOHN F. BERTLES,** New York.

Filamentous aggregation of deoxygenated molecules of sickle hemoglobin (Hb S) into parallel bundles appears primarily responsible for distortion of erythrocyte shape in sickle-cell anemia (Hb SS disease). As current interpretations of molecular organization within these filaments rely on inference and conjecture, the requirement is for structural information at the level of interatomic distances. Recent developments in the technical approach to Hb S filaments and Hb SS cells have permitted us to obtain distinctive X-ray diffraction patterns of deoxygenated Hb S in cells and in cell-free preparations. Filaments of deoxygenated Hb S were concentrated by ultracentrifugation, and Hb SS erythrocyte populations were fractionated by differential sedimentation and deoxygenated. Control preparations consisted of oxygenated cells (SS and AA), deoxygenated AA cells, and oxygenated hemoglobin solutions (S and A). Filaments, cells, and solutions were introduced separately into glass capillaries with particular efforts to maximize parallel orientation of filaments, whether in Hb SS cells or in Hb S cell-free preparations. Capillaries were sealed and X-ray diffraction patterns were recorded with a microfocus X-ray generator. The incident beam was collimated to approximately 100 μ to enhance resolution. Control preparations gave X-ray diffraction patterns similar to each other and to previously reported studies on normal hemoglobin, that is, several broad diffuse maxima. In contrast, patterns from deoxygenated Hb SS cells showed a series of sharp lines to a Bragg spacing of less 4 A. Deoxygenated filamentous Hb S provided additional detail consisting of a series of sharp layer lines of periodicity 64 A. The pattern is that of paracrystalline arrays composed of regularly packed helical rods, probably single coils, with the repeat distance along each rod being some integral multiple of 64 A. We report here the first precise information on molecular organization responsible for the sickling phenomenon. One can now predict that, through the use of well-established crystallographic techniques, problems of molecular interaction involving Hb S can be solved. (Research supported by grants from the National Foundation and NSF.)

314. Ouabain and Na^+-K^+ Effects on Cardiac Microsomal Adenylate Cyclase. MICHIHIKO TADA,* MADELEINE A. KIRCHBERGER,* JO-ANNA M. IORIO,* AND ARNOLD M. KATZ, New York.

Augmentation of myocardial contractility may be mediated by enhanced adenylate cyclase (ACase) activity, which increases intracellular adenosine 3',5'-phosphate (cAMP) levels, or by altered distributions of Na^+ and K^+ across the cell membrane. To elucidate the mechanism of cardiac glycoside action, the actions of ouabain and of Na^+ and K^+ on cardiac microsomal ACase were examined. ACase activity (pmoles cAMP min^{-1} mg^{-1}) of freshly prepared dog heart microsomes was assayed by the method of Bär and Hechter. ATP concentrations remained constant during incubation. Basal ACase (106±2 [SEM]) was activated by 120 mM KCl (144 ±2). In 100 mM NaCl + 20 mM KCl, activation was 2/3 that in 120 mM KCl (132±1). Ouabain (10^{-10} to 10^{-4} mole/liter) had no effect on basal ACase, nor on ACase in 100 mM NaCl + 20 mM KCl. These microsomes exhibit the essential features of myocardial ACase: V_{max} of basal ACase (170) was activated by 10^{-4} M L-epinephrine (1.7-fold) and 10^{-2} M NaF (2.9-fold), DL-Propranolol (10^{-4} mole/liter) abolished epinephrine stimulation. K_m for ATP, 0.05–0.1 mmole/liter, was unaffected by these substances. These findings provide no evidence that ouabain directly affects cardiac ACase. Because substitution of K^+ for Na^+ increases cardiac ACase, the inotropic action of cardiac glycosides cannot be attributed to ACase activation by increased intracellular Na^+, which would result from inhibited ($Na^+ + K^+$)-activated ATPase. If, however, the ACase site which distinguishes between Na^+ and K^+ is on the outside of the plasma membrane, K^+ which leaves the cell in exchange for Na^+ when the sodium pump is inhibited by cardiac glycosides, could enhance ACase. (Supported by grants from NIH and NYHA.)

315. Antibodies to a DNA: RNA Hybrid in Patients and Identical Twins with Systemic Lupus Erythematosus (SLE). NORMAN TALAL, San Francisco, Calif.

Patients with systemic lupus erythematosus (SLE) produce antibodies to single and double-stranded DNA and RNA that can be detected by a sensitive binding assay in which radioactive antigen-antibody immune complexes are retained on nitrocellulose filters. Electron microscopic and serologic evidence suggests the latent virus infection may be present in SLE. RNA-tumor viruses synthesize DNA: RNA hybrids and double-stranded DNA through the action of an unusual enzyme, an RNA-dependent DNA polymerase ("reverse transcriptase"). Sera from 118 patients with SLE were studied for antibodies to a radioactive DNA: RNA hybrid prepared by hybridization of polythymidylic acid with polyadenylic acid-^{14}C (dT · rA). 17 patients (14%) had antibodies to this hybrid nucleic acid. No binding was found in 26 normal control sera. 8 of the 17 positive sera also bound ^{14}C-labeled native DNA from KB cells, four also bound ^{3}H-labeled double-stranded RNA from reovirus, and five bound both radioactive DNA and RNA in addition to the dT·rA. The binding of radioactive dT·rA was inhibited 84% by prior incubation with nonradioactive dT·rA, 35% by native DNA, 40% by denatured DNA, and 47% by reovirus RNA. These results suggest some antibody specificity for the DNA:RNA hybrid combined with considerable cross-reactivity with DNA and RNA. In one patient undergoing an acute exacerbation of SLE, serum antibodies binding DNA, RNA, and dT·rA appeared simultaneously over a 2-wk period and then disappeared over the subsequent 3 wk. Considering the relative rarity of antibodies binding dT·rA, it is remarkable that 2 of the 17 positive sera were from identical twins, both with SLE. Both also had antibodies to reovirus RNA but not to DNA. Genetic factors in SLE may influence the type of anti-nucleic acid antibodies produced. Antibodies to dT·rA in SLE may represent immunization to DNA:RNA hybrids synthesized by a "reverse transcriptase," although it is equally likely that they reflect a generalized immunologic hyperresponse to nucleic acid antigens.

316. Host Resistance in Diabetes: Neutrophil Dysfunction. JAMES S. TAN,* CHATRCHAI WATANAKUNAKORN,* AND JOHN P. PHAIR,* Cincinnati, Ohio (introduced by Virginia Donaldson**).

17 of 31 nonketoacidotic diabetics studied had defects in phagocytosis and/or impaired intracellular killing. These defects can be differentiated by an assay which uses *S. aureus* as the test organism. After 2 hr of incubation of the neutrophil-bacterial suspension, lysostaphin is added to eliminate nonphagocytized bacteria. The number of *S. aureus* killed by lysostaphin equals the number of extracellular bacteria. Results (expressed as per cent of initial inoculum) of neutrophil function among 25 healthy volunteers concurrently studied, were: killed intracellular bacteria = 95.4 (SD = ±2.7), viable intracellular bacteria = 1.4 (SD = ±1.5), and extracellular bacteria = 3.1 (SD = ±2.0). In the total diabetic group, the mean percentage of killed intracellular bacteria was 77.6, viable intracellular bacteria was 3.7, and extracellular bacteria 18.7. Results in 14 diabetics were within 2 standard deviations of the mean of the control and were considered normal. Among the 17 abnormal diabetics, the mean percentage of killed intracellular bacteria was 63.6, of viable intracellular bacteria 5.5, and of extracellular bacteria 31.0. These defects were not related to blood sugar levels nor corrected by normal serum. 11 of the 17 had defective phagocytosis alone, 3 had mildly impaired intracellular killing, and 3 had both defects. Two of the latter had straphylococcal bacteremia and a marked intracellular killing defect (greater than 40% of phagocytized bacteria were not killed). The neutrophil dysfunction described may contribute to the alleged increased susceptibility of diabetics to infections. (Research supported by NIH.)

317. Metabolic Fate of Intravenously Administered Trioctanoin and Octanoic Acid. PHIENVIT TANTIBHEDHYANGKUL* AND SAMI A. HASHIM, New York (introduced by W. H. Sebrell, Jr.**).

The physiologic behavior of ingested trioctanoin differs markedly from that of long-chain triglycerides. Ease of hydrolysis, minimal reesterification, and increased water solubility of its component fatty acid are some of the characteristics that may render trioctanoin suitable for parenteral alimentation. Such potential use of trioctanoin has prompted the present study. Two groups of rats, one consisting of 27, the other of 29 animals, received intravenously either trioctanoin-carboxyl-^{14}C (38 mg/rat) or octanoate-carboxyl-^{14}C (40 mg/rat). Disappearance of ^{14}C-labeled lipid from the blood ($t_{\frac{1}{2}}$), oxidation rate as measured by expired $^{14}CO_2$ continuous monitoring, and ^{14}C distribution in blood, liver, muscle, and adipose tissue into CO_2, non-CO_2 aqueous metabolites (AM), and various lipid fractions by thin-layer chromatography were studied up to 200 min. $t_{\frac{1}{2}}$ was 26 min for trioctanoin and 2 min for octanoate. Both lipids were readily oxidized. In 60 min total radioactivty in expired air was 25% of administered dose for trioctanoin and 61% for octanoate. The corresponding values were 63% and 85% in 200 min. Appreciable quantity of octanoate was taken up by liver where negligible esterification took place. Muscle was the major site of octanoate oxidation. Trioctanoin was taken up readily by liver and muscle, followed by extensive hydrolysis into free octanoate and oxidation into CO_2 and AM. Uptake and esterification of ^{14}C in adipose tissue was small in both groups. After 60 min, in all tissues studied and in circulation, ^{14}C was present mostly in free fatty acid and AM fractions. In conclusion, intravenously administered trioctanoin was extensively hydrolyzed into octanoate which was readily oxidized into CO_2. (Supported by NIH Grant AM-08107.)

