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183. ACTH Release Induced by Metyrapone in Diphenylhydantoin (Dilantin)-Treated Patients. A. WAYNE MEIKLE,* WILLIAM JUBIZ,* SHIGERU MATSUKURA,* CHARLES D. WEST,** AND FRANK H. TYLER,** Salt Lake City, Utah.

After chronic diphenylhydantoin (DPH) therapy the conventional oral metyrapone test is known to produce subnormal responses. In this study we investigated whether DPH interferes with inhibition of 11β-hydroxylation induced by metyrapone, the release of ACTH, the absorption of oral metyrapone, or its splanchnic metabolism. With the standard oral metyrapone dose, 750 mg q. 4 hr 6 times, the decrease of plasma cortisol and the increases of 11-deoxycortisol, ACTH, and free metyrapone in plasma were all significantly less in the DPH-treated group. The double oral dose, 750 mg q. 2 hr 12 times, and the standard 5.0 g i.v. infusion produced normal steroid and ACTH responses. The disappearance of i.v. administered metyrapone was not significantly faster. We concluded that after chronic DPH treatment (1) ACTH release is normal, (2) oral metyrapone produces a lower plasma level of metyrapone, and (3) the splanchnic metabolism of oral metyrapone and/or gastrointestinal absorption may be altered.

184. The Pulsatile Uptake of CO in the Lungs. HAROLD A. MENKES,* ROBERT M. ROGERS,* KAZUAKI SERA,* RICHARD W. HYDE,* ROBERT E. FORSTER, II, AND ARTHUR B. DUBOIS, Philadelphia, Pa.

Although blood flow into pulmonary capillaries is pulsatile during the cardiac cycle, it is not known whether the capillary volume is also pulsatile. To investigate this, we measured the uptake of CO in two subjects seated in a water-filled body plethysmograph. The subject first breathed a gas mixture such that alveolar P_{O_2} and P_{CO_2} approached mixed venous levels. At this P_{O_2}, a volume of CO does not displace an equal volume of O_2 when it combines with hemoglobin. The subject then held his breath at FRC while mouth pressure, plethysmograph pressure, and EKG were monitored. After about 7 sec of breath holding, the subject did a brief Valsalva maneuver. The amplification of the mouth pressure signal was varied until the electrical sum of mouth pressure and plethysmograph pressure was flat during the Valsalva maneuver. Thus the effects of compression and decompression of gas in the lungs were abolished during breath holding. The inspired mixture was varied until there was a minimal change in lung volume produced by gas exchange during breath holding. After suitable controls had been obtained, the inspired gas mixtures were changed to include 2.0% CO and 1% Ne. The rate of CO absorption was found to be pulsatile. A peak uptake rate of 2 to 3 times the average rate occurred between 0.3 and 0.4 sec after the R wave of the EKG. A minimum uptake rate of less than one-half the average rate occurred before and during the QRS complex. Since the rate of absorption of CO is a function of pulmonary capillary blood volume, our data suggest that pulmonary capillary blood volume is pulsatile during the cardiac cycle.

185. Sexual Ateliotic Dwarfism and Diabetes Mellitus. THOMAS J. MERIMEE,* JUDITH G. HALL,* DAVID L. RIMOIN,* SAMUEL E. FINEBERG,* AND VICTOR A. McKUSICK, Baltimore, Md., and Boston, Mass.

Glucose intolerance and other metabolic abnormalities of diabetes mellitus were found to be common in dwarfs with a hereditary deficiency of human growth hormone (HGH). However, no retinal lesions were seen by indirect ophthalmoscopic examination. A comparative study was made between 38 sexual ateliotics, 52 healthy controls, and 38 non-obese, age-matched diabetics. The dwarfs were of two types: in type I, dwarfism was generally inherited as a recessive trait; in type II, as a dominant trait. The results are as follows: (1) Insulin was measured in 28 of the 38 diabetics. In 10, insulin antibodies prevented this determination. 19 diabetics exhibited insulinopenia. Mean maximal plasma insulin response: (*a*) to glucose: controls 77.3 ±2.9 μU/ml, diabetics 41.8 ±4.6 μU/ml., type I dwarfs 43.3 ±5.8 μU/ml; (*b*) to arginine: controls 90.4 ±8.6 μU/ml, diabetics 30.3 ±9.1 μU/ml, type I dwarfs 29.3 ±5.5 μU/ml. Similar variations occurred after glucose-beef meals. (2) Nine diabetics and all type II dwarfs had normal or increased plasma insulin responses to glucose. The mean maximal plasma concentrations of insulin after glucose were respectively 119.1 ±9.3 μU/ml and 153 ±29.9 μU/ml. Hyperinsulinism also occurred after arginine and after glucose-beef meals. (3) The mean increment of plasma glucose was greater after glucose-beef meals in both groups of diabetics and dwarfs than in controls. (4) A significant number of diabetics and dwarfs exhibited hypercholesterolemia and hypertriglyceridemia. (5) 40% of the diabetics had angiopathic or proliferative changes of the retina. No lesions were seen in the dwarfs. Carbohydrate, insulin, and lipid abnormalities similar to those seen in diabetes mellitus do not result in retinal abnormalities in subjects chronically deficient in HGH. (Grant support: NIH, NF-MOD.)

186. Solubilization and Partial Characterization of the Erythrocyte Phytohemagglutinin Binding Site. CHARLES F. MOLDOW* AND ROBERT SILBER, New York, N. Y.

The hemagglutinating and mitogenic properties of phytohemagglutinin (PHA) have been extensively investigated. Little is known about the cellular receptors for PHA. The present work describes the complete solubilization of erythrocyte ghosts with liberation and purification of a phytohemagglutinin binding site. Red cell ghosts were suspended in 4 M urea and their free amino groups eliminated by exposure to an excess of succinic anhydride. This treatment completely solubilized the ghosts as judged from the clearing of the suspension, its ability to pass through a 0.22 micron pore size filter, and its failure to sediment after centrifugation at 11,000 *g*. The solution contained 95% of the protein and all the membrane cholesterol. It retained the ability to inhibit hemagglutination by PHA. The PHA binding site, assayed in a hemagglutination inhibition system, is heat stable and nondialyzable. It is precipitated with 30% saturated ammonium sulfate and is stable to storage at 4°C over a 4 month period. After the removal of lipids by *n*-butanol or ethanol-ether, the material no longer inhibits hemaggluttina-

tion by PHA. A lipid may therefore be present at the binding site or affect the steric configuration of the receptor. The fraction containing the binding site has a molecular weight greater than 200,000 as determined by gel filtration and gradient ultracentrifugation. When ^{125}I-labeled PHA was mixed with this partially purified material, an increase in sedimentation velocity of the labeled PHA was noted on sucrose gradient ultracentrifugation. This observation suggests the formation of soluble PHA–membrane receptor site complexes and facilitates further kinetic and biochemical studies of the cell-PHA interaction.

187. Studies on the Regulation of Granulopoiesis. ALEC MORLEY* AND FREDERICK STOHLMAN, JR., Boston, Mass.

In order to study the mechanism by which granulopoiesis is regulated in response to demand, neutropenia was induced by irradiating mice in which one limb had been shielded. The neutrophil count fell to a minimum 4–6 days after irradiation. The number of transplantable colony-forming units or stem cells in the shielded limb did not change measurably. Serial changes in granulopoiesis in this limb suggested that neutropenia resulted in acceleration of the rate of release of segmented neutrophils from the marrow and an increase in the rate of differentiation of stem cells into the myeloblast-promyelocyte compartment (MPC). The MPC reached a maximum size of 4 times normal on days 4–6; cells that entered it subsequently proliferated and matured. Increase in size of the MPC on day 4 correlated better with neutropenia than with depletion of segmented marrow neutrophils, suggesting that the circulating neutrophil number regulates stem cell differentiation directly through a humoral mechanism rather than indirectly through the marrow store of segmented neutrophils. Erythropoiesis declined as granulopoietic hyperplasia developed and a greater increase in granulopoiesis developed in polycythemic animals, suggesting that erythropoiesis and granulopoiesis competed for stem cells. In contrast to the shielded limb, erythroid hyperplasia developed in the irradiated corresponding limb. Either the stem cells which migrated from the shielded area were or became qualitatively different from those remaining, or the shielded normal marrow exerted some influence over the direction of stem cell differentiation.

188. Effects of Cyclic 3′,5′-GMP (cG) on the Concentration of Cyclic 3′,5′-AMP (cA) in Fat Cells. FERID MURAD,* VINCENT MANGANIELLO,* AND MARTHA VAUGHAN, Bethesda, Md.

In addition to cA, other cyclic nucleotides probably are important in metabolic regulation. We had previously found that under certain conditions cG inhibited lipolysis stimulated by cA, theophylline, epinephrine, ACTH, or glucagon. We have now determined the concentration of cA in fat cells (plus medium) incubated for 50–60 min under conditions in which cG either stimulates or inhibits lipolysis. The cA content plus medium, 0.81 ±0.13 (mean ±SEM) mμmole/g cells (n = 20), was the same in Krebs-Ringer phosphate (KRP) or all Na^+ medium. In KRP medium 1 mM cG increased cA levels 7- to 20-fold. In the presence of theophylline cG produced a similar increase in cA at the same time that it inhibited glycerol production 50–70%. The concentrations of cA after incubation with cG were several times greater than the maximal levels attained with hormonal stimulation. In KRP, high concentrations of epinephrine, ACTH, and glucagon, singly or in combination, or theophylline resulted in a 2- to 3-fold increase in cA levels and maximal rates of glycerol production. The effect of cG on cA concentration increased with time of incubation (5–60 min) and with cG concentration (0.05–5 mM). The effects of cG on glycerol production or cA levels were not mimicked by 1 mM 5′-GMP, 2′-GMP, or 3′-GMP. In all Na^+ medium, 1 mM cG slightly stimulated glycerol production but increased cA concentration to the same high level as it did in KRP. Both these effects of cG were demonstrable also in the presence of theophylline, which itself increased glycerol production and cA concentration. Our observations raise two major questions: How does exogenous cG induce the remarkable elevation in cA concentration? How does it inhibit lipolysis in the presence of high levels of cA which are usually associated with stimulation of lipolysis?

189. The Stem Cell: A Functional and Morphological Identification by Electron Microscopy. MARTIN J. MURPHY, JR.,* ALBERT S. GORDON,* AND JOHN F. BERTLES, New York, N. Y.

The stem cell, despite numerous attempts at precise identification, has remained more a concept than an entity. We have utilized here the known ability of homologous marrow cells to prevent death in otherwise lethally X-irradiated mice by "colonizing" recipients' spleens. Those particular multipotential marrow cells from which splenic clones arise are, by definition, stem cells, capable of self perpetuation and diversified differentiation into erythrocytes, granulocytes, and megakaryocytes. The proportion of marrow cells functioning as stem cells peaks during posthypoxic polycythemia. Accordingly, CF-1 mice rendered hypoxic by exposure to 0.4 atm for 5 days were returned to ambient pressure and then given 10 μc each of ^{3}H-thymidine i.v. daily for 3 days. Their femoral and tibial marrow cells were layered on linear gradients of Ficoll (density 1.012–1.086) and centrifuged at 11,000 rpm for 20 min at 4°C in a Spinco SW-25.1 bucket rotor. Densities of fractions collected by gravity were calculated from refractive indices. $1–4 \times 10^6$ Ficoll-free cells from light (density 1.038–1.058) and heavy (density 1.064–1.076) fractions were injected through siliconized glass needles (I.D. 100 μ at tip) into the spleens of CF-1 mice which 48 hr previously had received 900 R of whole-body X-irradiation. Animals were sequentially killed from 15 min to 8 days later and spleens were prepared for radioautographic examination by light microscopy and electron microscopy. In radioautographs of preinjection cell preparations, labeled lymphoid mononuclears were predominantly cells displaying the specific morphologic characteristics of mammalian transitional lymphocytes. Light-fraction cells, over 90% transitional lymphocytes and devoid of erythroid elements, were 20 times more competent at colonizing spleens (erythrocyte, granulocyte, and megakaryocyte series) than were heavy-fraction cells. Radioautography of postinjection spleens showed labeled transitional lymphocytes early, labeled proerythroblasts later. Our studies are consonant with reso-

lution of the stem cell concept into an entity now designated the transitional lymphocyte. (Research was supported by grants from the NIH and the Whitehall Foundation.)

190. A Method for Detection and Localization of Myocardial Infarcts. JOHN MURRAY* AND NELL STEIN,* Minneapolis, Minn. (introduced by Richard Ebert).

After myocardial infarction, antibodies to myocardial proteins appear in the serum. It seemed reasonable to label antimyocardial antibody and test its capacity to localize in infarcts. Antibody was raised by injecting extracts of dog myocardium into rabbits in which immunological tolerance to other tissues had been produced. The serum was harvested and the IgG extracted and labeled with ^{131}I. The thyroid uptake of the dogs was blocked with potassium iodide, and myocardial infarction was produced by injecting glass spheres into the coronary arteries. 1 ml of labeled IgG was injected intravenously into the dogs at peak rise of LDH, and the animals were killed at various times later. Two control groups of dogs were treated similarly except that one received ^{131}I alone and the other IgG labeled with ^{131}I from nonimmunized animals. The hearts and other tissues were removed, the infarcts were separated, and radioactivity was determined in all specimens. All tissues, including blood, contained radioactivity, but there was preferential accumulation only in infarcts from animals receiving labeled IgG from immunized animals. The peak localization was 48 hr after injection, when 6 to 11 times indifferent tissue activity was present in infarcts. No such change was observed in controls or in noninfarcted myocardium. These findings suggest that it is technically possible to label and scan infarcts with short-lived high-activity isotopes such as technetium attached to appropriate antibody against myocardial protein.

191. Oxygen Transport during Upright Exercise in Patients with Angina Pectoris. JOHN A. MURRAY,* LORING BE ROWELL,* IRVING KASSER,* AND ROBERT A. BRUCE,** Seattle Wash.

To define the mechanisms of limited oxygen consumption ($\dot{V}O_2$) and circulatory oxygen transport in atherosclerotic heart disease with angina pectoris (AP), 10 men with coronary occlusive lesions (>50% stenosis) and AP exercised on a treadmill at increasing work loads to symptom-limited capacity (SLC). $\dot{V}O_2$, direct Fick cardiac output ($\dot{Q}$), heart rate (HR), and mean aortic blood pressure were measured at each work load and SLC. Mean values at SLC were: $\dot{V}O_2$, 1.92 liters/min, 22 cc/kg per min; HR, 139 bpm; stroke volume (SV), 89 cc; $\dot{Q}$, 12.4 liters/min; and arteriovenous oxygen difference (AVD), 132 ml/liter. Values in normal age-matched men are 38 cc/kg per min and 173 bpm for $\dot{V}O_2$ and HR, respectively. Reductions in $\dot{Q}$ were present at all levels of $\dot{V}O_2$ and before the onset of AP, primarily owing to reduced SV. $\dot{Q}$ at SLC was further reduced by a decreasing SV and 22% reduction in maximal HR. AVD was elevated at all levels of $\dot{V}O_2$. Mean aortic pressure rose slightly during progressive exercise. It is concluded that AP is preceded by premature but insufficient peripheral oxygen extraction to compensate for an impaired $\dot{Q}$. (Supported by grant-in-aid HE-09773 from the USPHS, National Heart Institute.)

192. The Effect of Exogenous RNA on Phytohemagglutinin (PHA) Transformation of Human Lymphocytes. PAUL NEIMAN* AND GAIL LARSON,* Seattle, Wash. (introduced by E. D. Thomas).

PHA induces DNA synthesis in vitro in the normal circulating lymphocyte along with many other morphological and functional changes. In chronic lymphocytic leukemia (CLL) this response is impaired. In this study the effect of exogenous RNA on the PHA response of normal and CLL human lymphocytes was tested. Isolated lymphocytes were incubated in hypertonic buffered sucrose for 15 min with RNA preparations and then washed free of RNA. Controls contained no RNA or RNA plus ribonuclease (RNase). When RNA uniformly labeled with ^3H-dimethyl sulfate was used to detect entrance into the lymphocytes, it was found that most of the radioactivity remained acid precipitable and alkali labile and was concentrated in the cell nucleus at the end of incubation. Polyacrylamide gel electrophoresis of the labeled RNA on both sides of the cell membrane demonstrated that smaller molecules were taken up more efficiently than larger ones. Incubated lymphocytes were cultured with PHA and the extent of their response was measured by ^3H-thymidine incorporation into DNA. Undegraded RNA from normal lymphocytes, dog liver, *E. coli*, and yeast enhanced the effect of PHA 2- to 5-fold over that of control PHA cultures. In addition, RNA accelerated the peak response to PHA by 24 hr. RNA itself was shown not to be a mitogen. Partial digestion of the RNA with RNase further enhanced the PHA response to about 10-fold that of controls, whereas complete digestion abolished the RNA effect. CLL lymphocytes did not respond to PHA even after incubation with RNA. These observations indicate a nonspecific enhancing effect of polynucleotides upon the PHA response of normal lymphocytes and suggest a role for these macromolecules in the modulation of the genetic apparatus.

193. The Metabolism of 5-Methyltetrahydrofolate in Patients with Pernicious Anemia. P. F. NIXON,* M. LEVITT,* E. KIRSCHNER,* P. O'BRIEN,* AND J. R. BERTINO, New Haven, Conn.

In order to study the metabolism of 5-methyltetrahydrofolate (MF) in B_{12} deficiency, a condition in which folate metabolism is known to be impaired, the active diastereoisomer of MF, labeled with ^{14}C (methyl) and ^3H (3',5'), was prepared by an enzymatic and chemical procedure. In three control subjects, 5 μg/kg was administered by a rapid intravenous injection. Both ^{14}C and ^3H disappeared from serum in two distinct exponential phases, a rapid initial phase lasting 15–20 min, and a slower exponential phase with a half time of 3–4 hr. Approximately 15% of the administered radiolabel was excreted within 6 hr, and only small amounts thereafter. When the same dose was given orally, the compound was well absorbed, a peak blood level being reached 1 hr after ingestion. An unchanged $^3H/^{14}C$ ratio and chromatography of the serum on DEAE-Sephadex indicated that the MF was absorbed unchanged. In these patients, a small amount of a folate compound (^3H but not ^{14}C labeled) could be displaced from body stores by the intravenous administration of 10 mg of folic acid or methotrexate. Unlike folic acid, which is cleared more rapidly from the plasma in B_{12}-deficient

patients than in normal subjects, both ^{14}C and ^{3}H were observed to disappear from the plasma more slowly than normal in two patients with untreated pernicious anemia. In addition, 2–3 times more radioactivity than that excreted by the controls was found in the urine in the corresponding time period. Chromatography of these urines indicated that the major folate form excreted was not unchanged MF, as it was in urine of control subjects. The folate form excreted in the B_{12}-deficient patients was doubly radiolabeled and was eluted from the column by buffer of a lower ionic strength than that which eluted MF. These findings support the hypothesis that the metabolism of MF is impaired in B_{12} deficiency; however, the nature of the folate form found in urine, and its significance, have yet to be determined. (Research supported by grants from the NIH and the American Cancer Society.)

194. Natural History of Herpesvirus Hominis Encephalitis and Treatment with Idoxuridine. David C. Nolan,* Mary M. Carruthers,* and A. Martin Lerner, Detroit, Mich.

14 patients with herpesvirus hominis (HSV) encephalitis were studied during 1968. This diagnosis was made when a patient with encephalitis had frontal and/or temporal lobe findings, had sterile CSF, and either had HSV isolated at brain biopsy, developed a 4-fold rise in HSV complement-fixing antibodies during the course of illness, or had a ratio of complement-requiring neutralizing antibody to non-complement-requiring neutralizing antibody of 4 or greater. The patients were 7 to 62 yr old with a predominance in the fifth decade. 11 were female. Headache, fever, and upper respiratory symptoms were common initial complaints. All showed depressed consciousness and nine became comatose. Confusion, disorientation, memory deficit, personality change, bizarre appetite, hallucinations, aphasia, ataxia, nystagmus, focal and grand mal seizures, hypesthesia, hemiparesis, and nuchal rigidity occurred. Coma and seizures were signs of a poor prognosis. Electroencephalograms often showed temporal or frontal lobe foci; CSF, bilateral carotid angiograms, and brain scans showed variable changes. Peripheral white blood cell counts ranged from 5800 to 21,100, usually with "shifts to the left." Intravenous idoxuridine (IDU) was administered to six patients; two died before the 5 day course of therapy was completed. Four of eight non-IDU-treated patients survived, but three of them retained incapacitating neurologic deficits. All four of the fully treated patients survived and were neurologically intact. Three of the treated intact survivors had been comatose. All four nontreated patients who became comatose died. IDU toxicity was transient and consisted of stomatitis, leukopenia, thrombocytopenia, and alopecia. (Research supported by grants from the NIH [AI-00261 and AI-05721] and from the Detroit General Hospital Research Corporation [M-828].)

195. Mechanism of Action of Estrogen II: Regulation of Growth and Gene Expression in Female Secondary Sex Tissue. Bert W. O'Malley,* Itamar B. Abrass,* Andreas C. Chrambach,* and Michael G. Rosenfeld,* Bethesda, Md. (introduced by Mortimer B. Lipsett**).

Estrogens control protein synthesis and growth of female reproductive tissues. We have previously shown that diethylstilbestrol (DES) stimulates chick oviduct growth, epithelial proliferation into three distinct new cell types, and the production of new specific proteins, i.e. ovalbumins and lysozyme. We have attempted to elucidate the mechanism of estrogen action by examining the changing populations of structural proteins concomitantly with quantitative and qualitative assay of messenger RNA species in the oviduct. Since the appearance or disappearance of proteins is a potential means for estimating the state of differentiation of a developing tissue, we have analyzed oviduct protein patterns obtained by polyacrylamide gel electrophoresis in single pores and linear pore gradients (5–15% acrylamide) by staining and radioautography. These analyses show that DES promotes the synthesis of a new population of major oviduct proteins during early growth and differentiation which differ quantitatively and qualitatively from those of the immature unstimulated oviduct. Using molecular hybridization (RNA-DNA), we find that DES also stimulates the production of nuclear polyribonucleotide sequences which are normally found only in the differentiated gland; however, at no time during estrogen-mediated oviduct growth are sequences of nuclear RNA "dropped out." Furthermore, oviduct nuclear "messenger RNA activity" assayed in vitro on ribosomes of a mutant strain of *E. coli* (RNase free) increases after DES administration coordinately with the changing populations of oviduct soluble proteins. Polyacrylamide gel analysis reveals differences in the peptides synthesized from mRNA before and after DES. Finally, DES does not increase oviduct adenyl cyclase or cyclic 3′,5′-AMP levels. In summary: (1) DES stimulates oviduct growth and the appearance of a new population of proteins; (2) DES stimulates production of new sequences of nuclear polyribonucleotides and the synthesis of mRNA; (3) DES does not act by stimulating adenyl cyclase. These results suggest that estrogen regulates oviduct growth by stimulating gene expression through transcription of new messenger RNAs.