318. Study of Hypercoagulability with a Clot Retraction and Lysis Assay. FLETCHER B. TAYLOR, JR., AND RICHARD H. CREECH,* Philadelphia, Pa.

There are instances of patients with clinical hypercoagulability with normal coagulation studies. Employing an assay of retraction and lysis of dilute whole blood clots, abnormalities have been revealed and defined in (*a*) paroxysmal nocturnal hemoglobinuria (PNH) with thromboembolism, and in (*b*) one case of hypercoagulability with a strong family history characterized by spontaneous thrombosis of the veins of arms, legs, and neck. Coagulation studies in both cases were normal. In the PNH patient, the clot retraction and

lysis assay was abnormal at pH 7.4 (2+ retraction, 36 hr lysis time) and normal at pH 6.5 (4+ retraction, 6–8 hr lysis time). PNH platelets washed in 0.2% Na_2 EDTA fused at both pH 7.4 and 6.5 upon addition of serum diluted 1:32, whereas normal platelets fused only at pH 7.4 with serum diluted 1:8. Anti-γM, β1c, and cold agglutinin aggregated Na_2 EDTA PNH platelets at dilutions 2-fold greater than normal platelets. It is concluded that these PNH platelets carried a membrane defect similar to the PNH RBC. In the second patient, clot lysis time was greater than 3 days. By recombination experiments using normal and patient's plasma supernatant and platelets, the patient's plasma was showed to contain the factor responsible for prolonged lysis time. Since plasminogen and plasmin inhibitor levels were normal and plasmin lysis of the patient's plasma clots was inhibited, it was concluded that the patient's fibrinogen-fibrin substrate was abnormal. The value of the clot lysis assay in the study of hypercoagulability lies in the fact that the effector systems (coagulation, complement, fibrinolytic, platelets) have been defined and that these systems are reacting in a biphasic milieu which most closely approaches that found in vivo. (Supported by NIH Grant HE 10-907.)

319. Response of Patients with Leukemia to Platelet Transfusions. FRANCISCO TEJADA,* WILMA B. BIAS,* GEORGE W. SANTOS,* AND PHILIP D. ZIEVE,* Baltimore, Md. (introduced by Reuben Andres**).

Nine aplastic leukemic patients received an average of 26 transfusions of randomly obtained platelet concentrates over a period of 5–32 wk. All nine developed lymphocytotoxic antibodies against an average of seven (range 1–28) HLA antigens from a panel of 40 cells within 3–16 wk after transfusions were begun. The number of different antibodies formed and the rapidity of response appeared to be dependent on the chemotherapy which the patients had previously received: three patients receiving prednisone at the time of transfusion had a diminished antibody response (antibodies reactive against less than five cells of the panel); two patients who had received infusions of cytoxan or cytosine-arabinoside within 48 hr before transfusion had an apparently enhanced response (12 and 28 different antibodies formed). Detectable antibodies markedly diminished in the serum of eight of these patients over a period of 16–20 wk despite continued occasional transfusion of platelets. Sera (diluted 1:40 in buffer) in which HLA antibodies were detected released endogenous serotonin from washed normal platelets when incubated at 37°C for 30 min (23±3% SEM released, $n = 31$, $P < 0.001$, compared to control cells incubated in buffer alone). In contrast sera from these patients, obtained at a time when HLA antibodies were not detected, released minimal amounts of serotonin (3±1%, $n = 29$) and sera from nine healthy subjects released none (0±1%). The results indicate frequent induction of HLA antibodies in patients receiving platelet transfusions and show that there is a positive correlation between the presence of these antibodies and the release of serotonin by serum from these patients. (Supported by an NIH grant.)

320. Enzyme Implantation: Acquisition of *"De Novo"* Uricase Activity by Alveolar Macrophages (AM). JAMES THEODORE,* JULIO ACEVEDO,* AND EUGENE D. ROBIN,** Stanford, Calif.

We have previously shown intracellular uptake of a macromolecule (ferritin) by alveolar macrophages (AM) without apparent functional impairment. This suggested that endocytosis of biologically active macromolecules (e.g., enzymes) could endow the cell with new biochemical functions. In the present studies AM acquired the ability to oxidize uric acid after extracellular exposure to uricase. Rabbit AM obtained by pulmonary lavage were exposed to 4 U of uricase in 5 ml of rabbit Ringer's (pH 7.4) at 38°C for 1 hr. After exposure, the cells were washed until no uricase activity could be detected in the extracellular phase. The cells were then incubated in Ringer's solution (pH 7.4) containing uric acid (50 μg/ml) and sequential measurements of uric acid were performed in the extracellular phase over 1 hr. Control studies involved incubation of AM not exposed to uricase with uric acid Ringer's. Uricase activity ($\Delta\mu$g urice acid $\times$ mg protein^{-1} hr^{-1}) in nonuricase-exposed AM was negligible (0.08±0.05 $\mu g \times$ mg protein^{-1} hr^{-1}) whereas uricase-exposed AM produced significant rates of uric acid oxidation (1.50±1.06 $\mu g \times$ mg protein^{-1} hr^{-1}, $P < 0.01$, six studies). Viability of uricase-exposed cells was demonstrated by the Eosin Y test and by persistent lactate production. Intracellular uptake (compared to nonspecific binding) was documented by increases in O_2 consumption during AM exposed to uricase (control $\dot{V}_{O_2} = 18.2 \pm 4.7$ $\mu l \times$ mg protein^{-1} hr^{-1}, uricase $\dot{V}_{O_2} = 23.9 \pm 4.1$ $\mu l \times$ mg protein^{-1} hr^{-1}, $P < 0.01$, six studies.) Parallel studies in nonendocytic cells (erythrocytes) showed negligible uricase activity after uricase exposure. This general approach offers a new method for the elucidation of biochemical control mechanisms. It raises the possibility of direct cellular replacement therapy for heritable deficiencies of regulatory macromolecules.

321. Relationship of Mixed Lymphocyte Culture Response to HL-A Histocompatibility Antigens: Effect of Allele Plus One Antigen Match. JOHN S. THOMPSON,* MICHAEL J. PARMELY,* RONALD J. FLINK,* MARILYN S. CANADY,* AND CHARLES D. SEVERSON,* Iowa City, Iowa (introduced by James Christensen**).

The influence of varying degrees of incompatibility for HL-A antigens on one-way mixed lymphocyte cultures (MLC) has been investigated in 16 large families. Reactions were compared to a simple expression of HL-A antigens, allele compatibility, and a proposal considering the potential influence of antigen matching in relationship to allele compatibility. As expected, HL-A compatibility was associated with nonstimulated cultures, but significant correlation was not observed when incompatibility was expressed in terms of HL-A antigen or allele mismatching. A second distinctive group was demonstrated, however, that shared one allele plus one antigen of the second allele. Within this group no stimulation, even with augmented culture conditions, was observed in some families, but there was no relationship with respect to whether the mismatched antigen was in the LA or four segregating series. Employing these same criteria, there was no significant difference in the MLC response in those groups that were incompatible for both alleles regardless of the number of matched antigens or the group that

shared an allele but differed by both antigens of the second allele. These results suggest that HL-A haplotype incompatibility, acting as a unit, is the primary stimulus of the MLC response, and that the immunogenicity of the haplotype also relates to whether or not one antigen is common to the stimulating and responding cell. (Supported by NIH Grant AI-09716-01 and the Veterans Administration.)

322. Differences in DNA Metabolism in Tumor vs. Host Cells; Predicting Patient Response to Chemotherapy. G. TISMAN,* V. HERBERT, H. EDLIS,* L. T. GO,* AND L. BRENNER,* New York.

Utilizing TdR-^{3}H and dU-^{3}H as labels, the *de novo* pathway of DNA synthesis was used 2- to 5-fold more than the salvage pathway by tumor cells, but host (normal marrow) cells used both paths equally in short-term (4-hr) suspensions of bone marrow vs. tumor cells from the same patient. In five (lymphosarcoma, colon, epidermoid, two lung) of seven tumors, preincubation with deoxyuridine (dU) (conc. 0.1 μmole/ml) enhanced subsequent incorporation of TdR-^{3}H into DNA by 30–60%; in all seven host cell suspensions, such preincubation reduced incorporation of TdR-^{3}H to less than 10% of controls. Since dU may be inhibitory to thymidine phosphorylase, there may be elevated thymidine phosphorylase in tumor cells, forcing them to prefer the *de novo* pathway, partly explaining clinical efficacy of inhibitors of the *de novo* pathway of DNA synthesis (5-fluorouracil; methotrexate). D-Penicillamine showed potent tumoricidal activity in vitro with little bone marrow toxicity, in five of seven patients studied. The panel of drugs used included methotrexate, 5–fluorouracil, vincristine, actinomycin D, cytoxan, CCNU, bleomycin, and D-penicillamine. Six patients were treated with the drug with best therapeutic index in vitro (i.e., relatively greatest in vitro toxicity to his tumor cells, and lowest to his marrow). In vivo daily dose was "best" dose per ml incubation medium in vitro $\times$ body wt(g) $\times$ 0.60 ml/g. Therapeutic improvement occurred in three, with predicted marrow toxicity in two and no predicted marrow toxicity in three. The results suggest "chemotherapy sensitivity tests" in vitro not only help select the "best" of a panel of drugs for induction therapy of the specific tumor in the specific host, but also help determine dosage level. (Supported by USPHS Grant 15163.)

323. Stimulation of Human Prolactin Secretion by Intravenous Infusion of L-Tryptophan. ROGER W. TURKINGTON AND JOHN H. MACINDOE,* Madison, Wis.

Previous studies in our laboratory have indicated that the human hypothalamus regulates the secretion of prolactin, and that this regulation is dependent primarily upon pathways leading to the synthesis of catecholamines in neural elements. We now present evidence for a new and additional biochemical pathway for hypothalamic regulation of prolactin secretion in man. Infusion of *l*-tryptophan, 5–10 g intraveneously over 20 min, resulted in a sudden release of prolactin after 45 min, and peripheral plasma levels of prolactin in 10 normal subjects rose from <2 to 110–150 ng/ml. This infusion did not significantly alter the plasma concentration of other amino acids, and the infusion of *d*-tryptophan or various mixtures of 19 other amino acids had no effect on prolactin secretion. The intravenous infusion of thyrotropin-releasing hormone, 500 μg, stimulated secretion of both prolactin and TSH. The infusion of *l*-tryptophan did not stimulate TSH release. Secretion of luteinizing hormone was slightly inhibited by *l*-tryptophan infusion, but growth hormone secretion was not affected. Prior treatment with methysergide maleate blocked these effects of *l*-tryptophan, suggesting that they depend upon conversion of tryptophan to serotonin. Infusion of *l*-tryptophan elicited no secretion of prolactin in four patients with panhypopituitarism, but did elicit prolactin secretion in seven patients with incomplete surgical hypophysectomy. These results indicate that infusion of *l*-tryptophan may serve as a sensitive test for evaluation of pituitary prolactin reserve and the completeness of surgical hypophysectomy, and support the concept that hypothalamic regulation of prolactin secretion in man is mediated by a balance between inhibitory catecholaminergic and stimulatory serotoninergic mechanisms. (Research supported by NIH Grant CA-12904-01.)

324. Human Prolactin Secretion and Puerperal Lactation. JOHN E. TYSON* AND HENRY G. FRIESEN, Baltimore, Md. and Montreal, Canada.

Pituitary prolactin is essential to the initiation of puerperal lactation in mammals; however, its role in human mammary physiology is unknown. The secretory dynamics of radioimmunoassayable human prolactin (HPr), have been studied in healthy parturient female volunteers. Although elevated at term (>150 ng/ml) mean basal HPr concentrations return to prepregnant levels 1 wk after delivery. During the first 2 months postpartum, suckling produces a 5- to 10-fold rise in HPr within 30 min. After 2 months, the rise is negligible. The ingestion of chlorpromazine (CPZ $-$ 25 mg) by four lactating women, 4 days postpartum, raised the mean basal HPr from 16.0 to 45.0 ng/ml; however the HPr response to suckling remained intact. To evaluate the relationship between puerperal HPr secretion and lactation, 100–500 μg of synthetic thyrotropin-releasing hormone (TRH) was injected intravenously into four lactating women 56–72 days postpartum. A 3- to 5-fold increase in plasma HPr was observed 30 min after the injection. Suckling had produced no HPr rise 30 min before TRH injection. Breast engorgement and lactation occurred 2.5 hr after TRH, well before the next scheduled feeding. In a fifth woman, intravenous TRH (500 μg) was given 4 days after weaning. HPR rose from 5.0 ng/ml to 37.0 ng/ml. Breast changes were similar to those previously mentioned. In summary, (*a*) the HPr response to suckling varies with the postpartum interval; (*b*) CPZ elevates basal HPr but fails to inhibit the suckling response; and (*c*) intravenous TRH induces a rise in HPr followed by breast engorgement and lactation. Thus, TRH may have a role to play in the establishment or maintenance of puerperal lactation.

325. Absence of Ventricular Escape Rhythm after Selective Suppression of the Sinus Node and AV Junction. FERDINAND URTHALER* AND THOMAS N. JAMES,** Birmingham, Ala.

It is widely believed that there are multiple potential automatic centers below the level of the His bundle, and that they

readily emerge when higher pacemaking centers fail. This belief is supported by the existence of spontaneous depolarization demonstrable in cells of false tendon and other isolated preparations of Purkinje tissue. In 15 dogs anesthetized with sodium pentobarbital (30 mg/kg) we cannulated the respective nutrient arteries of the sinus node and AV junction for direct experimental perfusion of those two centers of automaticity. Stable AV junctional rhythm was established by selective suppression of the sinus node with 2 ml of physostigmine salicylate (100 μg/ml). During the AV junctional rhythm, additional direct perfusion through the AV node artery with acetylcholine hydrochloride (1–10 μg/ml) or physostigmine produced immediate deceleration and cardiac arrest for up to 45 sec (average 19±9 sec). In 60 such experiments we have not observed a single example of ventricular escape beats or rhythm, and the asystole was always terminated by slow and erratic beats of either sinus or AV junctional origin (narrow QRS resembling that during AV junctional rhythm), initially with a high grade of AV block. Since neither acetylcholine nor physostigmine should have a chronotropic effect on ventricular Purkinje cells, and because the administration of the test substances was selectively into the AV junction in the septum, one may question whether idioventricular escape mechanisms exist in the intact canine ventricle and suspect that most if not all idioventricular rhythms are reentrant in nature. (Supported by NHLI Grants HE 11,310 and PH 4367-1441.)

326. Left Ventricular (LV) Response to Severe Exercise in Untethered Dogs. S. F. VATNER,* D. FRANKLIN,* C. B. HIGGINS,* R. W. MILLARD,* T. PATRICK,* AND E. BRAUNWALD, La Jolla, Calif.

It is generally held that the Frank-Starling mechanism contributes little, if at all, to the left ventricular (LV) response to severe exercise. To examine this concept, measurements of LV diameter (D) and pressure (P) were radiotelemetered from normal, healthy dogs as they ran spontaneously and unrestrained in the field at speeds exceeding 20 mph, for distances up to 6 miles. Effects of severe exercise were studied in eight dogs previously instrumented with ultrasonic D transducers on the LV epicardium and miniature P gauges in the LV and stimulating electrodes on the left atrium; dP/dt and dD/dt, the velocity of myocardial fiber shortening, were derived. Severe exercise increased heart rate from 89 to 296 beats/min, LV systolic P from 124 to 207 mm Hg, end-diastolic P from 7 to 19 mm Hg, dP/dt from 3520 to 11,690 mm Hg/sec, and velocity from 65 to 136 mm/sec, while end-diastolic D rose by 1.4 mm from a control of 59.1 mm ($P < 0.01$) and end-systolic D decreased slightly. With heart rate constant at 290 beats/min exercise caused similar increases in LVP, dP/dt, and velocity but increases in end-diastolic D were significantly greater (+4.2 mm). After propranolol, 1.0 mg/kg, severe exercise resulted in little inotropic response and rate increased to only 183 beats/min while the increases in LVD, end-diastolic (+2.0 mm) and end-systolic (+0.9 mm), were significantly ($P < 0.01$) greater than during the control exercise response. Thus, the left ventricle responds to severe exercise primarily with large increases in rate and contractility and, contrary to current concepts, the additional compensatory mechanism of an increase in initial LV fiber length (Frank-Starling mechanism) is utilized. When increases in heart rate or contractility are limited, the Frank-Starling mechanism assumes even greater importance.

327. Membrane Action of Glycosides. RICHARD T. VERNICK,* EDMUND H. SONNENBLICK, AND MICHAEL LESCH, Boston, Mass.

The effect of ouabain on the synthesis and turnover of phospholipids (PL) was studied in isolated rabbit atria. Left atrial strips were incubated in Krebs-bicarbonate buffer with 20 μCi of ^{32}P for 3 hr. PL were extracted, isolated by two dimensional thin-layer chromatography, and located with iodine. Specific activity was calculated from inorganic phosphate and ^{32}P content. Specific activity of the intracellular precurser pool (PP) was determined from TCA-soluble extracts. Ouabain (10^{-5} mole/liter inhibited ^{32}P incorporation into PL by 50–70%, while PP specific activity was decreased by 50%. This effect could be demonstrated at concentrations of as low as 5×10^{-7} mole/liter. The effect was present at the lower concentrations without the concomitant decrease in PP specific activity. Inactive analogs of digitalis had no effect even at concentrations of 10^{-5} mole/liter. These data demonstrated an alteration in PL metabolism brought about by ouabain, which may reflect subtle changes in membrane composition and structure. These changes could mediate the action of ouabain by altering the activity of membrane-bound enzyme systems which control both calcium and sodium ion transport as well as the binding and release of organelle calcium. The apparent dissociation of PP and PL specific activities at nontoxic ouabain concentrations also raises the possibility that toxic and pharmacologic effects may be mediated through different mechanisms. (This was supported in part by USPHS Grants HE11306 and HE09714.)

328. Hepatitis B Antigen (HB Ag) and Antibody (Anti-HB Ag) in Ugandan Patients with Cirrhosis and Hepatocellular Carcinoma. CHARLES L. VOGEL,* PETER P. ANTHONY,* F. SADIKALI,* AND LEWELLYS F. BARKER,* Kampala, Uganda, and Bethesda, Md. (introduced by C. Gordon Zubrod).

Persistent HB Ag (Australia antigen, HAA), presumably indicative of chronic infection with hepatitis B virus (HBV), occurs with increased frequency in Ugandan patients with cirrhosis and hepatocellular carcinoma (HC). To investigate further the relationship of HB Ag to cirrhosis and to HC, sera from 91 patients with cirrhosis, 106 with HC, and 224 controls were tested for HB Ag by counterelectrophoresis (CEP), complement fixation (CF), and radioimmunoassay (RIA), and for anti-HB Ag by passive hemagglutination (PHA) and RIA. Anti-HB Ag was present in approximately 30% of the cirrhosis, HC, and control patient groups, indicating a high rate of exposure to viral hepatitis, type B, in the population. However, 37% of 80 patients with macronodular cirrhosis and 40% of those with HC were positive for HB Ag by CEP and CF, in contrast with 3% of the controls and none of 11 patients with macronodular cirrhosis. Although additional HB Ag-positive sera were found by RIA, the significantly greater frequency of HB Ag in cirrhosis and HC patients was maintained, when compared with

controls. Alpha feto protein (AFP)-positive HC patients were positive for HB Ag more frequently than AFP-negative patients. Prospective studies of cellular and humoral immune responses in 20 patients with HC were essentially normal when compared with appropriate controls, suggesting that defects in host immune mechanisms probably were not responsible for HB Ag persistence. The results of this study support the hypothesis that chronic infection with HBV may play a role in the etiology of both macronodular cirrhosis and HC in Uganda. (Research supported by Grant PH 43-67-1343 with Chemotherapy, NCI, NIH.)