196. Studies on the Molecular Characteristics of Human Urinary Erythropoietin. Michael B. O'Sullivan,* Gerald J. Gleich,* and James W. Linman, Rochester, Minn.

Erythropoietin has not yet been isolated in pure form; however, the existence of a relatively specific bioassay has made possible the study of some of the molecular properties of this erythropoietic substance. Based on different techniques used singly (inactivation by ionizing radiation, ultracentrifugation, and gel filtration), estimates of the molecular weight of erythropoietin have ranged from 10,000 to 68,000. We have used gel filtration combined with density gradient ultracentrifugation to estimate the Stokes radius, frictional coefficient, and molecular weight of human urinary erythropoietin. Erythropoietic activity was quantified in mice with erythrocytosis induced by hypoxic hypoxia; ^{59}Fe incorporation in hemoglobin was the parameter of response. Urine from severely anemic patients collected with 0.1% phenol as preservative and concentrated by dialysis against polyethylene glycol served as the source of erythropoietin. Gel filtration on Sephadex G-100 columns calibrated with protein markers of known Stokes radii (ribonuclease cytochrome *c*, chymotrypsinogen, ovalbumin, and human serum albumin) yielded a Stokes radius of 33 A for erythropoietin. Radioiodinated

serum albumin and catalase were added as markers to the urinary concentrates, and ultracentrifugation was carried out in a 5 to 15% linear sucrose gradient. A sedimentation coefficient of 3.8S was obtained for erythropoietin. On the basis of these data and assuming a partial specific volume of 0.725, the frictional coefficient of erythropoietin was calculated to be 2.9 with a molecular weight of 52,000. (Research supported in part by a grant from the NIH.)

197. Limb Vascular Response to Intrabrachial-Arterial Magnesium Sulfate Infusion in Normotensive and Hypertensive Men. Henry W. Overbeck,* Robert M. Daugherty, Jr.,* and Francis J. Haddy,** East Lansing, Mich.

Intrabrachial-arterial infusions of isotonic $MgSO_4$ were made in 13 normotensive and 13 essential hypertensive men, because it has been suggested that defects in arteriolar Mg^{++} metabolism play a role in the genesis of essential hypertension, and abnormalities in vascular response to Mg^{++} might be evidence for such a defect. Limb blood flow responses to jet infusions (8 ml/min) composed of 0.25% $MgSO_4$ in isotonic NaCl (289 mOsm/liter) were measured by RIHSA dilution. No measurable systemic effects were produced. Limb venous $[Mg^{++}]$ increased by a mean of 3.47 mEq/liter. $MgSO_4$ decreased limb vascular resistance (mm Hg/ml flow per 100 cc limb volume per min) in all subjects: mean $\pm$SD = —12.14 ±9.12 (hypertensives), —5.46 ±4.04 (normotensives). Response in hypertensives was significantly greater ($P < 0.05$) than response in normotensives. Thus interpreted, these data would suggest hyperresponsiveness to endogenous Mg^{++} and a possible defect in vascular Mg^{++} metabolism. In addition, this hyperresponsiveness to endogenous vasodilators would oppose coexisting hyperresponsiveness to endogenous vasoconstrictors reported in hypertensives. However, there was a significant ($P < 0.01$) positive correlation between initial limb vascular resistance and magnitude of evoked resistance change. Data adjusted for this source of variation were (mean ±SD): —9.40 ±4.3 (hypertensives), —7.66 ±2.83 (normotensives); and there was no significant difference between hypertensives and normotensives ($P > 0.2$). Thus interpreted, these data fail to provide evidence for a defect in arteriolar Mg^{++} metabolism in essential hypertension. These data illustrate the fact that in studies comparing groups with different levels of vascular resistance, conclusions are dependent on method of interpretation. (Research supported by grants from the NIH and the VA.)

198. Absence of Potassium Loss in Hypoxic Rat Hearts. Ernest Page and Melvin E. Klegerman,* Chicago, Ill.

We have reexamined the classical observations that hypoxic cardiac cells lose K in exchange for extracellular Na. In isolated, working rat hearts perfused with Krebs solution at 37°C the concentration of oxygen was reduced from the control value of 95% to 20%, 10%, or 0 for 10–30 min or until contractions ceased. In hearts preequilibrated with ^{42}K before induction of hypoxia, the efflux of ^{42}K was measured during hypoxia. At the end of the hypoxic perfusion the sum (residual cardiac $^{42}K + {}^{42}K$ lost during hypoxia) was compared with the chemical K content of the heart. In separate experiments, intracellular concentrations of K, Na, and Cl ($[K]_i$, $[Na]_i$, and $[Cl]_i$) were determined using $^{35}SO_4$ and inulin to measure extracellular water. Hearts were not stimulated during hypoxia, but were allowed to slow spontaneously from control rates of 200–300/min to new, quasi stable rates of 50–80/min in hypoxia. In 45 experiments no net loss of K occurred and $[K]_i$ remained constant during hypoxic perfusion at the three oxygen concentrations tested and at all perfusion durations up to 30 min. Hypoxic perfusion for 20 min or longer produced a net uptake of Na and an increase in $[Na]_i$. These results contrast with the rapid net K loss observed * during hypoxic perfusion when rat hearts are stimulated to contract at 340/min instead of being allowed to slow down as in the present experiments. Our data suggest that under the latter conditions hypoxic cells may accumulate Na without concomitant K loss. (Supported by MIRU 43681334 and by a grant from the American Heart Association.)

* By Scheuer and Stezoski.

199. Evidence for a "Fourth Factor" Regulating Aldosterone Secretion. William P. Palmore,* Rosalind C. Anderson,* Patrick J. Mulrow,** and Norman C. Marieb,* New Haven, Conn.

ACTH, potassium, and renin influence aldosterone secretion. None of these factors explains the bilateral zona glomerulosa cell hyperplasia found in some patients with primary aldosteronism. This hyperplasia raises the possibility of a "fourth factor." We wish to present evidence from experimental studies in the rat which supports the existence of a "fourth factor." Bilateral nephrectomy for as long as 18 hr does not lower aldosterone secretion in sodium-deficient rats (normal 4 ±0.5, low sodium 19 ±2, nephrectomized low sodium 24 ±4 ng/min) (SE). Acute hypophysectomy (2–3 hr) plus nephrectomy also has no effect despite the marked decrease in corticosterone secretion. When rats are first nephrectomized and then sodium depleted by peritoneal dialysis, there is a 2-fold increase in aldosterone secretion as compared with similarly treated control rats that are sodium loaded (nephrectomized low sodium 10 ±1 ng/min, nephrectomized sodium loaded 4 ±1 ng/min). The increased secretion does not appear to be explained by changes in ACTH or serum electrolyte concentrations. In contrast, hypophysectomy prevents the response of aldosterone secretion to sodium depletion (hypox low sodium 1.4 ±0.5 ng/min). If rats are first sodium depleted, then hypophysectomized, aldosterone secretion in vivo and in vitro production are markedly decreased by 24–48 hr. Injections of ACTH or growth hormone alone, or thyroxine, cannot maintain the elevated response. ACTH and growth hormone together or rat pituitary gland extracts can maintain the elevated secretion. Rat pituitary gland extracts have growth hormone activity but no evidence of ACTH activity, thus indicating the presence of another factor which alone or in combination with growth hormone maintains aldosterone response to sodium depletion in hypophysectomized rats. In conclusion, these data suggest that another factor besides ACTH, potassium, and renin influences aldosterone secretion and that this "fourth factor" may originate in the pituitary gland. We speculate that this "fourth factor" may be playing a role in the pathogenesis of the bilateral zona glomerulosa cell hyperplasia that occurs in some patients with primary aldosteronism.

200. Acute Effect of NH_4Cl on Calcium Excretion in Man. A. M. PARFITT,* Brisbane, Australia, and Los Angeles, Calif. (introduced by M. H. Maxwell**).

The acute rise in urine Ca after NH_4Cl in dogs has been attributed to the concurrent rise in urine Na, although during chronic NH_4Cl loading in man, there is increased excretion of Ca but not Na. The relations between urine Ca, Na, and chloride (Cl) were studied during the short test of urinary acidification with NH_4Cl 100 mg/kg in 15 stone formers. The pooled 24 hr mean increments (Δ) were: Cl, 136 mEq ($P < 0.001$); Na, 61 mEq ($P < 0.001$); and Ca, 128 mg ($P < 0.01$). ΔCa showed significant positive correlations with control levels of both plasma and 24 hr urine Ca but not creatinine clearance, and with ΔCl and ΔNa. The subjects were classified into: (1) three with hypercalcemia, (2) five with hypercalciuria, and (3) seven with neither. The regression of Ca on Na in individual 2 hr periods was significant in only 5 of 15 cases and was significant ($P < 0.05$) but low in each group ($r = 0.393$ in 1, 0.454 in 2, 0.517 in 3). The slope (B) of the regression equation (μg/mEq) was 0.59 in 1, 1.03 in 2, and 0.87 in 3, and the intercept (A) (μg/min) was 439 in 1, 259 in 2, and 9 in 3. The corresponding values of r for the regression of Ca on Cl were 0.442 in 1, 0.451 in 2, and 0.626 in 3. In normals after mersalyl (Hg), $B = 0.439$ and $A = 69$ ($r = 0.99$), so that after NH_4Cl the values of r, both individually and collectively, are much lower and the values of either B or A, or both, are much higher than after Hg. These data indicate that (1) the response to NH_4Cl is conditioned by the subject's metabolic state; (2) the rise in urine Ca is not due only to the rise in urine Na; (3) the rise in urine Cl may be more important.

201. Isolation of a Zinc-Containing α_2-Macroglobulin from Human Serum. ALFRED F. PARISI* AND BERT L. VALLEE,** Boston, Mass.

Zinc in human serum occurs in concentrations comparable to those of copper and iron. The latter elements are each combined with specific proteins, i.e. ceruloplasmin and transferrin. Alterations in their states and/or concentrations are characteristic of diseases of copper and iron metabolism. In contrast, the relation of zinc to specific serum proteins is currently unknown, though decreased serum zinc concentration is a feature of a number of diseases. Advances in methods of protein fractionation and metal determination have now led to the identification of specific zinc proteins in human serum, one of which has been isolated. Macroglobulins (I) were separated from the smaller plasma proteins (II) by gel filtration on agarose beads. Zinc was detected in both fractions. A zinc-containing α_2- macroglobulin (α_2M) was separated from I and identified by immunoelectrophoresis. The zinc in II was associated chiefly with albumin and was not examined further. Several highly purified preparations of this α_2M from human serum or plasma consistently contained 400 to 700 μg zinc per gram protein. This α_2-macroglobulin is the first zinc metalloprotein to be identified and purified from human serum. Its functional properties are under study. The availability of this metalloprotein now affords the opportunity to examine pathological alterations of human serum zinc in terms of a discrete biochemical entity. (Supported by grant GM-15003 from the NIH.)

202. In Vitro Studies of Ventricular Asynchrony, Regional Ischemia, and Aneurysm, Utilizing the Cat Papillary Muscle. WILLIAM W. PARMLEY,* JOHN V. TYBERG,* AND EDMUND H. SONNENBLICK, Boston, Mass.

Although in the intact heart physiological asynchrony, and aneurysm with regional ischemia, have been noted, their impact on performance per se is unknown. Accordingly, 10 pairs of right ventricular papillary muscles were arranged in series in vitro so that tension and shortening of each muscle and their sum could be studied. The tension developed by the two muscles was less than the stronger, but more than the weaker, and the unloaded velocity was the sum of their individual velocities. At muscle lengths below the apex of the active length-tension curve, asynchronous stimulation (stimulation interval averaging 32 msec) produced greater tension (5.3 ±1.4% SEM) and rate of tension development (dP/dt) (9.4 ±2.4%) than did synchronous stimulation. Thus, the second muscle was stretched along its length-tension curve, increasing tension developed by the combination. Changes in force-velocity relations produced by asynchrony were characteristic of increased muscle length, i.e., isometric tension was augmented without changing maximum velocity of shortening (V_{max}). At the apex of the curve, this mechanical advantage was lost. A model of regional ischemia and aneurysm was produced by making one muscle hypoxic with N_2. After 20–30 min, tension and dP/dt of the hypoxic muscle fell (−60 ±2% and −57 ±4% respectively), with a smaller reduction in tension (−33 ±5%) and dP/dt (−32 ±6%) of the combination. The ischemic muscle contracted paradoxically, i.e., was stretched by the oxygenated muscle. If the hypoxic muscle was unstimulated, over-all tension deteriorated further (38 ±6%) and paradoxical motion worsened. After reoxygenation, recovery was virtually complete, although relaxation was prolonged immediately and only slowly returned to normal. Thus, limited asynchrony may produce extra force, and 30 min of ischemia with paradoxical motion was reversible. (Research supported by grants from the NIH and the American Heart Association.)

203. Pulmonary Extravascular Water Volumes. MORTON LEE PEARCE,* JAMES BEAZELL,* AND JILL ASHCRAFT,* Los Angeles, Calif. (introduced by Lucien B. Guze).

A problem central to the measurement of an extravascular water space in the lungs by the double isotope indicator-dilution technique is the equilibration of the water tag (THO) in the extravascular space in the time available before recirculation. The computation of this volume as the difference of mean transit times of an intravascular tag (RISA) and THO multiplied by flow needs no assumptions about the rate of mixing as long as all the THO can be accounted for. On the other hand, computation by the Newman "slope volume" method requires that the volume measured be almost instantaneously filled by the indicator and then washed out as a function of the rate of flow and the size of the volume which has been equilibrated. We find that the ratio of the transit time measurement to the slope measurement is 1.09 ±0.18 in control dogs, whereas it is 1.41 ±0.16 in dogs with pulmonary edema. When the transit time volumes were plotted against the slope volumes, the controls

showed a normal distribution. The edema values tended to fall into two groups, one close to the slope of the control values and the other considerably away from it. The recovery of THO is complete with respect to RISA in both cases. These data suggest diffusion equilibrium of THO in normal lungs, but not in pulmonary edema. Other experiments with $^{24}Na^{+}$ show complete recovery of this indicator in control dogs, but a significant loss in pulmonary edema, again suggesting that there may be a slowly equilibrated extravascular volume in pulmonary edema.

204. Insulin Stimulation of Amino Acid Transport in Bone. JAMES M. PHANG* AND THEODORE J. HAHN,* Bethesda, Md. (introduced by Gordon Zubrod**).

The regulation of bone metabolism by polypeptide hormones has recently received increased attention. In vitro studies of these hormones have demonstrated effects primarily on bone resorption. However, hormonal control of bone protein synthesis has not been emphasized. Although insulin has been shown to stimulate amino acid transport and protein synthesis in a variety of tissues, little is known of its action on bone. We now report that insulin markedly stimulates amino acid transport in fetal membranous bone. Fetal rat calvaria were isolated and incubated with ^{14}C-labeled amino acids by the method of Finerman and Rosenberg. In 30 min uptake studies we found that bovine crystalline insulin in the medium (0.2 U/ml) increased the distribution ratio (DR = [ICF]/[ECF]) of α-aminoisobutyric acid (AIB) by 37% ($P < 0.01$) and of proline by 35% ($P < 0.01$). Heat-denatured insulin was without effect on amino acid transport. Total tissue water and ECF space measured by ^{14}C-inulin were not changed by insulin. It was further found that stimulation of AIB uptake increased with longer exposure to insulin, did not require the presence of insulin in the medium after suitable insulin preincubation, and was abolished by puromycin. These findings suggest that insulin stimulation of amino acid transport is dependent on protein synthesis. In addition, insulin-stimulated increases in intracellular proline pools were accompanied by increased incorporation of proline into acid-alcohol–precipitable protein. Thus it appears that amino acid transport and protein synthesis in bone are both stimulated by insulin. The physiologic significance of these findings is not clear, but insulin may play a role in regulating protein and collagen synthesis in bone.

205. Appearance of Nondialyzable Chemotactic Activity after Intra-articular Injection of Monosodium Urate Crystals. PAULDING PHELPS,* Philadelphia, Pa. (introduced by Darwin J. Prockop).

Intra-articular injection of synthetic monosodium urate crystals into the canine joint results in a polymorphonuclear leukocyte (PMN)-dependent acute inflammatory response resembling gout. However, the mechanism by which PMN are attracted into the joint is not known. 3 min after intra-articular injection of a saline suspension of urate crystals into one knee of a dog and saline alone into the opposite knee, control aliquots were removed and compared with aspirates taken 90 min later for the presence of chemotactic activity, using a modification of the Boyden chamber technique. The animal's own PMN, obtained from venous blood by dextran sedimentation and suspended in phosphate buffer containing 20% canine plasma, were placed in the cell compartment, and the supernatants after centrifugation of the aspirates were added to the same plasma-buffer solution and placed in the opposite compartment. A 5- to 10-fold increase in cells migrating through the Millipore filter separating the two compartments was found when the 90 min aspirate from crystal-injected joints was compared with the 3 min aspirate. A significantly smaller increase was observed in comparable aspirates from saline-injected knees. When the 90 min aspirate from crystal-injected joints was added to both compartments, thereby abolishing the concentration gradient, PMN migration was reduced to control levels, thus establishing the presence of chemotactic activity. After overnight dialysis against cold tap water, potent chemotactic activity was still demonstrated. Significantly less chemotactic activity was present in saline-injected knees. The results of these studies in dogs indicate that a nondialyzable substance (or substances) chemotactic for PMN is present in the synovial fluid after intra-articular injection of urate crystals. (This study was supported by NIH grants AM-12593 and FR-107.)

206. Studies on a Hemolytic Factor of Cobra Venom. GERALD B. PHILLIPS, New York, N. Y.

That cobra venom (*Naja naja*) causes hemolysis of unsensitized sheep red cells in the presence of fresh guinea pig serum but not after the serum has been heated at 56°C for 30 min has been observed. The venom factor responsible for this activity has been reported to be a phospholipase A. In the present study, this hemolytic action of cobra venom was confirmed but did not appear to be attributable to phospholipase A activity. The following observations to support this conclusion were made. (1) Heating the venom at 100°C for 15 min inactivated the hemolytic factor but not phospholipase A. (2) Russell viper venom (*Vipera russelli*) did not contain the hemolytic factor but had potent phospholipase A activity. (3) The hemolytic factor and phospholipase A activities migrated differently on electrophoresis. (4) Heparin inhibited the venom hemolysis as well as immune hemolysis, but not the venom phospholipase A activity. (5) The hemolytic factor but not the phospholipase A became inactivated in buffer at pH 8.6. (6) Analysis of the red cell phospholipids indicated that the venom hemolysis, like immune hemolysis, was not the result of phospholipid hydrolysis, which is catalyzed by phospholipase A. "Direct lytic factor" activity of cobra venom was stable in buffer at pH 8.6 and to heating at 100°C for 15 min and could be identified in electrophoretically separated fractions which did not have hemolytic factor activity. The venom factor was not dialyzable and could be easily washed off the red cells. These results suggest that cobra venom contains a factor(s), other than phospholipase A or "direct lytic factor," which activates the serum complement system to cause hemolysis; this factor may be an enzyme similar in activity to one present in the activated complement system. (Research supported by a grant from the NIH.)

207. The Normal Ranges of Body Potassium: Effects of Age, Sex, and Fat, and the Development of a Reference Standard. Richard N. Pierson, Jr.* and James G. Hilton,** New York, N. Y.

Body potassium (TBK) is 97% intracellular, and defines both the limits and the integrity of the body cell mass, a compartment inaccessible to direct measurement. In hyperaldosteronism, diuretic administration, cirrhosis, and heart failure, great distortions of normal TBK are known or posited, but measurements are rarely used in diagnosis or management, owing to lack of normal reference standards. Liquid scintillator 4π counting of 1500 ambulatory subjects aged 1 wk to 96 yr discloses an age- and sex-specific curve of rapidly increasing TBK to peak at age 22 in women and 32 in men, followed by gradual reduction with increasing age. Men exceed women in TBK at all points after age 10–12. Body weight, height, body surface area, and other more complex functions have been used to normalize TBK for size. Since fat contributes largely to weight, and only slightly to TBK, it provides the major variable in any reference system. Direct measurement of fat by the Steinkamp anthropometric method, and calculation of the derived fat-free body as a reference, results in smaller and more useful standard deviations about the mean. Alternatively, reference to height rather than weight excludes the variable factor of fat, and also results in smaller standard deviations. The best solution to the reference standard question after computer solution of alternatives involves separate identification of organ K, fat-cell cytoplasmic K, and weight-obligated skeletal muscle K. A standard deviation less than 10% of the mean achieved by this method permits much clearer separation of abnormal from normal TBK than was previously possible. (Supported by a grant from the John A. Hartford Foundation.)

208. Degrees of Experimentally Induced Hyperketonemia and Secretion of Insulin in the Dog. F. Xavier Pi-Sunyer,* Robert G. Campbell,* and Sami A. Hashim,* New York, N. Y. (introduced by Theodore B. Van Itallie**).

Effects of varying magnitudes of hyperketonemia on insulin secretion were studied in dogs with cannulas in femoral artery and portal vein. Samples were obtained before, during, and after acute or constant (up to 3 hr) intravenous infusion of sodium β-hydroxybutyrate or saline. Serum was analyzed for glucose, ketones, and immunoreactive insulin (IRI). During 1 hr infusion of 7.0 mmoles/kg per hr of ketone (10 experiments), serum ketones rose from 1.0 $\pm$0.1 (SE) to 37.4 $\pm$3.2 mg/100 ml at 1 hr, while glucose fell to 65.5 $\pm$2.7% of control, both returning toward base line after completion of infusion. A 2.5-fold rise in IRI (12.4 $\pm$1.2 to 30.7 $\pm$4.7 μU/ml) occurred at 5 min, followed by gradual decline to base line achieved after cessation of infusion. 3 hr infusion prolonged the hyperketonemia, but hypoglycemia did not progress beyond the level reached at 1 hr, and subsequently IRI response was diminished. Infusion of 3.5 mmoles/kg per hr (10 experiments) was associated with smaller magnitude but similar pattern of change. Infusion of 1.0 mmole/kg per hr (5 experiments) caused no significant change in glucose or IRI, while ketones reached 3.7 $\pm$0.9 mg/100 ml. In rapid (1 min) infusion of varying amounts (16–79 mmoles) of ketone (5 experiments), prompt responses in IRI were elicited in proportion to quantity of ketone administered. In experiments in which portal venous blood was obtained, portal:arterial IRI gradients of 2–3:1 were observed. Results indicate a relation between degree of hyperketonemia, insulin response, and hypoglycemia, and suggest that hyperketonemia at levels encountered during lipolytic states may provide a feedback effect on adipose tissue mediated by insulin. However, the islet response to hyperketonemia may be limited by concomitant hypoglycemia. (Supported by a grant from the NIH.)

209. Metabolism of the Ether Linkage of Thyroxine (T_4) in Normal Man. Constance S. Pittman,* Virginia H. Read,* Joe B. Chambers, Jr.,* and Haruyoshi Nakafuji,* Birmingham, Ala. (introduced by Ben Friedman**).

Little is known of the metabolism of the carbon structure of T_4 in man; the animal data are controversial. This study was carried out to determine the integrity of the ether linkage of T_4 during thyroxine degradation in normal man. ^3H- and ^{14}C-labeled thyroxines were administered intravenously to each subject in total dose 27–98 μg/day for 7 days. During the subsequent 3 wk a total of 71–80% of the dose was recovered. To one control subject two radiothyroxines, each labeled on the alanine side chain, D,L-[α,β-^3H]-T_4 and D,L-[β-^{14}C]-T_4, were given simultaneously. The $^3H/^{14}C$ ratio of the dose was 5.27, and the average $^3H/^{14}C$ ratios of the daily serum, feces, and urine were respectively 4.85 $\pm$0.37 (SD), 5.89 $\pm$0.78, and 5.21 $\pm$0.69. The control data confirmed the stability of the ^3H labels of D,L-[α,β-^3H]-T_4 during the experimental manipulations. To each of the two experimental subjects two radiothyroxines labeled on the opposite sides of the ether linkage, D,L-[α,β-^3H]-T_4 and D,L-[phenolic ring-^{14}C]-T_4 were given simultaneously. The $^3H/^{14}C$ ratio of the dose given to the first experimental subject was 5.52, and the mean $^3H/^{14}C$ ratios of the serum, feces, and urine were respectively 8.28 $\pm$2.86, 4.38 $\pm$1.05, and 6.70 $\pm$1.06. The corresponding four ratios in the second experimental subject were 5.08, 6.63 $\pm$0.89, 5.49 $\pm$0.38, and 6.54 $\pm$0.98. Thus in the experimental subjects the rise of the urinary $^3H/^{14}C$ ratios over that of their respective doses also was small and questionable. Therefore, our data indicate that in man the major degradative pathways of T_4 leave the diphenyl ether intact. (This study was supported by grants AM-08181 and 2-MO1-FR-32 provided by the NIH.)

210. Albumin in the Nephron. Victor E. Pollak and Amadeo J. Pesce,* Chicago, Ill.

Most current literature favors the concept that glomerular filtration and proximal tubular reabsorption are controlling factors in the passage of proteins from serum to urine. As part of a study on the mechanisms of proteinuria, albumin distribution in the kidney was examined by immunohistochemical techniques. Kidneys of normal monkey and rat were examined, and renal biopsies from 25 subjects with a variety of renal diseases. The kidney tissue was frozen in isopentane at —160°C, substituted in acetone for 14 days, and then embedded in paraffin. Sections 0.5 μ thick were cut in a cryostat with a glass knife. The sections were incubated with

fluorescein isothiocyanate–conjugated antiserum specific for human (or rat) albumin; they were examined and photographed by fluorescence microscopy. There appeared to be no obvious diffusion artifacts. The sections were then fixed, and stained with PAS-hematoxylin. The identical field was rephotographed by light microscopy. A striking finding in the glomeruli was the frequent finding of albumin in the glomerular mesangium (13 of 19) and in visceral epithelial cells (10 of 19); albumin was rarely detected (3 of 19) in free Bowman's space. As expected, droplets of albumin were frequent (19 of 23) in the cytoplasm of proximal tubular cells. Unexpected was the demonstration of the presence of albumin diffusely in many cells of distal convolutions (19 of 22), loops of Henle (11 of 14), and collecting ducts (12 of 16). These findings strongly suggest the possibility that, in addition to proximal tubular reabsorption, albumin is transported through the cells of the distal tubules, loops of Henle, and collecting ducts. Albumin was found universally in interstitial connective tissue, both within (24 of 24) and outside (24 of 24) peritubular capillaries, confirming physiologic observations on the extravascular distribution of albumin in the kidney. (Research supported by NIH grant AM-10314.)

211. Are Bile Salt Micelles of Primary Importance in Fat Absorption? H. P. PORTER,* D. R. SAUNDERS,* O. BRUNSER,* AND C. E. RUBIN,** Seattle, Wash.

Bile salt micelles are believed to mediate the absorption of dietary fatty acids. How then do patients with bile fistula (BF) absorb most of their dietary fat? Luminal and mucosal phases of fat absorption have been examined in three BF and 11 controls by continuously infusing their proximal duodenum with an "Osterized" meal of saline, raw egg, and linseed oil (45% linolenic acid). From distal duodenum, biopsies were taken before and after infusion, and luminal contents were aspirated continuously from 30 through 75 min after starting the infusion. To assess composition of the "micellar phase," a portion of the aspirate was passed through a 500 A Millipore filter, which was the largest filter yielding optically clear filtrates. Free fatty acid (FFA) concentrations (0.78–27.7 mM) were directly proportional to bile salt concentrations (1.29–12.9 mM; $n = 11$) ($r = 0.899$; $P < 0.001$) in 500 A filtrates of normal duodenal contents. 7–38% of bile salts present in unfiltered aspirates were recovered in the 500 A filtrates of the five controls tested. 500 A filtrates of BF intestinal contents, obtained during infusion, contained no bile salts but did contain low concentrations of FFA (0.19–0.27 mM). Lack of bile salts did not impair lipolysis in two patients tested, because concentrations of FFA in unfiltered duodenal aspirates were similar during biliary diversion (39.2, 36.5 mM) and after restoration of biliary flow (27.4, 34.5 mM). Electron microscope examination of postinfusion biopsies from three BF showed that fat absorption was qualitatively normal, but quantitatively less than controls. Postinfusion biopsies from a BF, studied with and without biliary diversion, contained linolenic acid which was 98% esterified. In BF, FFA was absorbed, presumably from a molecularly dispersed luminal solution. Solution of FFA in luminal water, rather than formation of FFA–bile salt micelles, may be the fundamental mechanism for processing dietary fatty acids for absorption.

212. Effect of Intra-aortic Balloon Counterpulsation on Myocardial O_2 Consumption, Coronary Flow, and Ventricular Performance in the Dog. W. JOHN POWELL, JR.,* WILLARD M. DAGGETT,* JESUS A. BIANCO,* ALFRED E. MAGRO,* JOHN D. LAIRD,* PETER N. MADRAS,* MORTIMER J. BUCKLEY, JR.,* CHARLES A. SANDERS,* AND W. GERALD AUSTEN, Boston, Mass.

The effect of intra-aortic balloon pumping (IABP) with a 20 cc three-segment balloon (Avco Everett) upon myocardial oxygen consumption ($M\bar{V}O_2$), coronary flow (CF), and left ventricular performance was studied in 10 dogs on right heart bypass at constant heart rate and cardiac output. In the normotensive, non-CF-limited preparation, IABP produced a decrease in peak left ventricular pressure (LVP), in its peak first derivative (max LV dp/dt), and in $M\bar{V}O_2$. There was little steady-state change in CF, owing at least in part to coronary autoregulation. Left ventricular end-diastolic pressure (LVEDP) and circumference (LVEDC) fell if elevated, but exhibited little change if initially normal. However, in the hypotensive preparation, in which left ventricular performance was severely impaired by a decreased CF, initiation of IABP was followed by a striking increase in CF, an increase in $M\bar{V}O_2$ and max LV dp/dt, and little change in peak LVP. Elevated LVEDP and LVEDC fell substantially toward normal. Under these conditions directionally similar changes in max LV dp/dt, $M\bar{V}O_2$, LVEDP, and LVEDC could be produced by increasing CF alone. When CF was maintained constant in the hypotensive, CF-limited preparation, IABP produced a fall in peak LVP, max LV dp/dt, LVEDP, and LVEDC. These data suggest two mechanisms by which IABP may improve LV performance: (1) increase in CF with an accompanying increase in $M\bar{V}O_2$ in the failing, coronary flow–limited heart; and (2) restoration of a more appropriate length-tension relation in the failing LV which occurs independently of changes in CF and which may be accompanied by a decrease in $M\bar{V}O_2$. (Supported by American Heart Association grants 68-725 and 68-756, and USPHS grant HE-06664—HEPP.)

213. Zinc in Human Serum: Evidence for an Amino Acid–Bound Fraction. ANANDA S. PRASAD AND DONALD OBERLEAS,* Detroit, Mich.

Zinc (Zn) is an essential micronutrient for growth and gonadal functions of animals and man. Since the state of equilibrium of zinc in human serum is not well understood, the present investigations were undertaken. Ultrafiltrable (UF) ^{65}Zn was determined to be 2–3% in vitro after incubation of ^{65}Zn with normal sera. In similar experiments with pure predialyzed proteins, the Zn/protein molar ratio was determined to be highest for ceruloplasmin, although a significant amount of Zn was bound to albumin, transferrin, and IgG. Our data suggested the presence of two different binding sites for Zn on albumin, one having greater binding affinity for Zn. Four additional preparations were utilized for studies: (*a*) pooled native human serum (NHS); (*b*) predialyzed human serum (PDS) and predialyzed human albumin 4% solution (PDA); (*c*) PDS reconstituted with

dialysate; and (*d*) PDS reconstituted with ashed dialysate. Labeled ^{65}Zn with carrier Zn was added to achieve a Zn/albumin molar ratio of 0.33 to 2.5, and ultrafiltration was performed. UF ^{65}Zn was low (0.2–1.2%) in preparations (*b*) and (*d*), but in (*a*) and (*c*) it was 6–7 times greater at all tested Zn/albumin ratios, suggesting that organic dialyzable constituents affected the status of Zn in serum. In physiological concentrations, Na citrate, lactate, pyruvate, and oleic, stearic, and linoleic acid when added to PDS or PDA had no effect, but additions of amino acids increased UF ^{65}Zn severalfold. Proportionate increases in UF ^{65}Zn were noted when concentrations of amino acids were increased 2–3 times. Histidine, glutamine, threonine, cystine, and lysine showed the most marked effects in this respect. These studies show that in human sera a small fraction of Zn is bound to amino acids which compete effectively with proteins for binding of Zn. It is suggested that this fraction may have an important role in biological transport of Zn.

214. **Properties of Protein-Free Lung Surfactant.** R. A. Redding,* J. M. Steim,* T. Hauck,* and M. Stein,* Providence, R. I. (introduced by J. E. F. Riseman**).

The composition and role of lung surfactant is still controversial. We isolated and purified lung surfactant in a manner which would not fragment lipid from a second component, and determined its performance at physiological temperature, humidity, and concentration. Our methods employed an aqueous medium and differential centrifugation to separate crude dog surfactant from cellular debris, soluble protein, polysaccharide, and saline in alveolar lavages. The deionized crude surfactant was layered alternately on two sucrose solutions of different densities, separating surface-active phospholipids unattached to protein or polysaccharide from materials of other densities, which were not surface active. A representative 150 g dog lung contains 5% solution of surfactant within the alveolar fluid lining (150 mg surfactant distributed within 3 ml of fluid, 0.1 μ thick over 30 m^2 surface area). We compared physiological concentrations of lung surfactant with monolayer quantities upon a Wilhelmy balance at high humidity from 22° to 44°C. A 0.5% solution of surfactant with compressed surface area gave minimum surface tensions (γ min.) of 3 dynes/cm. At fully expanded surface area (γ max.) surface tension was 36 at 22°C. At 37°C, γ min. rose to 8, and γ max. diminished to 28. At 44°C, surface tension was independent of area at 25 dynes/cm. A surfactant monolayer (25 μg) gave γ min. of 4 and γ max. of 60 at 22°C. At 37°C, γ min. was 15 and γ max., 45. At physiological concentrations surface tension was unaffected by proteins and polysaccharide found in crude surfactant. However, when monolayer concentrations of surfactant were studied, protein and polysaccharide were observed to increase γ min. and decrease loop area. In summary, lung surfactant exists in dog lungs as lipids unattached to protein and polysaccharide, although the latter are present. High concentrations of surfactant are required to obtain optimal efficiency. Under conditions of body temperature and humidity, a physiological concentration of lung surfactant has less hysteresis than has been traditionally assumed.

215. **Chromosomal Aberrations in Rat Marrow Cells and Leukemic Cells after Intravenous Polycyclic Hydrocarbons.** E. Douglas Rees,* Lexington, Ky. (introduced by J. William Hollingsworth).

It is unresolved whether chromosomal aberrations are a cause or consequence of neoplastic disease. This problem was approached experimentally by means of the intravenous administration of emulsion containing 7,12-dimethylbenz(a)-anthracene (DMBA), a procedure shown by Huggins et al. to induce a high incidence of leukemia in rats. After DMBA injection, the most striking and distinctive observation is the presence of large chromatid and isochromatid breaks on marrow cell chromosomes. Breaks are present at 2 hr and reach a maximum incidence (35–45% of metaphase cells having chromosomes with breaks) in 6–24 hr. By the 4th day after initial injection, the incidence decays to the level of 0–3% observed in cells of control animals. Members of the largest terminal and largest subterminal chromosome pair have more breaks than expected on the basis of length; the group of median chromosomes is involved to a lesser extent than expected. The incidence of gaps is comparable to that of breaks. Other polycyclic hydrocarbons were injected to assess the relation between carcinogenic potency and ability to produce breaks and gaps. The ranking for breaks is: control (0–3%), chrysene (0–3%), benzo(e)pyrene (0–3%), 6-aminochrysene (19%), and 3-methylcholanthrene (30%, nearly comparable to DMBA). This is also the order of increasing carcinogenicity (mammary carcinoma induction) in the rat; sufficient time has not elapsed to determine leukemia incidence. Chromosomes prepared directly from marrow, liver, and spleen of some leukemic rats reveal a nearly pure stemline of cells with overt alterations (e.g. trisomy and/or aneuploidy); in other rats karyologic changes are minimal. Although no single morphologic chromosomal change is consistently related to rat leukemia, the high incidence of early, drastic aberrations produced by DMBA suggests that more subtle chromosomal changes may also be produced and these may be related to leukemia. (Supported by a grant from the American Medical Association, Education and Research Foundation, and a contract from the U. S. Department of Agriculture.)

216. **Serum Growth Hormone (HGH), Insulin (IRI), and Blood Sugar (BS) in Response to Intravenous Insulin, Arginine, and Glucose in Myxedematous Patients before and after Therapy.** Samuel Refetoff,* Peter H. Sönksen,* and Hugh H. Johnston,* Boston, Mass. (introduced by John B. Stanbury**).

Six patients (three males, three females) with severe myxedema (serum T4 < 1.2 μg/100 μl and elevated serum carotene, LDH, SGOT, and CPK) received a standard i.v. insulin, arginine, and glucose tolerance test. Serum HGH, IRI, and BS were determined on samples drawn at intervals for 3 hr. These studies were repeated 4 to 6 months after institution of thyroid hormone therapy, when a clinically euthyroid state was confirmed by normal laboratory tests. The following mean values were obtained and were significant at $P < 0.05$ (myxedema vs. euthyroid). HGH: Peak rise in response to insulin hypoglycemia was diminished (11.1 vs.

30.0 ng/ml), but was similar in response to arginine (9.0 vs. 7.8 ng/ml). BS: Fasting levels were lower (86.8 vs. 104.5 mg/100 ml). Fall in response to insulin was less marked (40.7 vs. 60.2 mg/100 ml drop from fasting), while in myxedema, arginine produced an exaggerated rise in BS (43.8 vs. 21.2 mg/100 ml) and subsequent fall (53.0 vs. 24.7 mg/100 ml). IRI: Exogenous insulin $T\frac{1}{2}$ was unchanged (6.9 vs. 6.9 min). Peak response to arginine was exaggerated (217.7 vs. 106.8 μU/ml). In both clinical states a linear correlation existed between (*a*) peak HGH and BS drop in response to insulin; (*b*) peak IRI and BS rise and fall in response to arginine. *Conclusions:* In myxedema (1) HGH response to arginine stimulation was unchanged. The blunted HGH rise with i.v. insulin was secondary to the less marked hypoglycemia. (2) The exaggerated IRI response to arginine was due to greater hyperglycemia, and was responsible for the reactive hypoglycemia. (3) These results indicate that pituitary and pancreatic function may be basically normal in myxedema but that their response to conventional stimuli might be masked by other features inherent in this condition. (Research supported by grants from the John A. Hartford Foundation and from the NIH, 5-MO1-FR-00088 and 5-F2-AM-3300307-02(e).)

217. Antibiotic Effect on Myocardial K^+ Transport and the Production of Ventricular Tachycardia.

T. J. Regan, M. I. Khan,* H. A. Oldewurtel,* and A. J. Passannante,* Jersey City, N. J.

Though in vitro studies have indicated that macrolide antibiotics can selectively alter ion transport, the effects on myocardium are unknown. Erythromycin, a major representative of this group, has been infused i.v. at 25 mg/kg for 10 min in the intact anesthetized dog. Net ion movement was assessed from serial arterial–coronary sinus plasma concentrations and coronary plasma flow. Enhanced uptake of K^+ occurred in the initial 60 min without significant change of Na or glucose transport. Coronary blood flow (^{85}Kr method), heart rate, and aortic pressure were unaltered. Concentration of K^+ at 72 ± 3 μEq/g wet weight of LV was increased over controls ($P < 0.01$), while sodium and water contents were normal. Since altered cell metabolism may reverse the directional effect on ion transport, interventions have been produced in animals receiving erythromycin, 30–40 mg/kg per day p.o. for 4 days. Group I then received acetyl strophanthidin 0.03 mg/kg, resulting in a net myocardial K^+ loss of 182 ± 9 μEq/100 g per LV, as compared with 93 ± 5 μEq in controls without antibiotic ($P < 0.001$). Though no arrhythmias were observed in controls, 11 or 12 in the antibiotic group had a ventricular tachycardia. To assess dependence on the interaction of two lactones, group II was studied during myocardial ischemia after 4 days of erythromycin. The anterior descending coronary artery was partially occluded by a balloon catheter and coronary flow measured by distal injection of ^{85}Kr. Reduction of blood flow by 50% resulted in no significant ectopic activity in nine controls, whereas seven of eight in the antibiotic group had ventricular ectopic beats. Thus, erythromycin at therapeutic doses can selectively enhance K^+ uptake acutely in the normal ventricle, but during the altered metabolism of ischemia or after digitalis, the antibiotic may enhance net loss of K^+ and produce ventricular arrhythmias. (Research supported by a grant from the NIH.)

218. Drug Induction of Organic Anion–Binding Protein in Rat Liver and Its Physiological Consequence.

Humberto Reyes,* A. Jonathan Levi,* and Irwin M. Arias, New York, N. Y.

We previously described two hepatic cytoplasmic proteins, Y and Z, which bind bilirubin and sulfobromphthalein (BSP), and presented evidence that these proteins are important in organic anion transfer from plasma into the liver. In the present study, the hepatic uptake of BSP was compared with the amount of Y and Z proteins before, during, and after phenobarbital administration. Adult Sprague-Dawley rats received daily injections of sodium phenobarbital (8 mg/100 g) for 6 and 21 days. The first-order rate constant for BSP removal from plasma (K_1) between 2 and 7 min after intravenous injection of 5 mg BSP per 100 g was used to estimate hepatic uptake of the dye. In control rats, K_1 was 0.192 ± 0.006 (SEM). BSP binding by Y and Z fractions after gel filtration of 100,000 *g* supernatant of liver homogenates was used to quantitate Y and Z proteins. In control rats, Y bound 0.59 ± 0.04 and Z bound 0.33 ± 0.02 mg BSP per 100 g body weight. In rats treated for 6 days with phenobarbital, mean K_1 increased 30% over basal values ($P < 0.01$), Y binding protein increased 117% ($P < 0.005$), and Z binding protein was unchanged. After 21 days of drug treatment, mean K_1 increased 47% ($P < 0.01$) and Y increased 153% ($P < 0.005$) over control levels; Z was unchanged. In other studies K_1 was also serially estimated in each of four additional rats at time 0, at 2 and 12 days during phenobarbital administration, and at intervals after discontinuing the drug. K_1 was unchanged in saline-injected control rats. In drug-treated rats, K_1 was 24% greater than control values after 2 days ($P < 0.025$) and 48% after 12 days ($P < 0.01$). After discontinuance of phenobarbital administration, K_1 returned to normal by 9 days, at which time normal amounts of Y and Z were found. The change in Y during and after phenobarbital administration correlated with the change in K_1 and emphasizes the importance of Y as an intracellular transport protein. Y but not Z was induced by phenobarbital. (Research supported by grants from the NIH, the Nuffield Foundation, and the New York Heart Association.)

219. Stabilization of Hemoglobin by Cyanide and Inhibition of Heinz Body Formation.

Ronald F. Rieder,* Brooklyn, N. Y. (introduced by L. W. Eichna**).