329. Ammonia Excretion in Experimental Renal Disease. JOHN WALLS,* HERBERT LUBOWITZ,* AND NEAL S. BRICKER, St. Louis, Mo. (introduced by William H. Daughaday**).

The development of metabolic acidosis in chronic renal disease is held to be due to the inability of the mammalian kidney to adequately increase ammonia excretion as renal function decreases. To examine this biological system in detail we studied *maximal* rates of ammonia excretion in rats with two forms of chronic renal disease: (*a*) glomerulonephritis (GN), a lesion in which single nephron glomerular filtration rate (GFR) (SGFR) decreases; and (*b*) a remnant lesion (R) in which SGFR becomes supernormal. The GN lesion was induced by a modified Masugi technique and the R model by partial renal infarction. Both groups of animals were acid-loaded with NH_4Cl. Over a range of GFR's varying from 0.13 to 1.54 ml/min in the GN rats and 0.31–1.67 ml/min in the R animals, a direct linear relationship between $U_{NH_4}V$ and total GFR was observed. In the GN rats ammonia excretion per unit of GFR ($U_{NH_4}V$/GFR) remained constant over the entire range of GFR's studied and no significant change was seen after glutamine infusion. However, $U_{NH_4}V$/GFR increased significantly in the R animals at low levels of GFR and this was accentuated by glutamine infusion. Thus, an adaptive increase in ammonia excretion per nephron was found in the R animals. By contrast, GN rats did not exhibit this adaptation. These data suggest that *maximal* rates of ammonia excretion are attuned to SGFR rather than to the biological requirements of the animal.

330. Presence of a Myelostimulatory Factor in Polycythemia Vera (PV) and Agnogenic Myeloid Metaplasia (AMM). HARRY P. WARD* AND WILLIAM A. ROBINSON,* Denver, Colo. (introduced by Matthew H. Block).

Polycythemia vera (PV) and agnogenic myeloid metaplasia (AMM) are characterized by hyperplasia of all three myeloid cell lines and reversion of hematopoiesis to extramedullary sites. The presence of a serum factor in these patients that stimulates the erythroid and granuloid compartments was evaluated by a modified erythropoietin (EP)-responsive assay and the in vitro agar method for granulocyte colonies. Hypertransfused CF1 female mice were injected for 3 days with test serum. EP (0.25 U) was given the 4th day and $^{59}FeCl_3$ on day 6. ^{59}Fe incorporation into RBC was determined on day 8 and compared with animals receiving normal serum or saline for 3 days before EP. The serum from five of five patients with AMM and five of seven with PV markedly enhanced the response to 0.25 U of EP ($P < 0.05$). All but two patients had insignificant levels of serum EP as evaluated by exhypoxemic mouse assay. The ability of sera to stimulate granulocyte colony-forming cells was determined by injecting test serum in CF1 mice for 3 days and plating 75,000 cells from the femur with a standard amount of human urine colony stimulating factor in soft agar. Numbers of colonies were determined after 7 days incubation and compared with animals receiving normal serum or saline. Serum of five of five patients with AMM and three of three with PV enhanced the number of granulocyte colonies measured by colonies/75,000 cells or colonies/femur ($P < 0.05$). PV and AMM serum contains a factor that increases EP-responsive stem cells and granulocyte stem cells. Studies have failed to find a similar factor in serum of normal subjects and patients with chronic granulocytic leukemia.

331. Suppression of Granulomatous Hypersensitivity after Administration of Soluble Antigens to Adult, Neonatal, and Prenatal Mice. KENNETH S. WARREN, LE MINH HANG,* AND DOV L. BOROS,* Cleveland, Ohio.

Granuloma formation around *Schistosoma mansoni* eggs is a manifestation of delayed hypersensitivity. Soluble antigens isolated from schistosome eggs (SEA) sensitize mice and guinea pigs to granuloma formation and delayed skin reactions. Granuloma formation has been suppressed by drugs, neonatal thymectomy, antilymphocyte serum, antimacrophage serum, and repeated intraperitoneal injections of eggs. In the present studies adult mice were injected intraperitoneally with a total of 2 mg (protein) of SEA, 21 and 7 days before intravenous injection of 2000 schistosome eggs. 16 days later the lungs were removed: mean granuloma diameter (MGD) in stained lung sections, 144 μ; control group treated with buffered saline (PBS), 193 μ. Groups of neonates were injected intraperitoneally with SEA within 8 hr of delivery, and 8 wk later challenged with eggs. 16 days later, MGD in recipients of 1 mg SEA, 110 μ; 100 μg SEA, 141 μ; PBS control, 178 μ; 10.0, 1.0, 0.1, and 0.01 μg SEA, 214 μ (sensitization). Pregnant mothers received a total of 1 mg SEA intravenously 1 wk before delivery. 8 wk later the weanling offspring received schistosome eggs: 16 days later MGD in 14 mice, 110 μ; 14 controls (mothers received PBS intravenously), 167 μ. Progeny from mothers with *S. mansoni* infections, MGD, 112 μ; controls, 168 μ. As the inflammation around schistosome eggs is followed by fibrosis, portal hypertension, and bleeding esophageal varices, prolonged specific suppression of the initiating lesion might prevent the occurrence of hepatosplenic disease. (Research supported by grant from NIH.)

332. The Anaerobic Threshold: Changes in Respiratory Gas Exchange Signaling Circulatory Insufficiency during Exercise. KARLMAN WASSERMAN AND BRIAN J. WHIPP,* Torrance, Calif.

When tissue O_2 is inadequate to meet energy demands during exercise, the proportion of energy supplied by glycolysis increases relative to that of aerobic energy producing metabolic pathways (anaerobic threshold). When this occurs, the rate of lactic acid production increases. It is possible to observe, by noninvasive respiratory gas-exchange measurements during graded work, the change from a balance be-

tween O_2 supply and demand to a state of relative O_2 lack resulting from the failure of the circulation to meet the O_2 needs of the cells. For work rates above the anaerobic threshold, the following changes in gas exchange are observed: (*a*) O_2 consumption ($\dot{V}_{O_2}$) steady state is delayed (> 2 min) with the difference between $\dot{V}_{O_2}$ at 2 and 5 min being proportional to the anaerobic metabolic rate; (*b*) the rate of CO_2 production increases relative to $\dot{V}_{O_2}$, i.e., gas-exchange ratio increases disproportionately; (*c*) ventilation rate assumes a nonlinear increase as work rate or $\dot{V}_{O_2}$ increases. Measurement of arterial blood lactate and acid-base measurements can also be used to detect the anaerobic threshold. However, the requirements of arterial blood and the time required for analysis by these methods are significant disadvantages. On-line display of computed respiratory measurements to signal anaerobic metabolism is of advantage to the investigator in permitting him to observe the evidence for anaerobic metabolism as the exercise test is being performed. (Research supported by Grant HE11907 from NIH.)

333. Effects of Tetracycline on Pancreatic Protein Synthesis and Secretion. PAUL D. WEBSTER, Augusta, Ga. (introduced by A. J. Bollet).

Tetracycline hydrochloride (THC) administration results in increased triglyceride content in liver, decreased iron transport by intestine, and decreased protein synthesis. Recent reports associate THC administration with acute hemorrhagic pancreatitis. These investigations examine effects of THC on pancreatic protein synthesis and secretion. Both in vitro and in vivo studies were performed. For in vitro studies, pancreatic slices were incubated in culture medium containing THC and effects on L-phenylalanine-^{14}C incorporation into protein and transport of amylase into medium determined. For in vivo studies, pigeons were given THC in doses of 500, 250, 125, and 62.5 mg/kg and killed after 2, 4, 12, 18, 48, and 72 hr; pancreases were then incubated in vitro and incorporation of L-phenylalanine-^{14}C into protein and tissue amylase content determined. THC inhibited L-phenylalanine-^{14}C incorporation into protein and amylase secretion in both in vitro and in vivo systems. Moreover, the pancreases of THC-treated animals were unresponsive to an in vivo secretory stimulus administered as bethanechol chloride. Impaired secretion seemed related to membrane dysfunction as evidenced by failure of phenylalanine-^{14}C labeled protein to migrate from interior to exterior of pancreatic cell. These studies suggest that gastrointestinal dysfunction associated with THC may, at least in part, be due to defective pancreatic protein synthesis and secretion. (Supported by NIH Grant AM-13131-04.)

334. Variation in Plasma Aldosterone Concentration in Recumbent Humans. M. H. WEINBERGER,* D. C. KEM,* C. GOMEZ-SANCHEZ,* D. R. ROSNER,* AND C. A. NUGENT,** Indianapolis, Ind., Dallas, Tex., and Tucson, Ariz.

Five normal subjects were studied, three during normal sodium (NS) and two during low sodium (LS) diets. Blood was sampled every 20 min during recumbent sleep between 2 a.m. and 8 a.m. for plasma renin activity (PRA), aldosterone (PA), and cortisol (PC) by radioassay determination. Two- to fourfold variations were seen in PRA, PA, and PC in each subject. These changes are greater than the coefficients of variation for PA, PRA, and PC of 5.5, 8.8, and 8%, respectively. During both NS and LS peak levels of PA occurred between 5 a.m. and 8 a.m. and were not consistently associated with peaks of PRA or PC. PA and PRA were higher during LS than NS while PC was unchanged. In the three subjects in whom the measurements were made no significant changes in serum potassium (K) or sodium (Na) were seen, and no significant correlation between K or Na and PA was observed. No significant positive correlation between PA and PRA was observed in the five subjects studied. Changes in PA in normal, recumbent humans during both NS and LS diets, were not consistently associated with changes in PRA, K, Na, or PC. It is not likely that alterations in metabolic clearance rate during these studies could account for the observed changes in PA. These studies strongly suggest intermittent secretion of PA, PRA, and PC. (Support by NIH Grants HE 06308, FR 00057, and HE 14159 and USA Tripler Research Funds is acknowledged.)