The increased heat precipitability of unstable Hb Köln is associated with abnormal heme binding and is prevented by cyanide. The heat lability of three other unstable hemoglobins and the formation of Heinz bodies seem to have a similar basis. Hemolysates containing unstable hemoglobins Gun Hill and Philly and another incompletely characterized mutant hemoglobin were incubated at 50°C in 0.05 M sodium phosphate, pH 7.0. After 20 hr of incubation 30–40% of the hemoglobin had precipitated. When normal hemolysates were heated, 5–20% of the hemoglobin precipitated. Prior addition

of sodium cyanide, 0.1 mg/ml, reduced hemoglobin loss in the normal and abnormal hemolysates to less than 5%. The effect of cyanide was less marked in the hemolysate containing Hb Gun Hill. This abnormal hemoglobin lacks heme groups on the beta chains. Erythrocytes containing Hb Gun Hill were incubated at 37°C with brilliant cresyl blue. Inclusion bodies were produced after 3 hr and were prominent after 24 hr. Addition of sodium cyanide, 0.05 M, completely blocked inclusion body formation. The addition of cyanide markedly suppressed Heinz body formation in red cells containing Hb Gun Hill and in normal erythrocytes during 1–24 hr of incubation with acetylphenylhydrazine. Cyanide did not prevent staining by crystal violet of spontaneous inclusions in erythrocytes of a patient with congenital Heinz body anemia. Heinz body formation and heat precipitation of hemoglobin are associated with decreased binding of heme to globin. Cyanide binds to the iron atom in methemoglobin and converts it from a high to a low electron spin state. This change is associated with displacement of the iron toward the plane of the heme group. This may result in increased stability of the heme-globin complex with inhibition of hemoglobin denaturation and inclusion body formation.

220. Opsonins after Immunization of Man with Meningococcal Capsular Polysaccharides. RICHARD B. ROBERTS,* New York, N. Y. (introduced by James G. Hirsch**).

Meningococcal infections continue to pose a serious medical and public health problem, owing in part to the emergence of sulfa-resistant organisms. Since antibiotics no longer are effective in eradicating the bacterium from the nasopharynx of carriers, protection of a population from clinical infection must be achieved by acquired immunity. Gotschlich recently isolated purified capsular polysaccharides from meningococci of groups A and C (polymers of mannosamine and sialic acid respectively). This report describes the interaction in vitro between human polymorphonuclear leukocytes and meningococci in the presence of sera from individuals immunized with these polysaccharides. Phagocytosis of meningococci did not occur in the presence of preimmunization sera. Group-specific opsonins were detected, however, in individuals immunized intradermally with 50 μg of either group A or group C capsular polysaccharide. Opsonic antibody titers ranged from 1:20 to 1:80 and persisted for at least 10 months. Opsonic activity was lost after absorption of these sera with either the group-specific bacteria or capsular polysaccharide. Meningococcal opsonins were present in both γM and γG immunoglobulins; furthermore, opsonic activity of both these classes of antibody was complement dependent. Studies of opsonins in sera of nasopharyngeal carriers and of patients recovered from meningitis showed antibodies with characteristics similar to the opsonins detected in immunized individuals. Meningococci were rapidly killed after ingestion by human granulocytes. These studies suggest that immunization with purified group A and group C polysaccharides may well protect individuals against group-specific meningococcal infections. (Research supported by contract DADA-17067-C-7008 from the U. S. Army Medical Research and Development Command.)

221. Triglyceride Formation in Intestinal Microvillous Membranes during Fat Absorption. SANDER J. ROBINS,* DONALD M. SMALL,* AND ROBERT M. DONALDSON, JR., Boston, Mass.

Electron microscope observations made during fat absorption have led to the concept that fatty acid (FA) and monoglyceride (MG) diffuse unchanged through microvillous membranes (MVM) and that triglyceride (TG) is resynthesized only in the subapical region of the cell. To examine this concept, we determined the lipid composition of MVM isolated from hamsters before and at 1 and 3 hr after intragastric instillation of pure FA and MG. Mean lipid/protein ratio in MVM increased from 0.9 in fasting hamsters to 1.2 in animals given fat. Fasting "nonpolar" lipids averaged only 21% (cholesterol 11%, FA 6%, TG 4%) of total MVM lipid; phospholipids and glycolipids accounted for the rest. Instillation of FA and MG increased the "nonpolar" fraction to 40% (cholesterol 8%, FA 15%, TG 17%) of total MVM lipid. Thus the increase in MVM lipid was due to a 2.5-fold rise in FA and a striking 4-fold rise in TG. Contamination of MVM with intracellular TG seemed unlikely since homogenization of intestinal scrapings with added TG did not increase TG content of subsequently isolated MVM. When hamsters were sacrificed 5 min after duodenal instillation of ^{14}C-oleic acid, 25% of radioactivity in isolated MVM was found in TG. Furthermore, when everted hamster intestine was incubated for only 30 sec in micellar lipid containing ^{14}C-oleic acid, 20% of MVM radioactivity was recovered as DG and 35% as TG. These results demonstrate that during fat absorption (1) FA is incorporated in the MVM; (2) TG content of MVM is markedly increased; and (3) radioactivity from FA appears in newly synthesized membrane TG. In contrast to current views of fat absorption, these findings suggest that at least some absorbed FA is reesterified to TG within MVM at the absorptive surface.

222. Bilirubin Production from Erythropoietic and Nonerythroid Sources in Experimental Iron Deficiency Anemia. STEPHEN H. ROBINSON,* Boston, Mass. (introduced by Howard H. Hiatt).

To assess ineffective erythropoiesis, formation of early-labeled bilirubin and hemoglobin ^{14}C-heme from 2-^{14}C-glycine was measured in rats with diet-induced iron deficiency anemia. Per cent incorporation of ^{14}C-glycine into bile ^{14}C-bilirubin was calculated for two intervals, 0–3.5 and 3.5–60 hr after glycine administration: the initial phase conforms to bilirubin production from nonerythroid (primarily hepatic) sources; the latter includes the erythropoietic component. Formation of late-phase bilirubin was increased in anemic rats (^{14}C-glycine incorporation 0.120 ±0.010% [SE] vs. 0.080 ±0.003% in controls), whereas production of erythrocyte hemoglobin ^{14}C-heme was depressed (0.28 ±0.02% vs. 0.52 ±0.04% in controls), an imbalance characteristic of ineffective erythropoiesis. Iron-deficient rats have unexplained reticulocytosis. Reticulocytes containing hemoglobin ^{14}C-heme were collected from anemic rats given 2-^{14}C-glycine 1 day earlier and transfused into normal recipients with external bile drainage. An average of 17.9 ±4.9% (SE) of transfused hemoglobin ^{14}C-heme was converted to ^{14}C-bilirubin over the

ensuing 3 days, as compared with only 1.6 ±0.2% with transfusion of labeled reticulocytes from normal donors. Thus, reticulocyte hemolysis accounts for a large part of the increase in erythropoietic bilirubin production in iron-deficient rats. A different pattern of early-labeled pigment formation was observed during treatment with iron-dextran. The erythropoietic enlargement began to diminish, whereas the initial bilirubin peak, which had been normal before therapy, rose substantially (^{14}C-glycine incorporation 0.036 ±0.005% vs. 0.015 ±0.001% in controls), consistently with increased bilirubin production from nonhemoglobin sources in the liver. These experiments illustrate the fact that bilirubin overproduction may arise from disordered erythropoiesis and altered hepatic heme metabolism, as well as from classical erythrocyte hemolysis. (Research supported by a grant from the NIH.)

223. Vitamin B_{12}-Dependent Methylmalonic Aciduria: Defective Cobamide Coenzyme Metabolism in Cultured Fibroblasts. LEON E. ROSENBERG, ANNE-CH. LILLJEQVIST,* Y. EDWARD HSIA,* AND FREDERICK M. ROSENBLOOM,* New Haven, Conn.

We described recently a 1 yr old boy with metabolic ketoacidosis whose urine contained massive amounts of methylmalonic acid (MMA) and whose leukocytes failed to convert ^{14}C-propionate to ^{14}C-succinate or $^{14}CO_2$. These findings indicated a block in the isomerization of MMA-CoA to succinyl CoA, an enzymatic reaction requiring a cobamide coenzyme formed from vitamin B_{12}. Although the child was not vitamin B_{12} deficient, he responded to parenteral administration of huge doses of vitamin B_{12} (1 mg/day) with a marked decrease in MMA excretion. In the present studies, skin fibroblasts grown in tissue culture were used to investigate the mechanism of this previously undescribed vitamin dependence. Initial experiments demonstrated that the mutant phenotype was expressed in cultured cells. Control fibroblasts, grown in conventional medium containing 25 picograms (pg) of vitamin B_{12} per ml, oxidized 50–60 nmoles of proprionate to CO_2 per 10^8 cells, whereas mutant fibroblasts oxidized only 10–15 nmoles/10^8 cells. Cobamide coenzyme concentration of these fibroblasts was determined by a specific enzymatic method using bacterial dioldehydrase. Coenzyme content in the mutant cells (0.44–0.67 ng/g wet weight) was only 5–10% of that noted in six control cultures (6.79 ±2.37 ng/g wet weight). When the concentration of vitamin B_{12} in the culture medium was increased to 25,000 pg/ml, however, cobamide coenzyme content of the mutant cells was increased to 4.2 ng/g, and propionate oxidation returned to near normal values. These findings suggest that this disease may be due to defective enzymatic conversion of vitamin B_{12} to one of its active coenzyme forms, rather than to a defect in the MMA-CoA isomerase apoenzyme. Such a biochemical mechanism has not been proposed previously to explain other human inborn errors demonstrating specific vitamin dependence.

224. Quantitative Antibody Studies in Autoimmune Hemolytic Anemia Due to Warm-Reactive Antibodies. WENDELL F. ROSSE, Durham, N. C.

A major obstacle in understanding the pathogenesis of hemolysis in autoimmune hemolytic anemia has been difficulty in quantitating immunological reactions at the cell surface. We have adapted the C′1a fixation and transfer test of Borsos and Rapp in combination with the antiglobulin technique to determine the amount and type of antibody in the serum and on the red cells of patients with immune hemolysis due to warm-reacting antibody. The rate of hemolysis is largely determined by the amount of IgG, warm-reacting antibody on the red cell and is conditioned by the presence of the spleen. In 16 unsplenectomized patients, 100–1500 C′1a-fixing (antibody) units per cell were found when the patients were anemic. With few exceptions, the severity of the anemia was correlated with the amount of antibody on the cell surface. 15 of 17 unsplenectomized patients with less than 100 units of antibody did not have evident hemolysis, indicating that small amounts of antibody may be present on the red cell without causing its destruction. After splenectomy, much greater amounts of antibody may be present on the cell without resulting in its destruction. In four patients who had been splenectomized the cells contained 200–1200 units of antibody at a time when little or no hemolysis was taking place. Anemia occurred in three of these patients only when the antibody titer exceeded 1200 units per cell. Red cells from the spleen at splenectomy in one patient had more antibody on their surface than circulating red cells. The administration of adrenocortical steroids appeared to have two effects: (1) Within 3–9 days, the amount of antibody on the cells decreased to less than 125 units per cell. If the amount on the cells was high initially, the titer of antibody in the serum increased transiently, suggesting that the ability of antibody to attach to cells had been altered. (2) Within 4–15 days, the amount of antibody in the serum decreased, usually to undetectable levels. The alteration of the hemolytic rate appeared to parallel the reduction of antibody present on the cells.

225. Albumin Synthesis Dependent on Tryptophan and Isoleucine. M. A. ROTHSCHILD, M. ORATZ,* J. MONGELLI,* AND S. S. SCHREIBER,* New York, N. Y.

Starvation or fasting rapidly inhibits protein production in vivo in the perfused liver, and amino acid incorporation by subcellular systems. Tryptophan administration causes polysomal reaggregation and stimulates the depressed hepatic microsomes from fasted animals. In 44 studies in the perfused rabbit liver the effects of excess amino acids on albumin production were examined. ^{14}C-carbonate was used to label the intracellular arginine pool and hence the urea carbon and the guanido carbon of albumin. Perfusions required 2½ hr to assure complete release of labeled albumin. Precursor specific activity was obtained from the specific activity of newly made urea. Albumin was isolated by preparative gel electrophoresis, and checked for purity by immunoelectrophoresis. Guanido carbon specific activity after hydrolysis was determined by consecutive treatment with arginase and urease. The livers from fed animals contained higher concentrations of amino acids. Livers from fasted rabbits synthesized 18.0 ±1.1 mg of albumin and 117 ±8 mg urea per 100 g per 2½ hr. The addition of 10 μmoles/ml each of

methionine, valine, leucine, lysine, and threonine had no effect. Upon the addition of isoleucine, 10 μmoles/ml, albumin production increased 83% to 32.9 mg. The addition of 0.05 to 10 μmoles/ml of tryptophan caused albumin synthesis to rise 138–175% to 42.8–49.4 mg. Urea synthesis increased to 183 mg. Tryptophan had no effect on livers obtained from fed animals. These results demonstrate that albumin production in the fasted state is extremely sensitive to tryptophan probably secondary to reaggregation of the ribosomes. A step in the urea enzyme cycle is also stimulated. Isoleucine, not required for reaggregation, and not influencing urea synthesis, probably exerts its action by another means. (Research supported by a grant from the NIH.)

226. Facilitation of Ribosome Assembly: A Factor Regulating Normal Lymphocyte Growth and the Possible Defect in Chronic Lymphocytic Leukemia (CLL). ARNOLD D. RUBIN,* New York, N. Y. (introduced by William Dameshek**).

After phytohemagglutinin (PHA) stimulation, increased ribosome synthesis appears to be a prerequisite for the transformation of resting lymphocytes into proliferating blast cells. In synthesizing ribosomes, proliferating cells transcribe a 45S RNA nucleolar precursor molecule which becomes methylated and then cleaves into 32S and 18S moieties. The latter moieties, already in combination with protein as ribonucleoprotein particles (RNP), proceed into the cytoplasm, where the 32S RNA further cleaves to 28S. RNP containing the 28S and 18S moieties subsequently bind to one another to form a stable ribosome. Through analysis of the sequential events in ribosome synthesis, we have now found that resting lymphocytes transcribe and methylate 45S and 32S ribosomal precursor molecules. However, as compared with proliferating blast cells, further processing into cytoplasmic ribosomal subunits containing 28S and particularly 18S RNA is greatly retarded. PHA treatment induces an immediate stimulation of methionine-methyl and uridine incorporation into lymphocyte RNA. This is followed in several hours by the facilitation of ribosome assembly before the first morphologic indication of cell growth. PHA-treated CLL lymphocytes, which show a greatly reduced and delayed growth response, synthesize methylated 45S and 32S RNA at an increasing rate for 2–3 days before a parallel increase in RNP containing 28S and 18S RNA species heralds the onset of facilitated ribosome assembly and cell growth. A series of pulse-chase studies suggested that in nongrowing lymphocytes, much ribosomal RNA precursor, particularly precursor to 18S species, becomes preferentially degraded into acid-soluble products, most likely because of the lack of available protein for packaging into RNP. These findings point to a basic control mechanism which maintains lymphocytes in the resting state by inhibiting ribosome assembly. PHA readily removes this inhibition in normal cells, while a defective control mechanism renders the CLL cell relatively insensitive to the usual proliferative stimuli. (Research supported by grants from the NIH, the AEC, and the Leukemia Society of America.)

227. Effects of Ethanol on Hepatic Mitochondrial and Microsomal Enzymes: Differences between Acute and Chronic Ethanol Administration. EMANUEL RUBIN,* PAOLA BACCHIN,* AND CHARLES S. LIEBER, New York, N. Y.

Hepatic ultrastructure after acute ethanol administration differs from that after chronic intoxication. The latter is associated with striking mitochondrial damage and proliferation of agranular endoplasmic reticulum, whereas comparable changes are absent after acute intoxication. To assess functional counterparts of these morphologic differences, we determined the activity of a mitochondrial enzyme involved in porphyrin synthesis, delta aminolevulinic acid synthetase (ALAS), and the activities of microsomal drug metabolizing enzymes (aniline and pentobarbital hydroxylase) after acute and chronic ethanol administration in rats. 3 hr after intragastric administration of 8 g/kg ethanol, hepatic ALAS was enhanced 3- to 5-fold ($P < 0.001$), as compared with controls given isocaloric sucrose or glucose. This increase in activity is much greater than any previously described change, and probably reflects enzyme induction, since it was prevented by simultaneous administration of puromycin or actinomycin. This rise in ALAS activity contrasted with unchanged activities of the hepatic microsomal enzymes. In vitro, however, aniline and pentobarbital hydroxylases were competitively inhibited by ethanol, whereas ALAS activity was unaffected. Contrasting with the effects of acute administration, chronic (15 days) ingestion of ethanol, isocalorically substituted for carbohydrate (36% of total calories), left ALAS activity unchanged, whereas aniline and pentobarbital hydroxylase activities, determined 18 hr after the last intake of ethanol, were significantly increased. In conclusion, these results indicate that acute ethanol administration induces mitochondrial ALAS but not hepatic microsomal detoxifying enzymes, whereas the opposite is true for chronic ethanol intake. These data may explain why acute inebriation aggravates porphyria, whereas chronic alcoholism is associated with increased resistance to a variety of drugs, especially sedatives.

228. Regional Distribution of Pulmonary Ventilation and Perfusion in Patients with Liver Cirrhosis. FRANCOIS RUFF,* JOHN J. PICKEN,* ALEX ARONOFF,* J. MILIC-EMILI,* AND DAVID V. BATES, Montreal, Canada.

Arterial hypoxia is known to be frequently present in patients with cirrhosis of the liver and has been attributed to intrapulmonary and portopulmonary shunts. Recent studies have shown that such venous admixture is insufficient to explain the observed degree of hypoxia, and it has been suggested that regional ventilation-perfusion abnormalities might also contribute significantly. Using radioactive xenon, we have measured the regional distribution of pulmonary ventilation and blood flow in eight men with liver cirrhosis. Subdivisions of lung volume, expiratory flow rates, and diffusing capacity were all within predicted limits. All measurements were made in the resting seated position. In every patient, regional ventilation distribution was either uniform or preferential to the upper lung zones, in contrast to the predominant lower-zone distribution in normal subjects. This

effect was diminished by the taking of a very slow inspiration. Pulmonary blood flow showed a decrease in the more dependent parts of the lung in every patient, and in three of them, blood flow distribution was much reduced to the lower half of the lung fields. These abnormalities in ventilation and perfusion distribution, which were observed in varying degree in every patient, are of sufficient magnitude to contribute materially to arterial hypoxia. They are believed to be explained by an increase in airway and vascular resistance in dependent parts of the lungs, and this in turn is due, at least in part, to perivascular and peribronchial edema caused by decreased blood colloid–osmotic pressure and possibly increased capillary permeability. (This work was supported by grants from the Medical Research Council of Canada and the John A. Hartford Foundation.)

229. Determinants of Infection in Experimental Enterococcal Pyelonephritis. E. A. RUTSKY,* J. R. CLAPP, AND R. R. ROBINSON, Durham, N. C.

The relative contribution of medullary hypertonicity and changes of ammonia concentration [NH_4] to heightened medullary susceptibility to infection remains uncertain. To further evaluate the possible contribution of these factors, susceptibility to infection (>10^5 organisms per g kidney) was assessed in 152 rats during the 10 day maintenance of normal antidiuresis (AD), water deprivation (WD) plus oral acid or alkali, water loading (WL), or WL plus acid or alkali. Bacteria (*Streptococcus faecalis*) were given i.v. on the 3rd day; rats were sacrificed on day 10. The physiological status of challenged rats was approximated by selected measurements (cortical and papillary [NH_4], plasma and urine osmolality, pH, and [NH_4]) on day 3 or 10 in 85 identically treated but nonchallenged control rats. Incidence of infection equaled 49% in AD, 93% in WD plus acid, 100% in WD plus alkali, 29% in WL plus acid, and 32% in WL plus alkali. Urine osmolality was similarly elevated (>1750 mOsm/kg) in both WD groups and during normal AD; it was similarly reduced in all WL groups (<538 mOsm/kg). Papillary [NH_4] was highest (9.3 mM) in WD plus acid rats, and lowest (3.0 mM) in WL plus alkali rats; similar values were observed in WD plus alkali and normal AD rats (5.2 and 5.4 mM, respectively). Greater per cent infection ($P < 0.01$) in WD than in AD rats (despite a similar UOsm in all, and similar papillary [NH_4] in AD and WD alkali rats) suggests that neither medullary hypertonicity nor [NH_4] is a major determinant of increased hematogenous infection during WD; other unidentified factors peculiar to WD are far more important. However, some protection from reduced tonicity and/or papillary [NH_4] cannot be excluded in view of an apparent trend toward ($P > 0.10$) decreased infection in WL.

230. Inducible Alterations in Cell Wall Structure of Methicillin-Resistant *Staphylococcus aureus*. LEON D. SABATH* AND MAXWELL FINLAND,** Boston, Mass.

The resistance of clinical isolates of *Staphylococcus aureus* to methicillin and other semisynthetic penicillins and cephalosporins appears to be independent of penicillinase (β-lactamase) production and function. The resistance is associated with an alteration in cell wall structure (difference in cell wall amino acid composition) that may be the basis for the resistance. The altered wall structure associated with resistance is a genetically stable trait that is inducible. Isolates taken directly from patients usually show only a small proportion of cells (often only 1 in 10^6) resistant to methicillin, but genetic analysis indicates that virtually all cells in that population have the potential to form abnormal walls and become resistant when inducer (e.g., methicillin or cloxacillin) is added. A phenotypic lag in the formation of the unusual wall noted under certain circumstances may represent time required to dilute out preformed normal wall. When resistant cells are removed from inducers, the vast majority revert to the methicillin-sensitive state with normal wall structure. The rate of reversion shows considerable strain-to-strain variation. Thus, the resistance of *Staph. aureus* to methicillin, like its resistance to penicillin G, is a rather stable trait widely distributed among the cells of a resistant population; in both instances the resistance is present mainly as a potential mechanism which is activated with an inducer. The result is the formation of unusual cell wall in the case of methicillin resistance, or penicillinase synthesis in the case of ordinary resistance to penicillin G. In both instances, cells are changed from the state of antibiotic susceptibility to that of resistance. (Aided by grants AI-23 and TO1-AI-86 and a Career Development award [to L. D. Sabath] from the National Institute of Allergy and Infectious Diseases.)

231. Quantitative Comparisons of Absorption and Excretion of Cholesterol and β-Sitosterol in Man. GERALD SALEN,* E. H. AHRENS, JR.,** AND SCOTT M. GRUNDY,* New York N. Y. (introduced by Morton Spritz).

Measurements of the intestinal absorption of cholesterol and the plant sterol β-sitosterol (which differs only in the addition of an ethyl substituent at C-24) were made by simultaneous isotope turnover and balance methods in three men hospitalized on a metabolic ward for 4 months. Body weights remained constant on formula diets containing both sterols (250 and 100 mg/1000 calories, respectively). Plasma concentrations of cholesterol and β-sitosterol were measured in 18 successive biweekly samples of gas-liquid chromatography; with cholesterol levels in the three men of 287 ±10, 287 ±9, and 226 ±12 mg/100 ml plasma, respective β-sitosterol concentrations were 1.02 ±0.05, 0.51 ±0.02, and 0.30 ±0.05 mg. Free/ester ratios of the two sterols were the same. After pulse labeling with β-sitosterol-22,23-^{3}H intravenously, specific activity-time curves conformed to a two-pool model, permitting calculation of daily turnover for β-sitosterol. However, since β-sitosterol is not synthesized in man, its turnover is equivalent to absorption. Thus, daily absorptions of β-sitosterol were calculated as 12.5, 7.5, and 6.5 mg/day (= 1.5–5.2% of daily intake). Simultaneously, cholesterol absorption was measured as the difference between dietary intake and unabsorbed dietary neutral steroids in feces. Daily absorption of cholesterol averaged 204 ±41, 307 ±64, and 307 ±62 mg/day (= 45–54% of daily intake). About 10% of absorbed β-sitosterol was converted to cholic and chenodeoxycholic acids; the remainder (as free sterol) was secreted into bile more rapidly than cholesterol. These data

demonstrate that β-sitosterol is absorbed from the intestine of man, but only 10% (or less) as effectively as cholesterol. This direct evidence of its limited absorbability validates our use of β-sitosterol as an internal standard in sterol balance studies for correcting losses of neutral sterols during their transit through the gut. However, once absorbed, β-sitosterol enters the same metabolic pathways as cholesterol, including its biotransformation to the primary bile acids of man. (Research supported by USPHS grant HE-06222-08 from the NIH, and by USPHS grant FR-00102 from the General Clinical Research Centers Branch of the Division of Research Facilities and Resources.)

232. Suppression of Glucagon Release by Hypoglycemic Sulfonylureas. E. SAMOLS* AND J. M. TYLER,* Augusta, Ga. (introduced by A. J. Bollet).

A new concept of the mode of action of the hypoglycemic sulfonylureas, and of homeostatic mechanisms, is introduced by demonstrating that these agents suppress pancreatic glucagon release in vivo and in vitro. Pancreatic immunoreactive glucagon was measured in vivo by radioimmunoassay using a specific antiserum. The biological competence of the methodology was confirmed in ducks, in which rapid or constant intravenous infusions of tolbutamide, in doses ranging from 0.05 to 15 mg/kg, suppressed circulating glucagon levels significantly ($P < 0.01$). In normal human subjects, and in patients (including subjects with functioning glucagonomas or insulinomas), plasma levels of insulin, glucagon, and various metabolic parameters were measured after intravenous or alimentary sulfonylureas. All studies strongly suggest that the dynamic changes in the relative concentrations of glucagon and insulin are important determinants of alterations in blood glucose, of clinical effects, and of some "extrapancreatic" effects of the sulfonylureas. In vitro studies of insulin secretion using isolated pancreatic islets showed that an increase in the glucose concentration of the incubation medium increased glucagon release ($P < 0.01$). Addition of tolbutamide caused an initial suppression of glucagon release, followed by a decrease in insulin release. It is concluded that these studies have an interesting and widespread relevance not only to the mechanism of action of hypoglycemic sulfonylureas, but to homeostatic mechanisms and to in vitro and in vivo studies using neutralizing antisera to insulin. (Supported in part by grants GRS FR-5365, AM-12961, and AM-12917 from the NIH.)

233. Effect of Neomycin on 7α-Dehydroxylation of Bile Acids by the Intestinal Bacterial Flora in Man. PAUL SAMUEL,* EDWARD MEILMAN,* AND IGNACY SEKOWSKI,* New Hyde Park, N. Y. (introduced by Herrman L. Blumgart**).

The oral administration of neomycin reduces serum cholesterol levels in man by increasing the fecal excretion of bile acids. Four patients were studied during control periods. Serial incubation of stool specimens with ^{14}C-cholic acid and/or ^{14}C-chenodeoxycholic acid, at 37°C in nitrogen atmosphere for 24 hr, indicated that 83–98% of the primary bile acids were converted to 7α-dehydroxylated compounds and were recovered as deoxycholic and lithocholic acids. Each patient then received 2 g of neomycin daily for 2–3 wk; by the end of 2 wk serum cholesterol levels fell by 17–30% in each patient. Biweekly incubation of stools with labeled bile acids during the 2–3 wk of neomycin administration showed virtual absence of conversion of the primary bile acids to their bacterial transformation products. Kinetic studies of bile acid conversion were performed. These experiments were reproducible within 10% variation on the same stool sample; in longitudinal studies, samples from the same individual showed little variation over a period of weeks. Oral administration of streptomycin, chloramphenicol, ampicillin, and sulfisoxasole showed no difference in the conversion of bile acids by fecal incubation during control and medication periods. These antibacterial drugs had no effect on cholesterol levels in man. The data suggest that the serum cholesterol-lowering effect of neomycin in man may be related to its action on bile acid-converting bacteria.

234. The Relation of Steroid Structure to Gene Expression in HTC Cells; A Unifying Concept. HERBERT H. SAMUELS* AND GORDON M. TOMKINS,** Bethesda, Md.

The glucocorticoid induction of tyrosine aminotransferase (TAT) in hepatoma tissue culture (HTC) cells provides a simplified system for investigation of the mechanism of steroid regulation of gene expression. From kinetic studies of the relation of steroid structure to TAT synthesis, we have separated a wide variety of steroids into four categories: (1) Strong inducers: C_{21} 11βOH steroids (aldosterone, corticosterone, cortisol, 6α-methyl-11βOH-progesterone, and dexamethasone) which induce TAT at concentrations close to the physiologic levels of natural glucocorticoids. The first four compounds induce to half maximal levels at about 5×10^{-8} M, and to maximal levels at 5×10^{-7} M, whereas the fluorinated steroids are as effective at one-tenth the concentration, but induce to the same maximal level. The dose-response curve suggests cooperative kinetics. (2) Weak inducers (inhibitors): C_{21} steroids (progesterone, 5α-dihydrocortisol, 17αOH-progesterone, 11βOH-progesterone, 11-deoxycorticosterone, 11-deoxycortisol) which even at very high concentrations induce only to submaximal levels characteristic for each compound. In addition, these compounds competitively inhibit induction by strong inducers and limit the induced level to that seen with the weak inducer alone. The dose-response kinetics appear cooperative. (3) Anti inducers (cortisone, 5β-dihydrocortisol, and derivatives of testosterone and estrogens). These compounds do not induce at any concentration, but competitively inhibit induction by strong inducers. (4) Inactive steroids, which neither induce nor inhibit. The differences in biological activity of these four groups cannot be accounted for on the basis of differential effects on steroid uptake, steroid metabolism, TAT turnover, or general protein synthesis. To account for inhibition, cooperative kinetics, and differences in inducer activity, we propose an allosteric steroid receptor which equilibrates between a functional and a nonfunctional state. Owing to a different affinity for each state, the action of a steroid is determined by its ability to affect the position of the equilibrium. The role of this receptor in the regulation of enzyme synthesis will be discussed.

235. In Vivo Studies of the Effect of Inflammation on the Metabolism of Synovial Fluid Hyaluronateprotein. JOHN SANDSON, New York, N. Y.

In the normal wrist joints of young calves, intra-articularly injected ^{3}H-glucosamine is incorporated into hyaluronateprotein (HP). The half-life of ^{3}H-HP in the normal wrist joint is about 30 hr. Synovitis was produced in the wrist joints of the calves by two different methods: either by the intra-articular injection of sodium urate (20 mg) or by the intra-articular injection of human fibrinogen (1 mg) in a calf previously immunized with human fibrinogen. Both methods produced a warm, swollen joint with an outpouring of white blood cells and serum proteins into the synovial fluid. ^{3}H-glucosamine was then injected into joints in which either a "urate" or an "immune" synovitis had been induced. The amount of ^{3}H-glucosamine incorporated into ^{3}H-HP was markedly increased in the inflamed joints. 2 hr after the injection of ^{3}H-glucosamine, the specific activity of ^{3}H-HP was 100 times higher in the inflamed than in the normal joints. After about 1 wk the rate of disappearance of ^{3}H-HP from the inflamed joints decreased markedly, suggesting local reutilization of ^{3}H-glucosamine (and possibly ^{3}H-HP) in the inflamed joint. Local reutilization of ^{3}H-glucosamine has not been demonstrated in the normal joint. These studies indicate that "urate" and "immune" induced synovitis may produce similar alterations in the metabolism of synovial fluid hyaluronateprotein. (This work was supported by grant AM-07343 from the NIH and contract I-157 from the Health Research Council of the City of New York.)

236. Cardiac Metabolism in Thiamine Deficiency. S. SCHENKER, C. HANSEN,* R. BUTCHER,* AND D. W. McCANDLESS,* Dallas, Texas.

Thiamine is an essential coenzyme of pyruvate decarboxylase and transketolase. Both enzymes, and especially the decarboxylase, participate in reactions which generate ATP, the ultimate source of energy for cardiac work. In this study, cardiac pyruvate decarboxylase and transketolase activity were correlated with the ATP concentration during development of and immediately after recovery from a thiamine-deficient state. Rats with diet-induced thiamine deficiency developed encephalopathy and significant cardiac hypertrophy ($P < 0.01$) at 5 wk. The encephalopathy was abolished fully in 24 hr after 15 μg of thiamine. Normally fed and pair-fed rats, which remained asymptomatic, served as controls. In the pair-fed controls, blood transketolase, cardiac transketolase, pyruvate decarboxylase, and ATP were slightly lower but the heart was not hypertrophied. Blood transketolase and cardiac transketolase and pyruvate decarboxylase activity in thiamine-deficient rats at 3 wk were 88, 64, and 61% below pair-fed values ($P < 0.001$). At 5 wk these values in thiamine-deficient rats were depressed by 89, 66, and 76% respectively ($P < 0.001$). Cardiac ATP in thiamine-deficient rats at 2 and 3 wk was not significantly decreased, but at 4 and 5 wk respectively was 17% and 33% below pair-fed control values ($P < 0.001$). Ventricle and auricle exhibited similar changes. At 24 and 72 hr after thiamine administration to symptomatic (5 wk) thiamine-deficient as compared with pair-fed control rats, blood transketolase was still depressed by 88 and 90% and heart transketolase by 58 and 58%, but heart pyruvate decarboxylase by only 44 and 23% respectively, and ATP not at all. Thiamine pyrophosphate in vitro markedly enhanced pyruvate decarboxylase activity of thiamine-deficient (5 wk) as compared with control heart but had no effect on cardiac transketolase. These data indicate that thiamine deficiency induces cardiac hypertrophy and a reversible depletion of heart ATP and suggest that this correlates better with a disturbance in cardiac pyruvate decarboxylase than in transketolase activity. (Research supported by grants from the NIH.)

237. Cytochrome P-450 of Human Liver Microsomes. J. B. SCHENKMAN,* H. L. GURTOO,* T. DONDERO,* AND D. G. JOHNS, New Haven, Conn.

Oxidative catabolism of many lipid-soluble drugs is catalyzed by mixed-function oxidases present in the endoplasmic reticulum of liver parenchymal cells. The terminal step in electron transfer by this system is the hemoprotein cytochrome P-450. Since little is known about the properties of the mixed-function drug oxidase system in man, this investigation was initiated to determine whether cytochrome P-450 could be demonstrated in human liver microsomes and whether it is involved in the oxidation of typical drug substrates. Necropsy specimens of human liver, obtained within 4 hr after death, were perfused with isotonic saline to reduce their hemoglobin content, and homogenized in sucrose, 0.25 M, followed by separation of subcellular components by centrifugation. The yield of microsomes ranged from 10 to 15 mg per g of liver. Suspensions containing 2 to 3 mg human microsomal protein per ml were examined by difference spectrophotometry. The presence of cytochrome b_5 was demonstrated by comparing NADH-reduced suspensions with aerobic suspensions, and the presence of cytochrome P-450 by comparing dithionite-reduced suspensions with dithionite-reduced suspensions to which carbon monoxide had been added. The two cytochromes appeared identical with those of experimental animals, but were present in lower concentrations: approximately 0.15 mμmole of cytochrome b_5 and 0.05 mμmole of cytochrome P-450 per mg human microsomal protein, in contrast to levels of approximately 1.2 and 1.5 mμmoles respectively in the rabbit, and 0.5 and 0.6 mμmole in the rat. The human liver microsomal suspensions metabolized aminopyrine, aniline, and benzpyrene. Enzyme activities and P-450 levels declined rapidly with time; in liver specimens obtained more than 5 hr after death, cytochrome P-450 could not be detected, and, of the substrates examined, only benzpyrene hydroxylase activity was still demonstrable. (Research supported by grants PRA-58 and T-506 from the American Cancer Society and CA-10748 from the NIH.)

238. Reversal of Digoxin Toxicity with Specific Antibodies. DONALD H. SCHMIDT* AND VINCENT P. BUTLER, JR.,* New York, N. Y. (introduced by Rejane M. Harvey**).

Antibodies capable of binding digoxin have been produced in rabbits and dogs immunized with digoxin–human serum albumin conjugates. This study was designed to determine whether digoxin-specific antibodies can reverse established

digoxin toxicity in the nonimmunized dog. 12 unanesthetized dogs were given 0.09 mg digoxin per kilogram intramuscularly for 3 consecutive days. All animals developed significant arrhythmias (atrioventricular block, ventricular premature contractions, and/or ventricular tachycardia). Five of these dogs received no serum or plasma; in these control animals arrhythmias persisted for 6 hr. Four of these dogs died within 24 hr of the last injection. Each of two additional control animals received 260 ml of normal canine plasma beginning 1 hr after the arrhythmia had become established. The arrhythmias persisted for 6 hr in each animal, and both died within 24 hr. The remaining five dogs were also given the toxic dose of digoxin as described above. After an arrhythmia had been recorded for 1–2 hr, these dogs were given varying amounts of canine plasma and/or rabbit serum containing digoxin-specific antibodies. The arrhythmia reverted to a sinus mechanism in four of the dogs within 30–90 min after the start of the infusion. These animals remained in sinus rhythm from 1 to 3 hr before reverting to a dysrhythmia which was abolished by administering more antibody. No animal received a total of more than 260 ml of antiserum. At the end of the 6 hr period of study all four dogs were in normal sinus rhythm. The fifth dog had ventricular tachycardia which was converted to atrial tachycardia with variable block and persisted 24 hr before reverting to a sinus mechanism. All five dogs survived. This study indicates that digoxin-specific antibodies can reverse severe established digoxin toxicity in the dog. (Research supported by grants from the NIH and the New York Heart Association.)

239. Inhibition of Cyclic 3′,5′-Adenosine Monophosphate–Activated Lipase. Paul H. Schreibman,* Dana E. Wilson,* and Ronald A. Arky,* Boston, Mass. (introduced by Sidney H. Ingbar).

Although antilipolytic agents are thought to act by lowering intracellular levels of cyclic 3′,5′-adenosine monophosphate (cAMP), we have shown in studies with both intact fat pads and isolated fat cells that the stimulation of lipolysis by added cAMP (10^{-3} M) was completely inhibited by the beta adrenergic blocker propranolol (10^{-5} M). To investigate whether the adipocyte membrane is required for this inhibition, cell-free extracts of both rat and human fat tissue were prepared. The clear infranatant obtained after sonification and ultracentrifugation was filtered through Sephadex 25 gel to remove nucleotides. The extract had lipolytic activity against a variety of glyceride substrates. It was essentially free of lipoprotein lipase, as evidenced by lack of inhibition by 1.0 M NaCl or of augmentation by serum proteins. In the presence of cAMP the extract exhibited greatest activity against a monoglyceride (monostearate) emulsion. Addition of ATP (10^{-7} M) potentiated the effect of cAMP. The optimal range of cAMP activation was limited, and actual inhibition was observed at concentrations exceeding 10^{-7} M. Mean activation (323 ±28% above basal) was not affected by 0.001–1.0 μU/ml of insulin, but was completely inhibited by propranolol (10^{-8} M), phentolamine (10^{-8} M), nicotinic acid (10^{-7} M), and prostaglandins E_1 and $F_1\beta$ (10^{-7} M). We conclude that (1) a cAMP-activated monoglyceridase is present in a cell-free extract of adipose tissue and ATP potentiates this activation; (2) the stimulation of monoglyceridase is directly inhibited by several antilipolytic agents at a site distinct from the classical alpha and beta adrenergic receptors; (3) this inhibition does not require the adipocyte membrane. (Supported by NIH grants AM-11176 and AM-5060.)

240. Immunofluorescence of Cutaneous Vasculitis. Arnold L. Schroeter,* Peter W. M. Copeman,* Robert E. Jordon,* W. Mitchell Sams, Jr.,* and R. K. Winkelmann,* Rochester, Minn. (introduced by Ward S. Fowler**).

Heretofore, the clinical and histopathologic features of dermal vasculitis have not been found to correlate well with definite direct immunofluorescence (IF) patterns, making it difficult to implicate an immunologic relation. The purpose of this study was to define immunofluorescence patterns of dermal vasculitis. Direct IF of necrotizing angiitis, livedo vasculitis, and facial granuloma was performed using fluorescein conjugates specific for IgG, IgA, IgM, beta lc/a, and fibrin. Patterns of staining were distinct for the entities studied. Control direct IF performed on 35 normal and pathologic tissues demonstrated no globulin or complement staining in vessels. A diffuse perivascular IF pattern of IgG, IgM, or complement was found in 15 of 24 cases of necrotizing angiitis. Livedo vasculitis (four cases) showed IgG, IgM, or complement staining of vessel walls only. Facial granuloma (three cases) demonstrated staining of IgG, IgM, IgA, or complement in a reticulated periadventitial pattern. Although IF patterns were correlated with definite vascular histopathologic changes, there was no association with severity of disease. This demonstration of the binding of immunoglobulin, and particularly of complement, implicates circulating immune complexes related to the disease process.

241. Separation of Liver Glycogen in Density Gradients of Sodium Iothalamate. Robert B. Scott,* Kathryn Hull,* Charlotte Hart,* and Laverne W. Cooper,* Richmond, Va. (introduced by G. Watson James, III**).

An ideal density gradient material for glycogen isolation would be one with high density, low viscosity, and low reactivity with glycogen particles. Sucrose solutions are limited in their usefulness by their limited density range, high viscosity, and interference with chemical determination of glycogen. Other substances, such as cesium chloride, allow gradients of high density to form, but provide a highly unphysiologic ionic environment. Sodium iothalamate, a radiopaque material for angiography, is perhaps more physiologic than cesium chloride solutions and can form density gradients of higher density than is possible with sucrose, with much lower viscosity. The density of the stock 80% solution is 1.499. The density varies linearly with refractive index, which is a convenient indicator for gradient determinations. Iothalamate does not interfere with the anthrone determination of glycogen. Starting with iothalamate solutions of density 1.45, gradients of iothalamate varying from 1.3 to 1.6 density are formed during extended centrifugation at 40,000 rpm in an SW-50 rotor. Rat liver homogenates are prepared in 0.01 M Tris, pH 8.0, and nuclei are removed at 800 *g*. A layer of 2.5 ml of iothalamate of density 1.45 is placed in a titanium 50 rotor tube and a thin layer of 70% sucrose is layered above it. The post-

nuclear supernatant is then added and the tubes are spun at 45,000 rpm for 3 hr. A band containing glycogen forms in the upper portion of the iothalamate, while the vast majority of the membranes and granules remain at the sucrose-homogenate interface. The iothalamate layer is then placed in the SW-50 rotor and the gradient formed during centrifugation. Isopycnic banding of glycogen occurs at a density of about 1.45 to 1.48. It can be recovered readily by collecting fractions sequentially from a needle piercing the bottom of the tube.

242. On the Mechanism of Renal Potassium Wasting in Patients with Renal Tubular Acidosis. ANTHONY SEBASTIAN,* ELISABETH MORRIS,* IRIS UEKI,* AND R. CURTIS MORRIS, JR.,* San Francisco, Calif. (introduced by Hibbard E. Williams).

In patients with renal tubular acidosis (RTA), renal potassium wasting (RKW) is generally considered a result of the renal acidification defect because correction of acidosis with alkali therapy can correct RKW. But after reduction of potassium supplements in four patients with RTA (RTA_{1-4}), persisting hypokalemia developed, yet urinary excretion of potassium (U_KV) ranged from 40 to 100 mEq/day despite sustained correction of acidosis with alkali therapy. Because urinary pH remained >7.4, RKW could not be explained as the result of a gradient limitation on H^+-Na^+ exchange; urinary excretion of aldosterone was not increased (RTA_{1-3}). In RTA_{3-7}, in whom RKW coexisted with Fanconi's syndrome (FS), a 15–40% reduction in tubular reabsorption of HCO_3^- ($THCO_3^-$) at normal plasma [HCO_3^-] indicated impaired HCO_3^- reabsorption in the proximal nephron. The severity of RKW correlated with the apparent rate of proximal $NaHCO_3$ rejection: (1) at normal plasma [HCO_3^-], U_KV and C_K/C_{inulin} varied directly with the magnitude of reduction of $THCO_3^-$; in $RTA_{4,5}$, C_K/C_{inulin} was >1.5 at serum potassium <3.8 mEq/liter; (2) when the plasma [HCO_3^-] was raised from 18 to 28 mmoles/liter, successive increments in filtered HCO_3^- were excreted quantitatively and U_KV and C_K/C_{inulin} increased strikingly, in direct proportion to ΔU_{HCO_3}-V. These findings support the hypothesis that RKW in patients with RTA-FS results from increased delivery of Na^+ and the relatively impermeant HCO_3^- ion to the distal nephron (DN). The findings predict that in patients with RTA-FS, RKW will invariably worsen and persist when acidosis is corrected with $NaHCO_3$ alone. Increased $NaHCO_3$ delivery to the DN might also explain persisting RKW in RTA_1, who had classic RTA; although $THCO_3^-$ was reduced <5%, increased delivery of $NaHCO_3$ to the DN was suggested by the finding that urine [HCO_3^-] and pH decreased significantly when urine flow was increased by water diuresis, at both normal and subnormal plasma [HCO_3^-]. (Research supported by a grant from the NIH.)