335. Stellate Vessel Chloride Concentration as a Function of Hydrogen Ion Secretion by the Rat Nephron. S. W. WEINSTEIN* AND J. SZYJEWICZ,* New York (introduced by M. Levitt**).

The reciprocal relationship between proximal tubular fluid concentrations of chloride and bicarbonate appears dependent upon hydrogen ion secretion, chloride reabsorption with sodium, and net filtrate reabsorption. These factors should change the chloride and water content of peritubular capillary as compared to systemic arterial blood. To test this hematocrits and plasma concentrations of chloride and inulin-carboxyl-^{14}C were determined in blood collected from cortical surface stellate vessels (SV) and the femoral artery of rats intravenously expanded 5% of body weight with isotonic NaCl, with or without acetazolamide, 20 mg/kg. The mean SV to femoral arterial ratio (SV/A) for chloride was 0.89 $\pm$0.013 SEM ($n = 17$). Filtration fraction (FF) calculated from hematocrits of these samples was 0.35$\pm$0.03 ($n = 17$). The SV/A for inulin, for separate samples, was 0.96$\pm$0.05 ($n = 10$). For these FF was 0.43$\pm$0.04 ($n = 10$). After acetazolamide SV/A chloride rose to 1.01$\pm$0.01 ($n = 18$) with a mean FF of 0.39$\pm$0.02 ($n = 18$). In summary (*a*) during control conditions chloride was reduced 11% in SV plasma; (*b*) within 95% confidence limits of the SV/A inulin mean, no greater than 14% dilution of SV plasma by reabsorbed water occurred; and (*c*) acetazolamide prevented the fall in SV/A chloride. We conclude from these data that most if not all filtrate reabsorption into SV blood for controls occurred concomitantly with exchange of sodium for hydrogen. The first 10–20% of the proximal convoluted tubule appears the most likely source for this reabsorbate since it contains the highest bicarbonate concentration and anatomically accompanies the efferent arterioles forming stellate vessels. (Supported by Veterans Administration and NIH funds.)

336. Membrane Glycoprotein Synthesis by Intestine: an Index of Cell Differentiation. MILTON M. WEISER,* Boston, Mass. (introduced by Kurt J. Isselbacher).

Cell surface glycoproteins have been implicated in the control and expression of cell turnover, differentiation, and

malignant transformation in tissue culture studies (Roseman, S. 1970. *Chem. Phys. Lipids.* **5**: 270). To test these concepts in a mammalian tissue, especially one having rapid turnover and differentiation, the characteristics of intestinal cell membrane glycoprotein synthesis were studied. A method was developed which permitted isolation of epithelial cells from different levels of the villus and crypt areas. These villus-to-crypt fractions were examined for their general properties of membrane glycoprotein synthesis using labeled sugar precursors. To test specifically for exposed plasma membrane enzyme-acceptor systems, intact isolated epithelial cells were examined for their ability to incorporate UDP-sugars. When labeled D-glucosamine, L-fucose, or D-galactosamine was injected intraperitoneally a sharp gradient of incorporation was noted with the highest specific activity in villus tip areas. Isolated epithelial cells incubated with glucosamine-^{14}C showed similar gradients of incorporation. Cell fractionation demonstrated that the highest specific activity was in the purified microvillus plasma membranes while the lowest specific activity was in the microsomal fraction and the cytosol. SDS-polyacrylamide gel electrophoresis of microvillus membranes showed peaks of incorporation which migrated with peaks of sucrase and alkaline phosphatase activity. In marked contrast, incubation of cells with UDP-*N*-acetylglucosamine-^{14}C, resulted in a 10-fold greater incorporation into crypt cells as compared to villus cells. These results suggest that: (*a*) the more differentiated upper villus cells have higher rates of membrane glycoprotein turnover (or repair) than the less differentiated lower villus or crypt cells, and (*b*) the plasma membrane of the crypt cell, rather than the villus cell plasma membrane, contains both glycosyltransferases and acceptor sites for sugar-nucleotide incorporation. (Research supported by NIH Grant AM-03014.)

337. Further Studies on Storage Pool Disease and Aspirin-Like Defects of Platelets. HARVEY J. WEISS AND RICHARD P. AMES,* New York.

We have recently found that the platelets of some patients with impaired collagen-induced platelet aggregation are deficient in both serotonin and the storage pool of adenine nucleotides which are essential for normal platelet aggregation (*Brit. J. Haematol.* 1970. **19**: 643; *Blood.* 1972. **39**: 187, and **39**: 197). In other patients, the storage nucleotides were normal, suggesting a defect in the platelet release mechanism (aspirin-like defect, APD). In the present study, ultrastructural evidence for such a defect was obtained. After collagen stimulation, there was no contraction of the marginal tubules or centralization of the platelet granules. A similar abnormality was observed in normal subjects after aspirin ingestion. No such defects were found in patients with storage pool disease (SPD), but their platelets were markedly deficient in the osmophilic dense bodies which are thought to be the storage granules for both serotonin and the adenine nucleotides involved in platelet aggregation. No increase in the number of dense bodies was obtained after incubating their platelets with 50 μM serotonin. The initial rate of uptake of serotonin-^{14}C into their platelets was normal, suggesting normal transport across the membrane, but the total uptake after 60 min was significantly decreased. The findings suggest that the storage granules are diminished or are functionally defective. Another constituent of the platelet granules, platelet anti-heparin activity (platelet factor 4, PF-4) was present in normal amounts in patients with SPD, but its release by both collagen or epinephrine was defective. These findings suggest that a normal storage pool of ADP may be necessary for the release of PF-4, but that the subcellular localization of these two substances may be different. (Supported by USPHS Grant 1-RO 1-HE 14595-01 and Career Scientist Award I-639 from the Health Research Council of the City of New York.)

338. Mechanism of Cyclic AMP-Mediated Inhibition of Lysosomal Enzyme Release in Tissue Injury. GERALD WEISSMANN, ROBERT B. ZURIER,* PI-KWANG TSUNG,* AND SYLVIA HOFFSTEIN,* New York.

Human peripheral blood leukocytes (PMN's) release lysosomal hydrolases by three mechanisms: (*a*) "regurgitation during feeding," when exposed to complexes of rheumatoid factor/heat-aggregated IgG (RF/aIgG), or zymosan, (*b*) "reverse endocytosis," on encounter with immune complexes coated on Millipore filters, and (*c*) "perforation from within," after ingesting crystalline monosodium urate (MSU). These constitute models for tissue injury in rheumatoid arthritis, vasculitis, and gouty arthritis, respectively. We now report that dibutyryl cAMP, cAMP + theophylline (10^{-3} mole/liter), and prostaglandins E_1 and A_2 ($> 10^{-6}$ mole/liter) block release of lysosomal hydrolases from PMN's in all three circumstances (e.g., to 40% of controls ingesting MSU or RF/aIgG) by interfering with an *intracellular* event subsequent to particle uptake. Evidence that this event is the regulation of microtubule function by a cAMP-dependent protein kinase follows. (*a*) Scanning and transmission electron microscopy show characteristic phagocytic "cups" in zymosan-treated PMN's and "projections" in cells exposed to RF/aIgG. PGE_1-treated cells retain these appendages, but particles remain subjacent to the cell periphery without undergoing tubule-dependent translation to the cytocenter. (*b*) When PMN's engage immune complexes on filters, selective extrusion of lysosomal β-glucuronidase is blocked by cyclic AMP, PG's, and colchicine without bulk phagocytosis: flow of primary lysosomes to the plasma membrane is inhibited. (*c*) A cAMP-dependent (10^{-6} mole/liter) protein kinase was vinblastine precipitable with microtubules from 100,000 *g* supernatants of disrupted PMN's. By DEAE-cellulose, the kinase (specific activity: 253 pmoles Pi/mg protein per min, or 400 × crude homogenate) was dissociated from the tubules; these can be phosphorylated by the kinase (Goodman et al. 1970. *Proc. Nat. Acad. Sci. U. S. A.* **67**: 652). Data suggest that release of inflammatory substances from lysosomes is regulated by intracellular cAMP, which activates a protein kinase to control, by means of microtubules, the flow of lysosomes to phagocytic vacuoles or the cell periphery.

339. Response of Normal and Leukemic Patients to Lymphoblast-Associated Determinants. MARC E. WEKSLER,* New York (introduced by Henry O. Heineman**).

Lymphocyte transformation stimulated by autologous cultured lymphoblasts (CLB) may serve as an in vitro model of immune surveillance. Out studies show that the stimula-

tory determinants carried on CLB are not affected by neuraminidase, are destroyed by trypsin and physical disruption of CLB, and are blocked by monovalent concanavalin A. As most CLB lines carry EB virus, additional evidence was sought for a lymphoblast-associated antigen. Incubation of human lymphocytes for 72 hr with phytohemagglutinin or concanavalin A results in transformation of more than 75% of cells into lymphoblasts. Less than 5% of lymphocytes in control cultures incubated without mitogen are lymphoblasts. Each mitogen was added at 72 hr to control cultures and all cultures washed with medium containing either fetulin (phytohemagglutinin cultures) or methyl-alpha mannoside (concanavalin cultures), irradiated, and mixed with fresh autologous lymphocytes. Mitogen-induced lymphoblasts markedly stimulate autologous lymphocyte transformation and could be differentiated from the minimal stimulation due to "carry over" of active mitogen by the kinetics of lymphoblast transformation and its insensitivity to specific inhibitors of mitogen action. The response of lymphocytes to autologous CLB and to phytohemmagglutinin was compared in patients with acute leukemia in remission and eight healthy volunteers. The normal response to CLB ranged from 71 to 165% of that to phytohemagglutinin while leukemic patients had a response to CLB of 28–46% of their response to phytohemagglutinin. Autologous plasma from leukemic patients did not depress the response of lymphocytes to CLB. Normal lymphocytes responded vigorously to CLB derived from leukemic patients. In summary, lymphoblasts in continuous or short-term culture possess a determinant recognized as "foreign" by autologous lymphocytes. Lymphocytes from patients with acute leukemia do not respond normally to this determinant. (Research supported by ACS.)

340. Factors Determining Carbon Dioxide Elimination in Diseased Lungs. JOHN B. WEST AND PETER D. WAGNER,* La Jolla, Calif.

It is often argued that CO_2 retention in patients with chronic lung disease is caused by "alveolar hypoventilation," and that ventilation-perfusion inequality and impaired diffusion across the alveolar wall are relatively unimportant in the transfer of this gas. We have analyzed CO_2 elimination in models of the lung in which it is possible to obtain quantitative information about the factors determining the elimination of this gas. Kelman's subroutines for the O_2 and CO_2 dissociation curves were used. In situations where ventilation and blood flow were suddenly mismatched, impairment of CO_2 transfer was often nearly as marked as that of O_2, and sometimes more so, depending on the pattern of ventilation-perfusion distribution. Fortunately, however, an increase in ventilation to the alveoli greatly improved the elimination of CO_2 even when ventilation-perfusion inequality was gross. When the time course of elimination of CO_2 along pulmonary capillaries was analyzed, we found that there was little difference between CO_2 and O_2 in their rates of equilibration with alveolar gas. As diffusing capacity was reduced, the transfer of CO_2 and O_2 were impaired by similar amounts. CO_2 elimination was particularly reduced in alveoli with high ventilation-perfusion ratios. The reason why CO_2 is affected so much is the disparity between the capacity of the blood for this gas and its rate of movement across the alveolar wall. We conclude that both ventilation-perfusion inequality and diffusion impairment can seriously reduce the elimination of CO_2 in chronic lung disease and that the term "hypoventilation" is misleading in this context. (Supported by NIH Grant HE 13687-01 and NASA Grant 05-009-109.)

341. Metabolic Control Mechanisms of Lipoprotein Lipase. THOMAS F. WHAYNE, JR.,* Columbus, Ohio (introduced by Palmer H. Futcher**).

Lipoprotein lipase (LPL) is crucial for circulating lipoprotein metabolism in man and other mammals. The enzyme hydrolyzes lipoprotein triglycerides (TG) present in activated form. My previous studies with native high-density lipoproteins (HDL) used for TG activation suggest heparin changes guinea pig (GP) LPL conformation, altering its kinetic properties. In this study, GP LPL activation by delipidated rat HDL and heparin concentration effect on GP and human LPL kinetics were tested. GP postheparin serum (PHS) contains low activity LPL but native rat HDL markedly increases lipolysis. Delipidated rat HDL and native rat HDL were added in increasing amounts to GP PHS assays with and without in vitro heparin. Delipidated rat HDL increased GP LPL activity as a Michaelis-Menten hyperbola, which heparin changed to an S-shaped curve. Native rat HDL produced a GP LPL activity curve following Michaelis-Menten kinetics; heparin addition, 0.8 U/ml, produced an S-shaped curve. Heparin was increased further and the S-shaped curve was shifted to the right with decreased V_{max}. Human very-low-density lipoproteins (VLD) used for TG activation produced human LPL activity curves following Michaelis-Menten kinetics; heparin addition, 3.2 U/ml, produced S-shaped curves. Further heparin increase shifted human LPL activity curves to the right with decreased V_{max}. All S-shaped curves followed the Hill equation. Thus, heparin alters kinetics of GP LPL activation by delipidated rat HDL and increases K in the Hill equation, tested with either GP LPL plus native rat HDL as activator or human LPL plus human VLD as activator. These data provide additional evidence for a heparin allosteric effect on LPL and suggest human LPL has similar metabolic control. This may be significant to further understanding of lipid transport. (Research supported by grant from NIH.)

342. An Investigation of the Hermansky-Pudlak Syndrome. JAMES G. WHITE AND CARL J. WITKOP, JR.,* Minneapolis, Minn.

The Hermansky-Pudlak syndrome (HPS) consists of albinism, hemorrhage due to defective platelets, and storage of ceroid-like pigment in macrophages. Investigation of two patients with HPS has clarified features of albinism and pigment storage, and has provided a new test for abnormal platelets. Electron microscopy of hair bulbs revealed pigmented premelanosomes in melanocytes. The presence of melanin indicates albinism in HPS is a tyrosinase-positive variety, rather than the classic tyrosinase-negative type. Electron microscopy of bone marrow macrophages revealed stages in the digestion of erythrocytes and cell debris yielding clear droplets which coalesced to form giant vacuoles filling the cytoplasm of some macrophages. Material in the vacuoles was morphologically similar to neutral lipids and

did not resemble ceroid. HPS platelets have a storage pool deficiency (SPD) characterized by decreased serotonin, adenine nucleotides of a nonmetabolic release pool, and the dense bodies which store these substances. On exposure to agents stimulating the release reaction, HPS platelets adhere normally, but secondary aggregation is reduced or absent due to inadequate secretion of ADP. Aspirin does not affect the storage pool of normal platelets, but prevents secondary aggregation by inhibiting the release reaction. In this study HPS citrate platelet-rich plasma (C-PRP) and C-PRP from normal donors after aspirin (A) ingestion were combined in ratios varying from 1:1 to 1:10, and the mixtures exposed to agents stimulating release on an aggregometer. Secondary aggregation did not occur when HPS or A platelets were tested alone, but secondary aggregation developed normally in samples containing approximately equal numbers of HPS and A platelets. The mutually corrective interaction of HPS and A platelets provides a new test for detecting platelet SPD syndromes, and separating them from drug-induced platelet dysfunction. (Research supported by Grants HE-11880, AI-05153, and AM-15317.)

343. Enhanced Cardiac Responsiveness to Norepinephrine after Prolonged Exposure to Thyroid Hormone In Vitro. KERN WILDENTHAL* AND JACQUELINE R. WAKELAND,* Dallas, Tex. (introduced by J. Donald Smiley).

When thyrotoxicosis is induced in vivo tachycardia and/or arrhythmias gradually develop. It has been suggested that the intrinsic responsiveness of the heart to catecholamines is enhanced by the hyperthyroid state. Definitive confirmation of this hypothesis has been difficult to obtain, however, largely because secondary alterations in neural and humoral factors may obscure any intrinsic changes in the myocardium itself. To study the direct action of thyroid hormone apart from secondary factors thyrotoxicosis should be induced in isolated hearts in vitro, but the slow onset of thyroid action plus the rapid deterioration of conventional in vitro preparations have precluded such experiments. Recently a method was developed for maintaining intact, spontaneously beating hearts from late-fetal mice in organ culture for several weeks. The hearts respond appropriately to norepinephrine throughout cultivation. When 10^{-7}–10^{-6} M *l*-triiodothyronine is added to the culture medium arrhythmias and/or tachycardia gradually appear after several days, just as in thyrotoxicosis in vivo. Accordingly, these "thyrotoxic" hearts were used in the present experiments to test for altered responsiveness to norepinephrine. Dose-response curves to norpinephrine were identical for hearts maintained for 2–3 hr in triiodothyronine-treated or control medium. After 2 days, however, the curve was shifted in triiodothyronine-treated hearts, so that 10^{-8} M norepinephrine raised the atrial rate by 20±6.1 (SEM) beats/min in hearts exposed to 10^{-6} M triiodothyronine and by 1±3.0 in control hearts ($P < 0.01$), and 10^{-7} M norepinephrine raised the rate by 79±22.3 in treated hearts vs. 17±9.8 in controls ($P < 0.01$). At maximal doses (10^{-6}–10^{-5} M norepinephrine), increases were identical (122±23.2 vs. 126 ±26.4), but multiple premature contractions were induced in 56% of treated hearts vs. 14% of controls ($P < 0.02$). Thus, in a precisely controlled environment free of alterations in neural and humoral factors, triiodothyronine can act directly on the heart to enhance myocardial sensitivity to norepinephrine.

344. Serum Dopamine-β-Hydroxylase Levels during Development of Various Forms of Hypertension in Rats. REDFORD B. WILLIAMS, JR.,* FRIEDHELM LAMPRECHT,* AND IRWIN J. KOPLIN, Bethesda, Md.

DBH activity in serum is mainly derived from sympathetic nerves and is an index of their activity. We have measured serum dopamine-β-hydroxylase (DBH) activity in the rat during development of elevated blood pressure (BP) levels associated with several models of hypertension to assess the role played by sympathetic nervous activity. In only one form, which induced by repeated immobilization stress, was there a significant ($P < 0.001$) *increase* in serum DBH activity. This apparent increase in sympathetic nerve activity is consistent with the increased catecholamine excretion associated with immobilization stress. In three other forms of BP elevation, however, serum DBH activity *decreased.* DOCA and salt resulted in significant ($P = 0.02$) BP elevations in treated animals and was accompanied by a significant ($P < 0.01$) decrease in serum DBH activity. Spontaneously hypertensive rats (SHR) attain BP levels averaging 200 mm Hg by the age of 10–12 wk. SHR have lower ($P < 0.001$) serum DBH levels than normotensive, age-matched controls of the NIH-Wistar strain which is apparent before the onset of hypertension (at 5–6 wk of age) and continues after development of hypertension. Rats (developed and kindly supplied to us by Dr. L. K. Dahl) which become hypertensive when fed a diet high in salt have lower DBH levels ($P < 0.01$) than control rats derived from the same strain. The lower serum DBH levels in these last three forms of hypertension suggest that the level of sympathetic nerve activity is diminished, possibly as a consequence of reflex compensation for the elevation of BP by mechanisms other than ones dependent upon sympathetic nerve activity.