243. Bile Salt Regulation of Fat Absorption Rate: The Mechanism of Steatorrhea in Blind Loop Syndrome. JOHN R. SENIOR,* M. L. CLARK,* F. CHENEY,* AND H. LANZ,* Philadelphia, Pa. (introduced by Truman G. Schnabel, Jr.**).

Discordant explanations for the steatorrhea occurring with excess proximal gastrointestinal bacteria ascribe it either to inhibition of fat absorption by unconjugated bile salts or to deficient concentration of conjugated bile salts. To resolve this question, we have infused micellar fatty acid solutions into duodenums of over 50 unanesthetized rats with lymph and bile fistulas, maintained in chronic and steady-state condition for up to 2 wk. We have found that deoxycholic acid (DC) does not inhibit absorption and esterification into lymph triglycerides of ^{14}C-palmitate or -oleate in the living animal, although marked inhibition occurs in fresh everted jejunal sacs with 0.5 mM DC, even with 15–30 mM taurocholate (TC). Recently we have shown that normal fatty acid absorption occurs in vivo despite 2–5 mM luminal DC, not because of selective proximal removal of DC. After infusing 1 mM palmitate in (15 mM TC) : (2 mM DC) with polyethylene glycol in isotonic buffer (pH 6.5), sampling at the ligament of Treitz, 10 and 20 cm distal, revealed palmitate absorption of 64, 75, and 92%, but DC absorption of only 27, 48, and 67%; without DC, palmitate absorption was 71, 83, and 94%. However, reduction of TC from optimal 15 mM led to gradually less fatty acid absorption, then sharp lessening below 8 mM as micellar solubilization was decreased. Further, a definite intracellular effect of bile salts on regulation of fat absorption was shown by prelabeling the intestine with ^{14}C-palmitate, then infusing alternately saline-glucose at 15 mM TC without fatty acid; despite constant lymph flow the TC caused 10- to 30-fold increase in release rate of ^{14}C-triglyceride into lymph. These data, in accord with recent clinical findings, allow earlier discrepant interpretations to be understood and reconciled, and show reduction in conjugated bile salt concentration to explain the steatorrhea of the blind loop syndrome.

244. Formation of an Artificial Lysosome In Vitro. GRAZIA SESSA* AND GERALD WEISSMANN, New York, N. Y.

Phospholipids form multicentric spherules (liposomes) in aqueous solutions of marker ions, glucose, or amino acids. Release of markers from liposomes is analogous to release of ions from erythrocytes or enzymes from lysosomes; furthermore, liposomes resemble biomembranes in response to steroids, polyenes, and lytic proteins. Heretofore it had not been possible to sequester enzymes in liposomes: a crucial step both for perfection of the model and if enzyme-containing liposomes are to be used for replacement of lysosomal enzyme deficiencies in heritable storage diseases, e.g. Hurler's syndrome or Pompe's disease. By swelling purified ovolecithin, cholesterol, and long-chain cations (stearylamine) or anions (dicetyl phosphate) in 0.29 M glucose containing 2.4 mg egg white lysozyme per 100 μmoles lipid, it was possible to trap the cationic enzyme (with glucose) in liposomes. Untrapped lysozyme was separated from liposomes by chromatography on Sephadex G-50 or G-75; enzyme-containing spherules trapped from 2.3 to 3.8 μg lysozyme per μmole lipid. When exposed to lysozyme substrate (*Micrococcus lysodeikticus*), liposomes were inactive until Triton X-100 was added; 85–98% of lysozyme was then released from lipid-bounded "latency." Nonspecific (ionic) interactions were excluded since (*a*) positively and negatively charged liposomes trapped lysozyme equally; (*b*) glucose was also trapped; this marker is retained only by intact liposomes; (*c*) when liposomes formed in glucose alone were chromatographed with added

lysozyme, no association was found; and (*d*) the amount of lysozyme and glucose rendered latent varied with the internal water spaces of liposomes (molar per cent of charged membrane components). Since lysozyme is a "drop of oil with a polar coat," it is likely that the enzyme (diameter, 45 A) is sequestered in aqueous channels between lipid lamellae of liposomes (crystallographic repeat unit, 76 A). These studies not only suggest that "latency" and other lysosomal functions can be duplicated by a model system, but suggest that clinical deficiencies of lysosomal enzymes can be approached by means of selective incorporation of enzymes in lipid spherules.

245. Ribonucleic Acid in Normal and Leukemic Granulocytes. GEORGE S. SHIELDS,* JOHN J. WILL,* HELEN S. GLAZER,* AND ELIZABETH TAYLOR,* Cincinnati, Ohio (introduced by Richard W. Vilter).

The ribonucleic acid content of rat chloroleukemic cells (10.0 picograms per cell) is greater than that of normal rat bone marrow (1.8 pg/cell) and splenic cells (3.0 pg/cell). To investigate whether this biochemical difference in chloroleukemic cells merely reflects greater immaturity or a more fundamental abnormality of these cells, selected pools of immature granulocytes from normal rat bone marrow were obtained by sedimentation in a sucrose density gradient. The morphologic appearance and maturity of granulocytes in the selected fraction of normal rat bone marrow were similar to those of the chloroleukemic cells. The RNA content of the normal granulocytes from the selected pool was 2.6 pg/cell. The nucleotide composition of the RNA of chloroleukemic cells and of normal splenic and bone marrow cells was the same. Nuclei isolated from the chloroleukemic cells contained 4.9 pg/nucleus while those of the normal splenic cells contained 1.2 pg/nucleus. Extraction of the RNA from the nuclei of these two cell types by the method of Drews and Brawerman and fractionation of the RNA on a sucrose density gradient demonstrated a higher level of RNA in each molecular weight class of the chloroleukemic cell nuclei. This difference was most marked in the heavier molecular weight (>28S) component of the RNA fraction isolated at pH 8.3 and 37°C. This component was the only RNA isolated from chloroleukemic cells which had a nucleotide composition ($G + C:A + U = 1.6$) different from that of a corresponding component isolated from splenic cells ($G + C: A + U = 1.0$). It is suggested that the ribosomal mass of chloroleukemic cells is increased both in the nuclei and in the cytoplasm and that the greatest difference from normal occurs in that fraction of nuclear RNA which is isolated with the DNA-like RNA. (This work was supported in part by NIH grant CA-07457 and American Cancer Society grant T-487.)

246. Two Pathways of Catabolism of Erythrocyte Phospholipid Fatty Acids (FA). STEPHEN B. SHOHET,* Boston, Mass. (introduced by David G. Nathan).

Phosphatides of erythrocyte membranes are renewed by two processes: (1) passive exchange with plasma phosphatides, and (2) active stepwise incorporation of plasma FA into lysophosphatides. To study the catabolism of FA incorporated by both routes, mature erythrocytes were incubated with phosphatidyl choline (PC), labeled with ^{14}C-FA in the β position, or with ^{3}H-FA bound to albumin. After incubation, surface radioactive FA was removed by washing with defatted albumin and replaced by nonradioactive FA. The cells were then reincubated in serum under various conditions. Chromatographs of serial lipid extracts of cells and serum were analyzed. Passively incorporated PC was removed during reincubation at 0.73%/hr, which approximated its incorporation rate of 0.66%/hr. PCMB, NaF, and reincubation in heated serum did not influence this process of PC exchange. Actively incorporated ^{3}H-FA in PC was discharged to serum primarily as FA at only 27% of its original incorporation rate, but there was no change in the total or the distribution of cell lipids. This implied transfer of the label from PC, and further analysis, confirmed that 48% of the PC FA transferred either to phosphatidylethanolamine (32%) or to neutral lipid (16%). Both discharge and transfer of incorporated FA were inhibited by NaF, PCMB, and reincubation in heated serum. Cells were doubly labeled by both ^{14}C-PC and ^{3}H-FA. The PC in the reincubation serum contained 88% ^{14}C and only 12% ^{3}H. The FA in the serum contained 93% ^{3}H and only 7% ^{14}C. Membrane phospholipid FA derived by active incorporation into lysophosphatides has a metabolic fate distinct from that obtained by passive PC exchange. It may transfer from one phosphatide to another or to neutral lipid before eventual discharge as FA. FA on passively exchanged phosphatide is primarily returned to serum intact. There is only slight mixing between these two pools of erythrocyte phospholipid FA.

247. Cephaloridine Nephrotoxicity: A Light and Electron Microscope Study in Rabbits. FREDRICK SILVERBLATT,* RUTH BULGER,* AND MARVIN TURCK,* Seattle, Wash. (introduced by Robert G. Petersdorf).

The antibiotic cephaloridine has been reported to cause renal tubular necrosis in man and in experimental animals. On the other hand, cephalothin, a closely related drug, is not associated with renal injury. In order to delineate further the site and morphology of the lesion, 15 rabbits were given 200 mg/kg cephaloridine. Their kidneys were fixed by intravascular perfusion at intervals from 1 to 48 hr. Light microscope examination showed alterations in the height and uniformity of the proximal tubular brush border at 1 hr. At later intervals, the proximal tubule became dilated and more acidophilic, and appeared shrunken. Frank necrosis was evident in 16 hr. With the electron microscope, early changes were observed to consist of loss of microvilli, and the disappearance of apical pinocytic invaginations and large apical vacuoles. Numerous uncoated vesicles appeared in the apex. At later stages, disorganization of lateral membrane interdigitations, condensation of the cytoplasm, and mitochondrial swelling were observed. Other regions of the nephron did not appear to be damaged. Necrosis did not result from administration of either 200 mg/kg cephalothin or 50 mg/kg cephaloridine administered to other groups of rabbits. However, proximal renal tubular lesions developed in two of three rabbits receiving 100 mg/kg cephaloridine. Serial sections revealed the site of injury to be limited to the second segment of the proximal tubule. Uptake of horseradish peroxidase was blocked in damaged tubules 1 hr after cephaloridine treat-

ment, indicating interference with pinocytic activity. These data demonstrate that in the rabbit cephaloridine produces a dose-dependent lesion of the second proximal tubule segment which results in early disruption of apical cell membrane structure and function. Similar changes may account for the renal injury reported in man.

248. Metabolism of Bile Salt in the Fetal Dog. Richard A. Smallwood,* Roger Lester, George J. Piasecki,* Helmut F. J. Rauschecker,* and Benjamin T. Jackson,* Boston, Mass.

Because of technical limitations, virtually nothing is known about bile salt metabolism in the fetus. Fetal cholate excretion was investigated in this study using advanced techniques for intrauterine surgery. Near-term fetal dogs were prepared in utero with indwelling jugular, carotid, and biliary cannulas. ^{14}C-cholate was infused intravenously into the fetus over 6 hr, and fetal bile and arterial blood samples were obtained over 10 hr. Fetal plasma disappearance, biliary excretion, tissue distribution, and placental transfer of infused material were measured. Bile salts were quantitated enzymatically, and identified by TLC and GLC. Fetal biliary excretion of ^{14}C label began within 30 min of the start of ^{14}C-cholate infusion, and reached maximum rates of 80% of the infusion rate. After 10 hr, distribution of the administered ^{14}C label was as follows: fetal biliary excretion, 58–68% (over nine-tenths as ^{14}C-taurocholate); fetal liver, 2–7%; fetal plasma, 1–2%; placental transfer, 2–6% (as intact ^{14}C-cholate); and small amounts in other fetal tissues, placenta, and amniotic fluid. Total recovery of ^{14}C label was 72–80%. The total fetal bile salt pool equaled 50 μmoles, and endogenous fetal bile salt excretion equaled 1.5 μmoles/hr during the initial 10 hr of biliary drainage. *Conclusions:* (1) Unconjugated cholate is cleared rapidly from plasma by fetal dog liver, conjugated with taurine, and secreted into bile; (2) there is little dependence on the placenta as an excretory organ for unconjugated cholate; and (3) the results provide the first direct demonstration that bile salt is excreted continuously into the fetal biliary system and intestine. In summary, the study reveals the presence of a remarkably "mature" mechanism for hepatic bile salt secretion in fetal dogs.

249. Identification of a Candida Clumping Factor and the Influence of the Immune Response on Candida Morphology and Infectivity in Rabbits. J. Kelly Smith* and Donald B. Louria,** New York, N. Y.

The capacity of human serum to reduce populations of *Candida albicans* in vitro is frequently lost during active candida infection. To study this experimentally, 14 rabbits were immunized with heat-killed *C. albicans* and their sera examined for agglutinating antibody to whole candida, precipitating antibody, and skin reactivity to a sonicated candida extract (S antigen), and for their ability to reduce candida populations in rotary experiments. Preimmunization sera reduced populations an average of 45-fold over zero hour controls; this reduction was associated with clumping of yeasts and mycelia, and could be duplicated by 15 mg/100 ml or more of a purified fraction of rabbit macroglobulin. The macroglobulin was of γ_1-β mobility, and had no precipitating or agglutinating activity in standard assays. Immunization resulted in a gradual loss in population reduction and clumping activity which paralleled the appearance of agglutinating and precipitating antibody and skin reactivity to S antigen. Activity was significantly altered only when agglutinating titers reached 1:80–1:160 or more, and could be restored by absorbing the sera with candida. Purified fractions of IgG containing high titers of agglutinating antibody vitiated the population reduction and clumping effects of normal serum or the macroglobulin fraction, whereas purified IgG from the same preimmunized animals did not. Hyperimmune IgG and serum promoted mycelial transformation in vitro, and appeared to enhance candida skin infection in rabbits. The results suggest that the ability of rabbit serum to reduce candida populations in vitro is related to the effects of a macroglobulin. The failure of this macroglobulin to clump candida in infection is due to the production and preferential binding to candida of IgG antibody present in sufficient quantity to produce antibody excess. This antibody may paradoxically promote candida infection. (Research supported by grants from the American Cancer Society, the National Institute of Allergy and Infectious Diseases, and the Health Research Council of the City of New York.)

250. Measurement of Clinical Blood Levels of Digoxin by Radioimmunoassay. Thomas W. Smith* and Edgar Haber, Boston, Mass.

Because of the lack of a clinically applicable means of measuring digoxin blood levels, a rapid, sensitive, and specific radioimmunoassay has been developed. Digoxin-specific antibody was raised in rabbits immunized with a human serum albumin–digoxin conjugate (kindly supplied by Dr. V. P. Butler). Tritiated digoxin (specific activity 3.2 c/mmole) bound by this antibody is displaced by unlabeled digoxin and separated from free glycoside by a modification of the coated-charcoal method of Herbert. No extraction of digoxin from serum is necessary. Serum levels are determined by comparison with a standard curve derived from addition of known amounts of digoxin. The sensitivity of the technique is at least 0.2 mμg/ml, well below usual therapeutic levels, and replicate determinations give a standard deviation of $\pm$4%. The determination is highly specific for the steroid nucleus of digoxin; little cross-reactivity is observed with digitoxin. There is no interference from cortisol, progesterone, 17β-estradiol, testosterone, dehydroepiandrosterone, or cholesterol, even when present in 10,000-fold molar excess. The serum level in patients with normal BUN and creatinine values without evidence of digitalis excess, receiving a daily oral maintenance dose of 0.25 mg, was 1.1 $\pm$0.3 mμg/ml (SD) (range 0.8–1.6 mμg/ml). Corresponding values for a 0.5 mg dose level were 1.4 $\pm$0.4 mμg/ml (range 0.9–2.4 mμg/ml). Considerably higher levels have been encountered in patients with diminished renal function and/or digitalis toxic arrhythmias. Since a determination can be done in 1 hr, the technique is highly applicable to the assessment of patients with arrhythmias requiring urgent management. It will also facilitate studies of the clinical pharmacology of digoxin, without the need for administration of radioisotopes to the patient. Promising preliminary results have been obtained with a similar rapid radioimmunoassay for digitoxin.

251. Synthesis of Transferrin by Human Peripheral Blood Lymphocytes. Henry D. Soltys* and Jerome I. Brody, Philadelphia, Pa.

The purpose of this investigation was to determine whether human peripheral blood lymphocytes synthesize transferrin. Observations that the bacteriocidal and opsonin-like effects of serum are destroyed when iron salts are added to saturate transferrin implied that this protein may be a component of an auxiliary antibody globulin system and a potential product of lymphoid tissue. Lymphocytes were separated from defibrinated venous blood by dextran sedimentation and differential centrifugation, and grown in duplicate cell cultures with the addition of a ^{14}C-labeled amino acid hydrolysate to one of the vials. After 72 hr, the cells were disrupted ultrasonically, the fragments centrifuged, and clear extracts lyophilized. They were redissolved in buffer, and $^{59}FeCl_3$ was added to the nonradioactive lymphocyte extract. To serve as a control marker, autologous serum also was combined with $^{59}FeCl_3$. Lymphocyte extracts, one carrying ^{59}Fe and the other ^{14}C-labeled amino acids incorporated during cell culture, were concentrated against Carbowax. These were analyzed for the presence of transferrin by immunoelectrophoresis, double diffusion, and radioautography. A single precipitin band, similar to that of the serum marker, was formed by reacting ^{59}Fe-tagged lymphocyte extract against antitransferrin as detected by radioautography and by conventional staining of an identically paired slide. More importantly, the development of a radioimmunoprecipitate, with extracts of ^{14}C-labeled cell protein and antihuman transferrin in double diffusion, supports the contention that lymphocyte transferrin biosynthesis actually did occur in vitro. Although the precise role of transferrin in human immunity remains undefined at the moment, its additional localization in the lymphocyte, and the occurrence of Gram-negative sepsis in conditions such as sickle cell anemia, hemochromatosis, and cirrhosis, in which the concentration of free transferrin is diminished, may implicate this iron-binding protein as a significant participant in host defense. (Supported by grants from the NIH.)

252. Impaired Glucose Tolerance in Progeny of Rats with Induced Latent Diabetes. Gabriel Spergel,* Leonard J. Levy,* and Martin G. Goldner,* Brooklyn, N. Y. (introduced by David M. Kydd**).

Latent diabetes was induced in Sprague-Dawley rats, a breed not noted for developing spontaneous diabetes. The rat colony was maintained under conditions of constant temperature, light, and humidity control. 10 normal animals served as original breeding stock. 60 g rats ($n = 26$) serving as the parent generation were given alloxan intraperitoneally in a dose of 150 mg/kg body weight. This dose caused no or minimal initial glycosuria, but persistent glucose intolerance when rats were challenged with an intracardiac glucose load of 1 g glucose per kg body weight (ICGTT). Age- and weight-matched littermates served as controls. Rats were allowed to mature and breed. Breeding was arranged as follows: group A, normal males $\times$ alloxan females; group B, alloxan males $\times$ normal females; group C, alloxan males $\times$ alloxan females; and group D, normal males $\times$ normal females. F_1 and F_2 generations were mated in all possible combinations. Litter size and birth weights were identical for all animals. 339 ICGTT were performed on F_1 and F_2 animals. The mean glucose disappearance rate ($-\%K$) for the alloxan-treated animals ($n = 26$) was 1.50 $\pm$0.10 (SEM), while for control animals ($n = 94$) $-\%K$ was 2.55 $\pm$0.09, $P < 0.001$. Control male animals had a significantly higher $-\%K$ than females (2.77 $\pm$0.14 vs. 2.32 $\pm$0.10). This difference was quenched in the alloxan-treated animals (1.60 $\pm$0.09 vs. 1.44 $\pm$0.16). F_1 offspring ($n = 232$) showed carbohydrate intolerance whether the treated parent was male or female (groups A and B), but most markedly in group C (male $-\%K = 2.13$ $\pm$0.13, female $-\%K = 1.60$ $\pm$0.08). F_2 generations ($n = 66$) showed persistence of abnormal ICGTT of similar severity. It thus appears that the alloxan-induced carbohydrate abnormality is transmissible to the offspring (F_1 and F_2) by either the male or the female parent. (This research was supported by grants from the NIH, National Institute of Arthritis and Metabolic Diseases.)

253. A New C′3 Activation and Conversion Mechanism Unrelated to C′3 Convertase in Patients with Glomerulonephritis. Roger E. Spitzer,* Enrique H. Vallota,* Judith Forristal,* Ann Stitzel,* Neil C. Davis,* and Clark D. West,* Cincinnati, Ohio (introduced by Edward L. Pratt**).

The sera of certain patients with glomerulonephritis contain a substance which, when reacted with a cofactor present in normal serum, produces an enzyme capable of directly activating human C′3. Neither of these factors nor the enzyme itself is identifiable as a component or product of the complement system. The activation of C′3 by this enzyme can initiate the hemolysis of normal human erythrocytes in the absence of both antibody and C′3 convertase. When C′3 is activated by this enzyme in the presence of an immune precipitate but in the absence of C′3 convertase, a portion of the C′3 molecules deposit in a form which is clearly distinct from C′3i. The C′3 which does not deposit is lysed in the fluid phase into at least two inactive products, β1A and α2D. This enzyme has therefore been called C′3 lytic nephritic factor (C′3LyNeF). The factor in nephritic serum, C′3 nephritic factor (C′3NeF), and the cofactor in normal serum which are the precursors of C′3LyNeF are both pseudoglobulins. The combination of these two substances to produce C′3LyNeF occurs rapidly and is dependent on the presence of Mg^{++} but not Ca^{++}; once formed, however, C′LyNeF acts in the absence of both Mg^{++} and Ca^{++}. C′3LyNeF has a broad pH optimum (pH 6–9) but a narrow temperature range, with maximal activity at 37°C. Studies based on the destruction of the B antigen of C′3 demonstrate typical enzyme kinetics, 80% of the C′3 in normal human serum being broken down by C′3LyNeF in 20 min at 37°C. The role of this enzyme in the pathogenesis of glomerulonephritis or hemolytic anemia is not clear. (Research supported, in part, by fellowship FO3-AM-36243 from the National Institute of Arthritis and Metabolic Diseases.)