345. Threshold for Feedback Suppression of Adrenocorticotropin Hormone (ACTH) Secretion in Cushing's Disease. ADA R. WOLFSEN* AND WILLIAM D. ODELL, Torrance, Calif.

Cushing's disease is currently believed to be a functional defect associated with decrease in sensitivity to cortisol (F) feedback suppression of ACTH at a hypothalamic and/or pituitary level. We attempted to compare the dose response relationships between F and ACTH in normal, Addisonian, and Cushing's patients. A sensitive (1 pg) radioreceptor assay for ACTH was used (mean for normals at 8:00 a.m. = 74 pg/ml [range 30–100]; at 4:00 p.m. = 54 pg/ml [range 10–100]); CBG assay was used for F. The design of the study was as follows. A constant intravenous infusion was administered for 72 hr. Saline or compound F infusion was adjusted to give constant serum F concentrations over three 24-hr periods beginning at 8:00 p.m. Serum F in Addisonians was maintained at 0, 15, and 45 μg/100 ml on study days 1, 2, and 3; in adrenalectomized Cushing's patients F was maintained at 0, 50, and 100 μg/100 ml. ACTH and F were monitored at 30-min intervals from 6:00 to 10:00 a.m. and 4:00 to 6:00 p.m. throughout the study. Five Addisonians, one normal, and three Cushing's patients have been studied

to date. All patients demonstrated pulsatile ACTH secretion during day 1. ACTH was suppressed to ≤100 pg/ml and pulses diminished, in normals and Addisonians on day 2 (F ≃ 15 μg/100 ml). ACTH was unchanged in Cushing's patients on day 2 (F ≃ 50 μg/100 ml). On day 3 ACTH was suppressed to ≤100 pg/ml in Cushing's patients (F ≃ 100 μg/100 ml). We conclude: (*a*) the threshold for F suppression of ACTH secretion in Cushing's disease is 3–5 times that in normals, and (*b*) partially suppressive levels of F decrease pulsatile ACTH release in Addisonians. (Research supported by Grant 5 R01 NB08676-03 from NIH.)

346. Cardiovascular Adjustments to Acute and Chronic Increases in Oxyhemoglobin Affinity. MICHAEL WOLK,* PHILIP LIEBSON,* NUSEN BEER,* ANTHONY CERAMI,* AND THOMAS KILLIP, New York.

Sodium cyanate (NaCNO) is being evaluated as a possible useful agent in the treatment of sickle cell disease and is known to increase the affinity of hemoglobin for oxygen (p50). To evaluate the influence of increased p50 on cardiovascular performance, cyanate has been administered to dogs in both acute and chronic experiments. Measurements were obtained 3 hr after acute i.v. administration (200 mg/kg) in eight dogs and compared with a control saline infusion in five animals. Cyanate lowered p50 from 28.8 to 22.9 mm Hg, raised coronary sinus oxyhemoglobin saturation from 21% to 54%, decreased myocardial oxygen extraction from 77% to 46%, and depressed mixed venous P_{O_2} from 43 to 34 mm Hg ($P < 0.05$ for all). Simultaneously, peak ventricular systolic pressure fell from 172 to 143 mm Hg ($P < 0.05$), cardiac output declined from 4.7 to 3.3 liters/m ($P < 0.01$) and lactate utilization decreased from +51% to +24% ($P < 0.01$). Similar alterations were not observed in the control animals. Chronic oral administration of 180 mg cyanate/kg per day over 10 days to eight dogs depressed the p50 to 22.4 mm Hg, a level comparable with that found with acute infusion. When compared with 18 control studies, chronic administration of cyanate had little effect on myocardial utilization of oxygen or cardiac function. Although stroke volume fell ($P < 0.05$) cardiac output was maintained by an increase in heart rate. Peripheral oxygen delivery was influenced by a decrease in systemic oxygen extraction (24.0% vs. 17.5%, $P < 0.05$) and a higher hemoglobin (16.1 vs. 14.2 g/100 ml) than the control animals. When the chronic animals received supplemental i.v. cyanate (150 mg/day) they behaved in a manner similar to that of the acute dogs with a significant rise in coronary sinus oxyhemoglobin saturation as p50 fell further to 20.3 mm Hg. Thus the complex cardiovascular effects of cyanate may depend on a direct effect on myocardial function as well as the rapidity of change of the increased affinity of hemoglobin for oxygen. (Supported in part by contract #PH 43-67-1439 from the National Heart and Lung Institute.)

347. The Rosette-Forming Cell as an Indicator of Successful Transfer Factor Therapy in Immunodeficiencies. JOSEPH WYBRAN,* LYNN E. SPITLER,* ALAN S. LEVIN,* AND H. HUGH FUDENBERG, San Francisco, Calif. (introduced by Gilbert S. Gordan**).

Some human peripheral blood lymphocytes from unimmunized people will bind, in vitro, to sheep red blood cells forming rosettes. Our previous studies indicate that such cells (RFC) are thymus derived. Further evidence for this hypothesis is presented here: namely, patients with Wiskott-Aldrich syndrome (WA) who respond to transfer factor (TF) therapy show an increase in their RFC and patients with cellular immunodeficiency have a low number of RFC. Normal individuals, tested at repeated intervals by a modification of the original technique, show a stable number of RFC (normal: more than 15%). Four patients with WA were treated with TF. Their RFC were very low (3%, 5%, 1.5%, and 3%). After treatment with TF, three patients showed evidence of induction of cellular immunity (conversion of skin tests and production of migration inhibition factor [MIF]) and at the same time showed a rise in their RFC. A fourth patient who showed no response in any criteria and no clinical improvement did not show an increase in his RFC. Two other patients, siblings with chronic mucocutaneous candidiasis, were repeatedly treated without success with TF. Tested at different times during a 10-month period, their RFC remained stable (5% and 10%); it is of interest that the patient with 10% RFC had previously received a thymic graft and produced MIF, in contrast to his sister who did not. We conclude that the increase of RFC in patients with immunodeficiencies after TF therapy is correlated with the induction of cellular immunity and clinical improvement. (Research supported by ACS.)

348. Mechanism of Uremic Hemolytic Anemia: Acquired Hexosemonophosphate Shunt Deficiency. YOSHIHITO YAWATA,* ROBERT HOWE,* AND HARRY JACOB, Minneapolis, Minn.

A serendipitous observation that RBC from splenectomized, nephrectomized patients frequently contain Heinz bodies suggested a mechanism for uremic hemolysis—that of oxidative RBC damage as in G6PD deficiency. A rapid (ascorbate) screening test documented RBC hexosemonophosphate shunt deficiency more severe than in Caucasian (total) G6PD deficiency in 43% of 100 uremics. In four patients with normal screening tests, ^{51}Cr-labeled RBCs survived normally despite severe uremia requiring regular dialyses. Conversely, similarly uremic patients with abnormal screens manifested 50–70% reduced RBC survivals with predominant splenic sequestration; they developed explosive Heinz body hemolytic disease (^{51}Cr half-survivals ≐ 3 days) if given primaquine. Defective RBCs sluggishly recycled glucose-2-^{14}C metabolites through the hexosemonophosphate shunt; oxidants (methylene blue, ascorbate) completely failed to foster their usual 20-fold stimulation of this pathway. The metabolic error was: (*a*) intrinsic to uremic RBCs; (*b*) uncorrected by normal plasma; (*c*) obvious 1 wk after anuria; and (*d*) absent 1 wk after transplantation. Normal RBCs, briefly incubated in affected uremic's plasma, became defective, especially if such plasma was predialyzed against hemodialysis bath fluid. Analogously, the defect progressively worsened in patients during prolonged hemodialysis, leading to methemoglobinemia (20% in some, especially children), glutathione decrement (30%), and ultimately Heinz body accumulation. Deionizing hemodialysis water, lessened, albeit incompletely, these abnormal-

ities. The defect was uncorrelatable with: (*a*) RBC transketolase or G6PD; (*b*) renal pathology; or (*c*) uremia severity. We conclude significant hemolysis occurs only in some uremics—those with acquired, defective hexosemonophosphate shunt metabolism. Predictably, hemolysis worsens with oxidant drug ingestion (a previously unsuspected hazard in uremics). The RBC defect is induced by a plasma constituent which is: (*a*) nondialyzable; (*b*) of extrarenal origin; and (*c*) potentiated by trace metals in hemodialysis baths. Screening for hexosemonophosphate shunt deficiency should detect uremic patients vulnerable to sulfonamides and other oxidant drugs.

349. Defective Regulation of Glucuronyltransferases in Liver Microsomes from Gunn Rats. D. ZAKIM, D. A. VESSEY,* J. GOLDENBERG,* AND P. K. HOGUE,* San Francisco, Calif.