254. Deficiency of Immunologic Tolerance in Murine Systemic Lupus Erythematosus (SLE). Parker J. Staples,* Alfred D. Steinberg,* and Norman Talal, Bethesda, Md.

NZB and B/W F_1 mice spontaneously develop an autoimmune disease resembling human SLE. Autoimmunity (loss

of self tolerance) is preceded by an inability to develop tolerance to foreign antigens. In previous work, adult control mice (C3H and C57B1) were rendered completely tolerant to 5 mg of ultracentrifuged bovine gamma globulin (BGG), whereas up to 24 mg of BGG failed to induce tolerance in 6–8 wk old NZB and B/W mice. We have now induced tolerance in weanling NZB and B/W mice by administering BGG at 2½ wk of age. Tolerance was transient (30 days) in NZB and B/W mice and long lasting (over 160 days) in control strains when antibody was measured by hemagglutination. Tolerance was induced and partially maintained in B/W mice by 10 biweekly injections of 10 mg BGG starting at 5 days of age. These mice produced little antibody 30–80 days after challenge, although they were not so tolerant as C3H control mice. Tolerance was also induced in utero by injecting 60 mg BGG systemically into term pregnant NZB mice. At 8 wk of age (30 days after challenge), the offspring produced almost no antibody. A lymphocyte abnormality probably explains this resistance to tolerance. NZB serum injected with BGG into C3H mice did not prevent tolerance. Biological filtration of BGG through NZB and Balb/c mice failed to produce tolerance in other NZB recipients, although Balb/c mice were rendered tolerant. This early escape from tolerance to BGG suggests a similar mechanism for the autoimmune disease. The maintenance of partial tolerance to BGG may mean that similar treatment with self antigens (e.g. DNA) might reduce autoantibody formation and ameliorate the autoimmune disorder.

255. Limited Cation Excretion after Diuretic-Induced Volume Depletion in Man.

THOMAS H. STEELE,* Baltimore, Md. (introduced by David P. Rall**).

Rapid shrinkage of the extracellular fluid (ECF) volume might attenuate the renal response to diuretics. Normal subjects received either ethacrynic acid or furosemide intravenously, and in 10 studies urinary losses were not replaced (volume depleted). In 10 others, body weights were maintained within 0.15 kg of control values by isotonic saline infusions (saline repleted). Base-line values for inulin clearances (GFR) and sodium, calcium, and magnesium excretion were similar in the two groups, and responses to the two diuretics were comparable in each group. During the 1st hr after diuretics, GFR decreased an average of 11% in the volume-depleted subjects. Mean fractional excretion rates of sodium (FE_{Na}) and ultrafilterable calcium (FE_{Ca}) and magnesium (FE_{Mg}) increased at least 7-fold, but remained similar in the depleted and repleted groups. During the 2nd hr after diuretics, GFR decreased 12% in both groups. The volume-depleted subjects lost an average of 2.4 kg body weight, and FE_{Na} decreased from a mean 1st hr value (±SEM) of 0.22 ±0.02 to a 2nd hr value of 0.08±0.01, while changing from 0.27 ±0.02 to 0.18 ±0.02 in the saline-repleted group. Similarly, FE_{Ca} decreased from 0.25 ±0.02 to 0.10 ±0.01 in the depleted subjects, but only from 0.29 ±0.03 to 0.19 ±0.02 in the repleted individuals. FE_{Mg} fell from 0.28 ±0.02 to 0.13 ±0.01 in depleted subjects, and from 0.30 ±0.02 to 0.20 ±0.02 in the repleted group. The ultrafilterable fractions of plasma calcium and magnesium remained stable (±3%) during each experiment. Likewise, PAH clearance remained a constant multiple of GFR. GFR decreased similarly in both groups after the 1st hr, but the excretory rates of sodium, calcium, and magnesium decreased more rapidly in the volume-depleted subjects ($P < 0.05$) during the same time interval. Thus, ECF volume depletion appears to play an important role in limiting the magnitude of the renal response to potent diuretics.

256. Hepatic Function in Acute Intermittent Porphyria (AIP).

JEFFREY A. STEIN,* JOSEPH R. BLOOMER,* PAUL D. BERK,* AND DONALD P. TSCHUDY, Bethesda, Md.

Bromsulphalein (BSP), indocyanine green (ICG), and ^{14}C-bilirubin clearances were measured in patients with AIP. Other standard tests of liver function were within normal limits in the 10 patients (five asymptomatic, five symptomatic) at the time of study. BSP (5 mg/kg) was injected intravenously and samples were obtained for 120 min. The 30 min retention value for the patients was 16.5 ±6.6% (mean ±SD) (range 7.0–26.6). The 30 min level was 19.2 ±7.5% for symptomatic patients and 13.9 ±5.1% for those without symptoms. These results were significantly different ($P < 0.01$) from the value of 3.7 ±1.0% (range 2.0–5.3) in normals. Over the 2 hr period of sampling, the BSP clearance curve could be approximated by two exponentials. The first exponential component (K_1) of the curve, thought to represent hepatic uptake, was not significantly different in patients (0.164 ±0.023 min^{-1}) and normals (0.157 ±0.026 min^{-1}). The increased BSP retention in AIP reflected abnormalities in the second exponential (K_2), which is thought to represent hepatic conjugation and excretion of the dye. K_2 for the patients (0.021 ±0.013 min^{-1}) was significantly different ($P < 0.01$) from that of the normals (0.043 ±0.019 min^{-1}). The difference was most marked in the symptomatic patients ($K_2 = 0.013 \pm 0.006$ min^{-1}). ICG (0.5 mg/kg) was administered intravenously to six patients (three asymptomatic, three symptomatic) and was cleared from the blood normally with a half time of 3.0 ±0.6 min. ^{14}C-bilirubin clearance was also normal in these six. The findings suggest that patients with AIP may have a defect in the hepatic conjugation and/or excretion of BSP which is more pronounced during attacks. Certain steroid metabolites are known to cause BSP retention in normal livers and also can induce aminolevulinic acid synthetase (the enzyme increased in AIP). Thus, it is postulated that BSP retention which occurs in AIP is the consequence of metabolic effects and does not reflect structural liver disease.

257. Median Eminence Stimulation of Growth Hormone (GH) and Thyrotropin (TSH) Secretion and the Pituitary Adenyl Cyclase System.

A. L. STEINER,* G. T. PEAKE,* R. UTIGER, AND D. KIPNIS, St. Louis, Mo.

The present study was undertaken to examine the role of the adenyl cyclase system in the secretion of anterior pituitary hormones and the stimulatory effect of hypothalamic releasing factors. The effects of median eminence extracts (SME) and theophylline on tissue cyclic AMP (cAMP) content and GH and TSH release (measured by immuno-

assay) were determined with rat hemipituitaries incubated in vitro. Theophylline (2.5 mg/ml) caused a 300–500% rise in pituitary cAMP and a 150–350% increase in hormone release. These effects occurred concomitantly and were evident within 3 min after addition of theophylline. SME produced a 260–644% rise in cAMP content with a corresponding increase in GH (250–360%) and TSH (178–320%) release. Maximal effects were obtained with the equivalent of two median eminences per four hemipituitaries. Cerebral cortex extracts and lysine vasopressin (0.15 U/ml) did not affect either the cAMP level or hormone release. When Ca^{++} was omitted from the incubation medium, cAMP levels were still increased by SME and theophylline, but stimulation of hormone secretion was markedly reduced. Pituitary adenyl cyclase activity, determined by the conversion of ^{14}C-ATP to ^{14}C-cAMP, was stimulated 29–70% by SME and 200–250% by 10 mM NaF, but was not affected by the following amines (10^{-5} M): norepinephrine, epinephrine, 5-OH-tryptamine, melatonin, and acetyl choline. Pituitary cAMP phosphodiesterase activity, assayed as the rate of disappearance of ^{3}H-cAMP, was not affected by SME. These data indicate that (1) increased levels of pituitary cAMP are associated with accelerated GH and TSH release, (2) SME stimulates adenyl cyclase activity and increases pituitary cAMP levels, (3) the effects of SME on adenyl cyclase and hormone secretion can be dissociated, and (4) extracellular Ca^{++} facilitates hormone release.

258. Defect in Urinary Acidification Induced In Vitro by Amphotericin B. PHILIP R. STEINMETZ* AND LOIS R. LAWSON,* Boston, Mass. (introduced by Howard S. Frazier).

Impaired urinary acidification has been observed in patients receiving amphotericin B (AMB), a polyene antibiotic. In the present study a defect in acidification was induced in the turtle bladder in vitro by AMB, and the nature of the defect was explored. Net H^+ secretion was measured by means of the pH stat method at different pH gradients with and without 15 μg/ml AMB in the mucosal solution (M). Normally, net H^+ secretion into M decreases as M pH is lowered from 7.4 to 4.4, and reaches zero at an opposing gradient of about 3 pH units, back flux becoming equal to forward flux. When both sides of the bladder are isohydric at 7.4 and short circuited, passive forces are close to zero and net H^+ secretion approaches the active component of flux. In the absence of passive forces, AMB had little effect on H^+ secretion, the control rate being 1.44 ± 0.14 μmoles/hr and the rate 10 to 40 min after AMB being 1.24 ± 0.15 μmoles/hr (SEM). At concentration gradients of more than 2 pH units (M pH < 5.4), AMB abolished net H^+ secretion. However, by raising M pH, net secretion could be restored in AMB-treated bladders. Increased passive flux of H^+ for a given concentration gradient indicated increased permeability of the M membrane to H^+. AMB also increased the permeability to two other cations, ^{42}K and ^{22}Na. We suggest that the defect in acidification induced by AMB in the turtle bladder is caused by increased back flux of H^+ rather than by impairment of the active transport system. (Research supported by a grant from the American Heart Association.)

259. Pathogenesis of Hyperkalemic Periodic Paralysis. DAVID H. P. STREETEN,* HERBERT FELLERMAN,* AND THEDORE G. DALAKOS,* Syracuse, N. Y. (introduced by Paul A. Bunn**).

In a patient with familial hyperkalemic periodic paralysis, potassium chloride (156 mEq by mouth) reproducibly caused severe hyperkalemia and flaccid quadriplegia, whereas in two normal subjects of comparable size, the same dose of KCl induced a smaller rise in plasma K and no measurable weakness. ACTH-gel (80 U) consistently caused hyperkalemic paralysis in the patient, which could be prevented by simultaneous administration of metyrapone, and could be reproduced by various glucocorticoids (cortisol, dexamethasone, triamcinolone, 6-methyl-prednisolone) but not by mineralocorticoids (aldosterone, DOCA). Since cortisol produced a greater increase in urinary K excretion in the patient than in normal subjects, excessive renal retention of the K extruded from cells did not contribute to the patient's hyperkalemia. In all attacks of paralysis, there were decreases in plasma Na and Cl which correlated significantly with the simultaneous rises in plasma K ($r = -0.81$ and -0.56 respectively, $n = 73$, $P < 0.001$). The magnitude of these changes implied rapid exchange, between extra- and intracellular fluid, of 2 Na^+ and 1 Cl^- with 1 K^+ as paralysis developed. Such exchanges confirm the expectation (Creutzfeldt et al.) that the fall in membrane potential during episodes of hyperkalemic periodic paralysis results from a change in Na permeability as well as a rise in extracellular K concentration. Spontaneous and KCl-induced paralyses were not preceded by a demonstrable rise in plasma corticoids. Treatment with a mineralocorticoid (fludrocortisone) and acetazolamide protected the patient from KCl-induced paralysis, abolished spontaneous attacks of severe paralysis, and strikingly reduced the incidence of spontaneous attacks of mild weakness. *Conclusions:* In this type of paralysis, spontaneous and glucocorticoid-induced attacks were associated with excessive rise in plasma K and fall in plasma Na, independent of abnormalities in urinary K excretion, probably due to excessive muscle K-Na exchange, causing prolonged muscle membrane depolarization.

260. Tissue Iodoprotein Formation: A New Pathway in the Metabolism of the Thyroid Hormones. MARTIN I. SURKS,* HAROLD L. SCHWARTZ,* AND JACK H. OPPENHEIMER, Bronx, N. Y.

We have previously demonstrated in man and rat the formation of plasma iodoproteins during the peripheral metabolism of β-ring ^{125}I-labeled L-thyroxine (T_4-β) and L-triiodothyronine (T_3-β). The presence of tissue iodoproteins was examined by determining the concentration of non-ethanol-extractable ^{125}I (NE^{125}I) in various tissues after the injection of T_3-β and T_4-β in groups of rats with iodide-blocked thyroid glands. 3 days after injection of T_3-β and 7 days after T_4-β the concentration of NE^{125}I in the liver and kidney was 5–10 times greater than in plasma. Smaller but significant concentrations of NE^{125}I were also demonstrated in skeletal and cardiac muscle. The major portion of the hepatic NE^{125}I was in the microsomal fraction. Treatment with phenobarbital (PB) produced an increase in metabolic clearance of T_3-β

(30%) and T_4-β (100%) and a highly significant increase in the $NE^{125}I$ concentration in liver and plasma. The increase in hepatic $NE^{125}I$ was primarily due to the hepatic microsomal component. After simultaneous injection of α-ring ^{125}I-labeled T_4 (T_4-α) and T_4-β, the concentration of NEI derived from T_4-α in plasma, tissues, and hepatic subcellular fractions exceeded that from T_4-β. Phenobarbital treatment also resulted in an increase in $NE^{125}I$ from T_4-α in hepatic microsomes. During incubation of hepatic microsomes with T_4-β and T_3-β, $NE^{125}I$ formation was proportional to deiodination. $NE^{125}I$ was the principal product of microsomal T_4-α metabolism. In vitro deiodination and NEI formation for all labeled iodothyronines were increased when microsomes from PB-treated rats were used. Thus, thyroid hormone metabolism results in the formation of structural tissue iodoproteins as well as plasma iodoproteins. The rate of iodoprotein formation in liver and plasma is related to the rate of hepatic hormone metabolism. It appears possible that chemical modification of microsomal membranes may also be an initiating step in hormonal action. (Supported by a USPHS grant and a U. S. Army contract.)

261. Studies of C-6 Hydroxylation of C_{21} Steroids in Human Placenta. T. TABEI* AND P. TROEN, Pittsburgh, Pa.

Although classical steps in steroid biosynthesis in the human placenta have been well studied, data are sparse concerning control mechanisms and the presence or significance of newer steroid metabolites. The present study concerns the in vitro metabolism of 7α-^{3}H-pregnenolone by the human placenta at term. Five placentas obtained at repeat caesarean section were used. From three placentas, 1 hr incubations were done with minced tissue; from two placentas, organ cultures were performed for 6 hr and 24 hr. 21 experiments were performed to determine the effect of human chorionic gonadotropin (HCG), human placental lactogen (HPL), and heat-inactivated HCG (one experiment). As expected, the major radioactive product was progesterone (40–60%); unchanged pregnenolone accounted for only 5–15% of the radioactivity. ^{3}H-6β-OH-progesterone was found and rigorously identified. In control experiments, 6β-OH-progesterone was 2–4% of the radioactivity. In the presence of HCG there was a significant ($P < 0.02$) 2- to 3-fold increase of ^{3}H-6β-OH-progesterone in the 1 hr mince incubations and the 24 hr organ cultures. There was no increase in ^{3}H-6β-OH-progesterone with HCG after 6 hr organ cultures, with heat-inactivated HCG, or with HPL. These findings provide additional data showing that HCG affects steroid metabolism in the human placenta. In addition, ^{3}H-6α-OH-progesterone was found and rigorously identified in yields of approximately 0.5–1%. The effect of HCG on 6α-hydroxylation was not determined. This is the first demonstration of 6α-hydroxylation of C_{21} steroids by human tissue. This extends our previous report of 6α-hydroxylation of phenolic steroids by human placenta. The significance of these 6-hydroxylated compounds is not yet known. (Supported by NIH grant AM-08375.)

262. A Clinical Radioimmunoassay for Bradykinin. RICHARD C. TALAMO,* EDGAR HABER, AND K. FRANK AUSTEN, Boston, Mass.

Activation of the kinin system is believed to play a part in circulatory regulation. Definition of its role has been difficult because of lack of a specific assay. Problems in the existing assay include the lability of the components of the system and the inadequacy of the bioassay for the end product, bradykinin. A method for processing blood has been devised which avoids nonspecific activation of the kinin-forming enzyme, kallikrein, and inhibits the kinin-destroying enzymes, kininases. It was demonstrated through use of ^{14}C-2,3-probradykinin that there is minimal destruction of bradykinin at each step in the procedure. Blood is drawn without glass contact, and plasma is prepared by centrifugation in tubes containing 400 μg hexadimethrine and 1 mg disodium ethylenediaminetetraacetate per ml of blood. Protein is precipitated immediately by addition of 20% trichloroacetic acid, and the bradykinin in the supernatant is isolated and purified with Amberlite IRC-50. Storage of plasma at 4°C for 18 hr, or freezing and thawing before the acid precipitation step, produces up to a 30-fold spurious increase in the value obtained for plasma bradykinin. Purified bradykinin is measured by radioimmunoassay employing rabbit antibradykinin antibody and ^{125}I-tyr^8-bradykinin of high specific activity. Dextran-coated charcoal is used to separate the free radiolabeled hapten from that bound to antibody. Per cent inhibition of binding by the test sample is compared with standards; this method detects 0.1 to 10 mμg bradykinin. Plasma bradykinin levels in 16 normal young adult controls were below 3 mμg per ml. The advantages of the improved processing of blood and the radioimmunoassay for bradykinin over previous methods include full recovery, specificity, reproducibility, and ease of performance. (Supported by NIH grant AI-07722.)

263. Effect of Heart Rate, Left Ventricular End-Diastolic Pressure, and Diastolic Pressure on the Preejection Period as an Index of Myocardial Contractile State. ROBERT TALLEY,* JERRY MEYER,* AND JOHN MCNAY,* Atlanta, Ga. (introduced by Leon Goldberg).

Preejection period (PEP) (time from Q of EKG to second heart sound, minus left ventricular [LV] ejection time) is used as an external estimate of myocardial contractile state (MCS). Studies were undertaken to compare the effects of heart rate (HR), LV end-diastolic pressure (LVEDP), and diastolic pressure (DP) on PEP and two internal indices of MCS: (1) maximum change in LV pressure with time (max dp/dt), which is reportedly affected by both afterload and preload; (2) time from Q of EKG to max dp/dt (t max), which is reportedly not affected by afterload or preload. HR was varied in two pentobarbital-anesthetized dogs by atrial pacing. Max dp/dt, DP, and LVEDP were maintained constant by regulation of blood volume and infusion of dopamine and angiotensin. LVEDP was varied by manipution of blood volume in four chlorolose-anesthetized dogs pretreated with propranolol and atropine while DP and HR were kept constant. DP was varied by angiotensin infusion in two hexamethonium-pretreated chlorolose-anesthetized

dogs while HR and LVEDP were kept constant. The influence of DP was studied in 10 pentobarbital-anesthetized dogs pretreated with hexamethonium. DP of 60, 90, and 120 mm Hg were maintained by infusions of pressor (angiotensin, dopamine) and depressor (nitroglycerine, isoproterenol) agents. MCS varied widely at each DP owing to the different cardiac effects of the vasoactive drugs. HR had no effect on PEP when t max, max dp/dt, LVEDP, and DP were constant. Increasing LVEDP from 2 to 24 mm Hg with DP and HR constant did not change t max, but increased max dp/dt by 100%, and decreased PEP by 300%. Increasing DP during constant HR and LVEDP did not change t max, but increased both max dp/dt and PEP. Multiple regression of the three indices in the 10 dog group revealed that (1) the correlation of PEP with t max ($r = 0.99$; $P < 0.01$) was not significantly affected by DP; (2) the correlation of PEP with max dp/dt ($r = 0.70$; $P < 0.01$) was systematically affected by DP ($P < 0.01$). LVEDP was excluded as a factor in this differential effect. We conclude that PEP, when referred to t max as an index of MCS, is unaffected by HR, markedly affected by LVEDP, and minimally affected by DP.

264. Ultraviolet Light and Antibodies to DNA. Eng M. Tan, Pier G. Natali,* Robert G. Freeman,* and Richard B. Stoughton,* La Jolla, Calif., and Houston, Texas.

It was recently reported that rabbits immunized with DNA irradiated with ultraviolet (UV) light produced serum antibodies reactive only with UV-irradiated DNA. This was shown by immunodiffusion, complement fixation, and immunofluorescence. Employing immunofluorescence and specific antiserum, UV-altered DNA was demonstrated in epidermal cell nuclei of hairless mice exposed to UV light from germicidal or sun lamps. These studies have been extended to determine whether similar alteration of cellular DNA occurred in man. Lumbar skin 1 cm^2 from three human volunteers was exposed to UV light. With the sun lamp, 10 times minimal erythema dose (MED) was delivered. This would have produced a moderate sunburn on untanned human skin. In areas where the horny layer of skin had been stripped with Scotch tape, the sun lamp produced extensive alteration of DNA in epidermal cell nuclei. Unstripped skin showed less involvement. Germicidal irradiation produced altered DNA in extensive areas of epidermal cell nuclei whether skin was stripped or unstripped of horny layer. Since little or no UV light below 295 nanometers is detected in sunlight on the earth's surface, it was important to determine whether UV present in sunlight could produce changes in cellular DNA. Hairless mice were irradiated with an UV monochromator calibrated to deliver 10 times MED at specific wavelengths. Altered DNA was detected in epidermal cells at 295, 300, and 305 nanometers but not at 310 nanometers. These studies show that potentially antigenic DNA was produced in tissues by UV spectra present in sunlight. It is possible that under certain circumstances, altered tissue DNA might induce formation of antibody, and that such a mechanism of autoantibody formation might play a role in systemic lupus erythematosus and other diseases associated with photosensitivity. (Research supported by grants from the NIH.)