Evidence from in vitro studies indicates that the activities of several different isoenzymes of hepatic microsomal glucuronyltransferase (GT) can be regulated by modification of enzyme-phospholipid interactions in the microsomal membrane. In normal Wistar rats the activity of GT with *o*-aminophenol (OAP) as aglycone is increased fourfold by treatment of microsomes with Triton X-100 and twofold by phospholipase A (PLA). With *o*-aminobenzoate (OAB) as aglycone these treatments increase activity twofold. In contrast, the activities of GT with OAP and OAB were unaffected in liver microsomes from homozygous Gunn rats treated with PLA or Triton X-100. Although the rates of glucuronidation of OAP and OAB are abnormally low in these rats, the lack of stimulation of activity by PLA or Triton X-100 was not a nonspecific reflection of abnormal enzyme function since the activities of GT with OAB and OAP as glucuronyl acceptors were raised to near normal levels by treatment of microsomes with diethylnitrosamine. Also, despite the reported normal level of GT activity with *p*-nitrophenol (PNP) as aglycone in Gunn rats, the regulatory functions of this isoenzyme of GT were abnormal in these animals. Thus, treatment of hepatic microsomes from normal Wistar or heterozygous Gunn rats with PLA and Triton X-100 increased the rate of PNP glucuronidation 12- and 20-fold respectively; but in homozygous Gunn rats GT activity with PNP as glucuronyl acceptor was unaffected by these agents. It is not yet certain that the defect in the Gunn rat is limited to faulty regulation of GT; but these studies suggest that the decreased activities of these enzymes in Gunn rat liver microsomes result from abnormal tertiary and/or quaternary structures rather than alterations of the primary structure in the region of the active site. It is significant that a qualitatively similar defect occurs in at least three different isoenzymes of GT.

350. Effect of Prostaglandin E_1 (PGE_1) on Toad Bladder Adenyl Cyclase; the Critical Role of Magnesium. ZOLTAN ZARDAY,* JUNE GOUAUX,* AND RICHARD M. HAYS, New York.

The rate of cyclic AMP formation within many cells is regulated by hormonal stimulation and by the action of the prostaglandins. In toad bladder epithelial cell homogenates, vasopressin produces a 10-fold stimulation of adenyl cyclase activity; it has not been possible, however, to demonstrate the anticipated inhibitory effect of PGE_1 on the vasopressin stimulated enzyme, despite the sharp inhibition of water movement by PGE_1 in the intact tissue. We wish to report a significant regulatory role for magnesium in determining the extent and direction of the effect of PGE_1 on adenyl cyclase. Formation of cyclic AMP[32] was determined in phosphate-buffered epithelial cell homogenates, in the presence of maximal (10^{-7} M) vasopressin stimulation. When 5 mM magnesium was present in the medium, PGE_1 (10^{-6} M) had no effect on adenyl cyclase activity. Decreasing magnesium to 2.5 mM resulted in a small (7%), but significant inhibition by PGE_1 ($\Delta = -27 \pm 7$ pmoles/mg protein per 30 min; $P < 0.01$). A greater (11%) inhibition of enzyme activation by PGE_1 occurred at 1 mM magnesium ($\Delta = -57 \pm 21$pmoles, $P < 0.05$). Increasing magnesium to 20 mM had the opposite effect: PGE_1 significantly increased vasopressin stimulation of adenyl cyclase ($\Delta = +38 \pm 11$ pmoles; $P < 0.05$). The regression line for all experimental points was highly significant ($P < 0.001$). We would conclude that the magnesium concentration at or near the cell membrane is critical in determining the interaction of PGE_1 and adenyl cyclase in this, and possibly other tissues, and that a strictly maintained magnesium level may be essential for the regulation of hormone action. (Research supported by grants from NIH.)

351. Radioimmunoassay of IgE Antibody to Ragweed Antigen E and Total IgE and Their Relationship to Leukocyte Sensitivity of Ragweed-Sensitive Patients. C. R. ZEISS, JR.,* J. PRUZANSKY,* AND R. PATTERSON,** Chicago, Ill.

The experimental measurement which correlates best with the clinical sensitivity of allergic patients is leukocyte sensitivity. This is the minimum quantity of antigen inducing release of 50% of the histamine of washed leukocytes. 10 allergic patients were selected whose leukocytes released at least 50% of their histamine in the presence of optimal concentrations of antigen. Two parameters which could affect the degree of leukocyte sensitivity were determined: (*a*) serum IgE antibody against ragweed, which sensitizes normal mast cells and besophils in vitro and (*b*) total serum IgE, which might compete with specific IgE for surface receptor sites. A new solid-phase assay was developed for measuring IgE antibody to ragweed. Polystyrene tubes were successively coated with purified myeloma IgE and rabbit anti-IgE. To the tubes were added preincubated mixtures of a constant volume of patient serum with increasing quantities of trace-labeled antigen E. The counts bound to the tubes are directly related to the antigen binding capacity of the IgE antibody. Sensitivity of the leukocytes of the 10 *untreated* patients varied over a 180-fold range while serum IgE antibody to antigen E varied 120-fold. There was no correlation between values for each patient. Similarly total serum IgE varied 40-fold, but there was no relationship between this parameter and leukocyte sensitivity. The ratio of specific to total IgE, which combines both parameters, was also unrelated to cell sensitivity. The relationship between cell surface and fluid phase antibody is apparently complex and may involve heterogeneity of cell receptors for IgE.

(Research supported by grants from NIH [AI 00057, AI 6139] and Bazley Trust.)

352. Pulmonary Function with Dialysis of Patients in Chronic Renal and Heart Failure. A. ZIDULKA,* P. DESPAS,* J. MILIC-EMILI,* AND N. R. ANTHONISEN,* Montreal, Canada (introduced by D. V. Bates).

In six seated patients with chronic renal failure and concomitant pulmonary vascular congestion we have measured alveolar-arterial oxygen differences (A-a D_{O_2}) and the regional lung function using xenon-133. In four patients the measurements were also made after hemodialysis. Before dialysis all patients had decreased ventilation to the lung bases and in 4/5 patients the basilar lung regions were also hypoperfused. Three patients had grossly elevated regional residual volumes at the lung bases, suggesting gas trapping. These patients had an increased "closing volume" and (A-a) D_{O_2}. After dialysis 3/4 patients studied increased both the ventilation and perfusion to the lung bases. This resulted in a distribution of regional ventilation to perfusion ratios similar to that before dialysis and their (A-a) D_{O_2} did not change. In the fourth patient the basilar ventilation but not the perfusion increased, resulting in further mismatching of ventilation and perfusion distribution and (A-a) D_{O_2} increased. The "closing volume" decreased with dialysis in the two patients in whom this parameter was elevated. We conclude that some patients with chronic renal and heart failure have decreased basilar ventilation and perfusion with a concomitant increase in (A-a) D_{O_2} and basilar gas trapping. These abnormalities can be at least partially reversed by dialysis and therefore are presumably caused by interstitial pulmonary edema. (Research supported by the Canadian Tuberculosis and Respiratory Disease Association and the Medical Research Council of Canada.)

353. Immunologic Studies in Tropical Splenomegaly Syndrome. JOHN L. ZIEGLER* AND RAMON KHIROYA,* Kampala, Uganda (introduced by Paul P. Carbone).

Tropical splenomegaly syndrome (TSS) is characterized by idiopathic splenic enlargement, hypersplenism, polyclonal macroglobulinemia, and frequently, Kupffer cell hyperplasia with sinusoidal lymphocytic infiltration in liver biopsies. Epidemiologic and serologic evidence suggesting a malarial etiology is supported in part by clinical improvement after prolonged antimalarial prophylaxis. This study reports the results of immunologic investigations designed to test the hypothesis that TSS is produced by prolonged stimulation of the reticuloendothelial (RE) system by circulating immune complexes. Sera obtained from 10 patients with TSS, and from 10 neighbor controls without splenomegaly matched for age, sex, and tribe, were analyzed. All TSS sera had anticomplementary activity, hypocomplementemia (C3), elevated levels of IgM, high titers of rheumatoid-like factor, and cryoglobulinemia, differing significantly from the findings in control sera. Analysis of cryoglobulins in Ouchterlony plates using a panel of antisera revealed mixed IgM-IgG in six, IgM alone in two, and IgM, IgG plus complement in two, the latter being present in individuals with the largest spleens. No precipitin lines were observed between cryoglobulin and sera containing high titers of malarial antibody, or purified *Plasmodium falciparum* antigen. Immunofluorescent studies in liver biopsies obtained from eight patients with TSS have revealed gamma globulin within the Kupffer cells. We conclude from these preliminary data that: (*a*) patients with TSS have circulating cold-precipitable immune complexes the nature of which are yet unknown; (*b*) malarial antigen or antibody could not be detected in the cryoprecipitates by the present techniques; and (*c*) gamma globulin appears to be phagocytosed by Kupffer cells and may account primarily for the RE hypertrophy resulting in TSS. (Research supported by Contract No. Ph 43-67-1343 from the NIH.)

354. Interaction of Complement, Platelets, and the Blood Coagulation System. THEODORE S. ZIMMERMAN* AND HANS J. MÜLLER-EBERHARD, La Jolla, Calif.

The coagulant activity of native platelets is largely unavailable for the coagulation process. Recent investigations have provided evidence that the action of complement makes platelet coagulant activity available and that failure of this action is responsible for the coagulation abnormality in C6-deficient rabbit blood. Our investigations now indicate that complement-induced evolution of platelet coagulant activity is primarily a result of complement protein-platelet membrane interaction. It is not the consequence of the irreversible platelet aggregation which may also be induced by complement. Activation of complement in normal platelet-rich rabbit plasma by the recently described C3 proactivator pathway with inulin or endotoxin results in the evolution of platelet coagulant activity which exceeds by greater than 20-fold that resulting from ADP, collagen, or kaolin treatment. In contrast, evolution of platelet coagulant activity does not occur after activation of complement in C6-deficient platelet-rich plasma, though partial aggregation, probably caused by C3 binding, does occur. However, subsequent addition of small quantities of highly purified C6 results in development of clot promoting activity as well as maximal and irreversible aggregation. Prostaglandin E_1, in quantities exceeding those required to completely block ADP triggered aggregation, has no appreciable effect on the evolution of clot promoting activity though it markedly retards aggregation. These studies indicate that the alterations produced in platelets by early complement components are of lesser significance to the coagulation process. However, subsequent reaction with C5, C6, and C7, and possibly with C8 and C9, produces profound alterations in the platelet membrane resulting in the availability of platelet coagulant activity as well as irreversible aggregation.