265. Hemoglobin CC_{Harlem}: A New Hemoglobin Combination Associated with Hemolytic Anemia and Sickling, and Complicated by Hematuria. Kouichi R. Tanaka, Y. Stuart Wong,* and Lowell H. Greenberg,* Torrance, Calif.

The heterozygous state for a new sickling variant, hemoglobin C_{Harlem} (Hb C_H), was reported recently by Bookchin et al. This report describes the first instance of the combination of Hb C and Hb C_H resulting in hemolytic anemia, sickling, and spontaneous hematuria. The propositus is a 40 yr old Negro man with marked splenomegaly, hemoglobin 11.1 g/100 ml, packed cell volume 32%, RBC 5.15×10^6 per mm^3, reticulocytes 4.4%, MCV 62 μ^3, many target cells on smear, decreased osmotic fragility, sedimentation rate 1 mm/hr, bilirubin 1.6 mg/100 ml, normal autohemolysis, and ^{51}Cr $T\frac{1}{2} = 10.5$ days. Glycolytic and pentose phosphate pathway enzymes in the red cells were normal or increased. The red cells sickled with deoxygenation or with sodium metabisulfite. The sickling tendency was similar to that of sickle cell trait, but sickling was typical and reversible. Hemoglobin electrophoresis by cellulose acetate with Tris-EDTA-borate buffer at pH 9.1 demonstrated two separate components, Hb C_H migrating in a position slightly but definitely cathodal to Hb C. The mother of the propositus has a pattern of Hb AC; the father was not available for study. Both children of the propositus have Hb AC_H, and one of them (daughter) also has G-6-PD deficiency. Renal scan, IVP, retrograde pyelogram, and arteriograms were unremarkable except for displacement inferiorly of the left kidney, which was the site of the bleeding. Serum creatinine and creatinine clearance were normal. The propositus was unable to concentrate urine above 462 mOsm/kg H_2O with dehydration or after vasopressin. Both of his children with Hb AC_H were also unable to concentrate urine above 585 mOsm/kg H_2O after a 12 hr fast. Red cells with Hb C_H showed some sickling in vitro when exposed to saline concentrations above 800 mOsm/kg H_2O. Hyposthenuria may be related to hypertonicity of sodium chloride, increased viscosity, decreased blood flow, and sickling. In summary, the first patient with a combination of Hb C and Hb C_H is described. The clinical features of hemoglobin CC_{Harlem} appear to be similar to those of Hb CC except for sickling and its related complications. (Research supported by a grant from the NIH.)

266. Value of Plasma Volume Measurement in Hypertensive Diseases. Robert C. Tarazi,* Harriet P. Dustan,** Edward D. Frohlich,* and Ray W. Gifford, Jr.,* Cleveland, Ohio.

Plasma volume judged in relation to arterial pressure levels may suggest important clues in evaluation and treatment of various forms of hypertension. Plasma volume (RIHSA) was measured in 59 normal subjects and 119 hypertensive patients without cardiac or renal failure, and results were expressed in ml/cm height to minimize age and weight differences. Sex groups were considered separately because normotensive men had larger plasma volumes than women (18.7 vs. 15.4, $P < 0.001$). Of the 43 essential and 7 renovascular hypertensive men, only those with dia-

stolic pressure <110 mm Hg had normal plasma volume, whereas patients (21 essential and 5 renovascular) with higher pressure had either of two abnormalities: a significant volume reduction was found in all renovascular and 14 essential (15.8 and 15.9 respectively, $P < 0.01$ for both), contrasting with an expanded volume in the remaining 7 essential hypertensives (20.6, $P < 0.05$). All the latter 7 had pressure reduction to near normal levels with spironolactone therapy alone or combined with thiazide diuretics. Hypertensive women (21 renovascular and 17 essential) also had low average plasma volume (14.3 and 14.2 vs. 15.4, $P < 0.05$) but with no relation to pressure elevation. In contrast, plasma volume correlated directly with diastolic pressure level in all 13 hypertensive men and women with renal parenchymal disease. Plasma volume was contracted in 11 patients with pheochromocytoma, 8 men (16.8, $P < 0.025$) and 3 women, whereas it was normal in 5 and expanded in 2 patients with primary aldosteronism and high diastolic pressure. In conclusion, plasma volume varies according to type of hypertension. In the absence of renal parenchymal disease, an inappropriately normal or expanded volume despite marked diastolic pressure elevation may be an indication of some types of hypertension responsive to spironolactone and/or sodium and water depletion. (Supported in part by grants from the Heart Association of Northeastern Ohio and the NIH [HE-6835].)

267. Histogenesis of Bone Marrow in Phenylhydrazine-Induced Chronic Hemolysis. M. Tavassoli,* Alice K. Maniatis,* and W. H. Crosby,** Boston, Mass.

Histogenesis during the repair of marrow consists of a series of well defined steps which accomplish a total reconstruction of the marrow's adventitia preliminary to restoration of hemopoiesis. It can be studied intramedullarily after mechanical disruption or extramedullarily by autotransplantation of the marrow. Hypertrophy of marrow occurs in response to increased requirement for blood cells; for example, in chronic hemolytic disease, areas of normally atrophic marrow become hyperplastic. Histogenesis of marrow during chronic hemolysis was compared with histogenesis in the normal state. In rats the intraperitoneal injection of phenylhydrazine, 0.5 mg/100 g body weight 3 times a week, induced chronic hemolysis with a sustained reticulocytosis of 15 to 20%. Pieces of bone marrow were removed from the femur and implanted subcutaneously. Regeneration of marrow in the implants as well as in the femoral cavity was followed periodically by histological sections. In more than 30 rats with chronic hemolysis the process of intramedullary marrow regeneration showed no difference from the histogenetic response in 50 control animals. In the implants the rate of regeneration and the ultimate size of the implants showed no difference in the two groups of animals. It therefore appears that the process of bone marrow histogenesis is not accelerated in the chronic hemolytic state induced by phenylhydrazine, nor do implanted bits of marrow undergo hypertrophy. The stimulus to increased erythropoiesis seems not to affect the behavior of adventitial elements of the marrow. (Research supported by grants from the AEC and the NIH.)

268. A Qualitative Description of Factors Involved in Lysis of Diluted Whole Blood Clots and Fusion of Platelets. Fletcher B. Taylor, Jr.,* and Hans J. Müller-Eberhard, Philadelphia, Pa. (introduced by Earl Barker**).

A study was undertaken to determine the requirements for lysis of clots formed by the addition of 1 U of thrombin to whole blood diluted 1:10 in phosphate buffer (μ 0.082, pH 7.4, 4°C). Removal of platelets inhibited normal clot retraction and lysis. Addition of antisera specifically directed against purified γM globulin (19S cold agglutinin), complement components C′4, C′3, and plasminogen inhibited normal clot retraction and lysis. Antisera to γG globulin and albumin did not inhibit. Washed platelets were preferentially agglutinated by antisera to γM, C′3, and C′4. Photomicrographs of clots formed in the presence of γM antisera had less platelet fusion than control samples. Serum-induced fusion (viscous metamorphosis) of these washed platelets was also inhibited by pretreatment of the serum with anti γM, or cobra factor, or with hydrazine (selectively inactivates thrombin, C′3, and C′4). The ability of the hydrazine-treated serum to support platelet fusion was restored upon addition of thrombin, C′3, and C′4. These findings suggest that (1) platelets, γM, complement components C′1, 2, 4, 3, and plasminogen facilitate lysis of dilute clots, and that (2) thrombin, γM, and complement components C′1, 2, 4, 3 facilitate clot retraction and fusion of platelets. The relevance of these findings to clinical pathologic states is shown in observations made on three patients with thrombasthenia (Glanzman's). The clots of these patients failed to retract or lyse and the platelets failed to fuse upon addition of normal serum. Associated with the above defects was the relative lack of γM and complement components on the platelet membrane as compared with normal controls.

269. Interaction of Angiotensin and Catecholamines. Gurdarshan S. Thind* and Lysle H. Peterson,** Philadelphia, Pa.

Previous work from our laboratory demonstrated the presence of specific receptor sites for angiotensin in the rabbit aorta. A study was undertaken to investigate the interaction of angiotensin and catecholamines in very small to submaximal threshold doses in the rabbit thoracic aorta strips. The response to the combined dose of angiotensin and catecholamines was always less than the sum of the responses to the same dose of angiotensin and catecholamines administered separately. Therefore, the results of the combined dose are expressed in per cent of the largest control response (100%) to the same dose of either angiotensin or catecholamines. The mean ±1 SE response to angiotensin-norepinephrine combination, 0.001 μg/ml or 0.002 μg/ml of each, was 71.9 ±10.8% and 72.2 ±7.7% respectively, a significant drop ($P < 0.05$). There was also a drop in the response (79.8 ±9.8%) to angiotensin-epinephrine (0.001 μg/ml of each) combination ($P > 0.05$). An additive effect (up to 22%) was obtained when angiotensin-norepinephrine or angiotensin-epinephrine (0.005 to 0.01 μg/ml of each) was used. Our findings suggest that the angiotensin and catecholamine responses are additive in relatively large doses; however, an antagonism is unmasked when smaller amounts are employed.

270. Organ Culture of Human Small Intestine. JERRY S. TRIER AND THOMAS H. BROWNING,* Albuquerque, N. M., and Boston, Mass.

In vitro experiments on small intestinal mucosal function and metabolism utilizing excised tissue have been limited to a few hours by rapid epithelial cell necrosis which occurs with current incubation methods. We describe a method for maintaining human mucosal biopsies for at least 24 hr employing organ culture methodology, and demonstrate its potential application to studies of mucosal function. Peroral biopsies were placed in organ culture plates and maintained with modified Trowell's medium in 95% O_2–5% CO_2 at 37°C for 6–24 hr. To study cell proliferation, 2 μc of ^{3}H-thymidine was added per ml of medium. To study fat absorption, biopsies were exposed to micellar solutions of linolenic acid, monoolein, and taurodeoxycholate in Krebs-Ringer buffer for 15 min after maintenance in vitro for 24 hr. After 24 hr of culture, villi were shorter and wider. Cells in the lamina were reduced. Light and electron microscope morphology of epithelial cells compared favorably with those of control biopsies except in occasional areas of partial necrosis. Some absorptive cells were more cuboidal and contained more lysosomes; many appeared entirely normal. Most crypt cells appeared normal; some contained increased glycogen and lysosomes. Mitoses were present. Labeled cells were abundant in crypts of biopsies from normals and two celiac sprues after 6 hr incubation with ^{3}H-thymidine. By 24 hr, labeled cells migrated to the base of villi in normals but reached the surface epithelium in sprue biopsies. Sprue surface cell morphology reverted toward normal after culture for 24 hr. When biopsies maintained in vitro were subsequently exposed to micellar lipid, numerous lipid droplets were identified in the cytoplasm of absorptive cells. Thus, after 24 hr in vitro under these culture conditions, human small intestinal epithelial cells maintain near normal morphology, epithelial cell proliferation proceeds, and fat absorption occurs. Initial in vitro studies of cell proliferation in celiac sprue biopsies indicate that epithelial cell renewal is accelerated in this disease.

271. Defective Cell Differentiation and Abnormal Methylation of Transfer RNA in Breast Cancer Cells. ROGER W. TURKINGTON,* Durham, N. C. (introduced by Joseph C. Greenfield).

In order to test the ability of neoplastic breast cells to differentiate into cells producing specialized cell products, explants of C3H mouse normal breast and breast carcinomas were cultured in medium containing insulin, hydrocortisone, and prolactin. These hormones induced normal breast epithelial cells to differentiate into nondividing cells which synthesize casein, α-lactalbumin, and lactose synthetase galactosyltransferase. However, C3H carcinoma cells failed to produce significant increases in these specialized proteins. This failure was unrelated to rate of proliferation per se, since the C3H carcinoma cells proliferate at a rate similar to that of normal cells. These results support the concept that cells which form C3H carcinomas cannot limit the cell population size through the normal sequence of hormone-dependent cell differentiation. An alteration in the control of genetic expression could potentially involve altered regulation of protein synthesis at the translational level by transfer RNA (tRNA). Modification of tRNA structure was studied by assaying the enzymes which methylate specific bases in tRNA. Normal breast cells contain a characteristic, tissue-specific profile of enzymes which are also present in similar amounts in the neoplastic cells. However, the neoplastic cells contain three specific methylases which are not detectable in normal breast cells. Hypermethylation of tRNA by these apparently derepressed enzymes may alter the functional activity of tRNA in these cancer cells.

272. Ileal Electrolyte Transport in Normal Man. L. A. TURNBERG,* F. A. BIEBERDORF,* AND J. S. FORDTRAN, Dallas, Texas.

Using a triple-lumen perfusion method, we observed the following: (1) Net movement of chloride, bicarbonate, sodium, and hydrogen occurred against existing electrochemical gradients. (2) During perfusion with a plasma-like electrolyte solution, the ileum generally absorbed, but sometimes secreted. A chloride/bicarbonate exchange was obvious when sodium movement was zero. Increasing rates of sodium absorption were associated with decreasing bicarbonate secretion rates and finally bicarbonate absorption. Even when bicarbonate was absorbed, ileal contents were alkalinized (by contraction of luminal volume). (3) Net chloride movement was found to be sensitive to bicarbonate concentration in ileal fluid. For instance, chloride was absorbed when bicarbonate was 14 or 44 mEq/liter, but was secreted when ileal fluid contained 87 mEq/liter bicarbonate. (4) Although the ileum usually alkalinizes its contents, acidification resulted during perfusion of a chloride-free solution (sulfate). Hydrogen secretion in these studies was not accompanied by a change in PD or potassium secretion, and sodium absorption, which continued even in the absence of chloride, most likely occurred in exchange for secreted hydrogen ions. These results suggest that ileal transport occurs via a simultaneous double exchange: Cl/HCO_3 and Na/H. In this model, neither exchange causes net ion movement, which is a consequence of the relation between hydrogen and bicarbonate (to CO_2 and H_2O). No other unitary model explains and predicts all the following observations: (*a*) Ileal transport is essentially nonelectrogenic. (*b*) The ileum can secrete as well as absorb. (*c*) Ileal contents are alkalinized during absorption of or during secretion into a plasma-like solution; and (*d*) the ileum acidifies its contents when sulfate replaces chloride. The model predicts the hydrogen loss in congenital chloridorrhea and the composition of diarrheal fluid in other diseases, such as cholera. Acetazolamide experiments support the model. (Supported by USPHS grants T1-AM-5490 and T1-AM-06506.)

273. Biosynthesis of the Carbohydrate Moieties of Immunoglobulin and Its Intracellular Transport. JONATHAN W. UHR, DANIEL ZAGURY,* ISAAC SCHENKEIN,* JAMES D. JAMIESON,* AND GEORGE E. PALADE,* New York, N. Y.

Immunoglobulin G (IgG) is a heteropolymer with carbohydrate moieties covalently linked to one of its polypeptide

chains, the heavy (H) chain. It has previously been shown that light (L) and H chains are formed on different classes of polyribosomes and that assembly of L and H chains occurs on the H chain polyribosome. The purpose of the present study was to investigate the route of intracellular transport of the assembled immunoglobulin and the site(s) of addition of the carbohydrate moieties. Cell suspensions prepared from plasma cell tumor LPC_1 of Balb/c mice were pulse labeled in vitro with ^{3}H-leucine, galactose, or glucosamine, and the following studies were performed. (1) The partition of labeled immunoglobulin between intracellular and extracellular compartments (secretion) was determined at various times after labeling. By comparison with leucine-labeled immunoglobulin, it could be established whether the two sugars were incorporated at the same time as protein synthesis, or later in the intracellular life of the molecule (i.e., nearer to the time of its secretion). (2) The effect of pretreatment with puromycin (6×10^{-4} M) on immunoglobulin synthesis and secretion was measured using the above mentioned labeled precursors. At this concentration of puromycin, protein synthesis was completely prevented; therefore, it could be determined whether labeled sugars were incorporated on H chains that had been completed and released before the addition of puromycin. (3) Ultrastructure radioautography was performed to establish the subcellular site(s) of protein and carbohydrate biosynthesis as well as the route of transport of the molecule to the outside. The results obtained from each of these three approaches are in agreement and indicate that glucosamine is incorporated predominantly on polyribosomes attached to the endoplasmic reticulum, whereas galactose is incorporated in the Golgi apparatus. The route of transport is from rough endoplasmic reticulum to Golgi apparatus, across the cytoplasmic matrix, and across the plasma menbrane.

274. Decreased Acute Renal Conservation of Sodium in Primary Myxedema. Carlos A. Vaamonde,* Mario J. Sebastianelli,* Liliana S. Vaamonde,* Richard S. Watts,* Eugene L. Klingler, Jr.,* and Solomon Papper,** Albuquerque, N. M., and Miami, Fla.

We have previously reported that patients with primary myxedema eliminate an acute salt load as well as euthyroid patients, despite a reduced filtered load of sodium (FL_{Na}). Since this observation suggested an abnormal tubular handling of sodium in myxedema, the excretion of urinary sodium ($U_{Na}V$) after stimuli for rapid sodium conservation was studied in six untreated patients with primary myxedema and in four euthyroid control subjects. All subjects were in sodium balance on a 155 mEq sodium diet. $U_{Na}V$ was measured from 8 a.m. to 3 p.m. on two separate days. The stimuli employed were (*a*) a change from recumbent to sitting posture from 10:30 a.m. to 1 p.m., and (*b*) the ingestion of 2 mg 9α-fluorohydrocortisone (9α-F) at 10:30 a.m. Glomerular filtration rate (Ccr) was lower ($P < 0.025$) in patients with myxedema (85 ±3 ml/min) (mean ±SEM) than in controls (122 ±16). Prestimuli $U_{Na}V$ was not different as between the groups. Thus FL_{Na} was lower in myxedema patients before the posture ($P < 0.025$) and 9α-F stimuli ($P < 0.05$). After the stimuli the per cent decrease in $U_{Na}V$ from prestimuli $U_{Na}V$ was significantly less in myxedema patients in response to posture (27 ±7) and 9α-F (49 ±5) than in controls (posture, 61 ±11, $P < 0.025$; 9α-F, 73 ±8, $P < 0.05$). This decreased response in myxedema patients occurred despite a FL_{Na} that was 3000 to 4100 μEq/min lower than in controls at the time of maximal response to the stimuli. Two patients with myxedema were restudied while euthyroid; one had a 75% improvement in his response to 9α-F, while the other showed no change. Both did increase their responses to posture, by 64% and 75%. This abnormal response, occurring in the presence of a reduced FL_{Na} and mineralocorticoid administration, suggests a decreased tubular reabsorption of sodium in myxedema under these experimental conditions. This abnormality may be reversible with treatment.

275. Coronary Dynamics during Eating and Digestion in the Conscious Dog. Stephen F. Vatner,* Dean L. Franklin,* and Robert L. Vancitters,* Seattle, Wash., and La Jolla, Calif. (introduced by Eugene Braunwald).

Postprandial angina pectoris is a classical clinical observation which dates back to Heberden. The pathophysiological basis for postprandial angina is thought to reflect reflex coronary constriction, a shift in flow distribution away from the coronary circulation in favor of the mesenteric, or increased work of the left ventricle. We studied eight intact, unanesthetized, resting dogs 1 to 4 wk after implanting pulsed ultrasonic, Doppler ultrasonic, or electromagnetic flow probes on the ascending aorta, left circumflex coronary, mesenteric, iliac, and renal arteries, and miniature blood pressure gages in the central aorta. When the fasted dogs were presented with food, there was a general sympathetic response, including increased coronary blood flow (CBF), cardiac output, heart rate, and blood pressure (up to 2× control levels), which returned to base-line levels within ¼–½ hr and remained there for up to 6 hr. Similar, but less marked, responses occurred when muzzled dogs were shown food but not allowed to eat. Within ½–1 hr after a heavy meal, when mesenteric dilation was maximal (up to 4× control) and when iliac constriction was maximal (decrease as much as 20%), coronary blood flow remained at control levels. The increases in CBF which accompanied excitement or exercise were not altered postprandially. Although moment-to-moment fluctuations up to 10% mean coronary blood flow occurred, both pre- and postprandially, at no time did a sustained constriction in the coronary bed occur. Thus, in the normal conscious dog there is a redistribution of blood flow after eating that did not appear to embarrass the coronary circulation, nor was there any evidence for reflex coronary constriction. It appears more likely that postprandial angina is related to the observed generalized sympathetic response and accompanying increase in myocardial oxygen requirements.

276. Effect of Hepatectomy on Renal Glucose Balance. Alfonso Villasenor* and Leonard L. Madison,** Dallas, Texas.

Previous studies from this laboratory have shown that after an overnight fast the kidneys utilize glucose (3.1

mg/mm) rather than supply it to other tissues. This indicates that the liver is the sole source of glucose in the postabsorptive state. However, it has long been known that nephrectomy superimposed on hepatectomy results in a more rapid fall in blood glucose than that caused by hepatectomy alone. The present studies were designed to define and quantify changes in renal glucose balance after hepatectomy. In 10 dogs, renal venous blood was obtained via a catheter passed deep into the right renal vein. Four to seven integrated arterial and renal venous blood samples were drawn in each study. Quintuple glucose determinations were made enzymatically on each Somogyi filtrate (each renal A-V difference was calculated from 10 glucose determinations). These data show that after hepatectomy renal glucose balance changed from uptake to output of glucose. Renal glucose output occurred in each study and averaged 4.5 mg/min (range 2.2–11.0). Since total glucose utilization averaged 21.2 mg/min for these hepatectomized dogs, the kidneys were supplying 11 to 44% of their glucose needs. These data indicate that after hepatectomy the kidneys become a quantitatively important source of glucose for the organism, supplying an average of 23% of the glucose needs. (Research supported by a grant from the NIH.)

277. Enhancement of the Toxicity of Heavy Metals as the Result of Phagocytosis. HENRY N. WAGNER, JR., AND FRANK P. CASTRONOVO, JR.,* Baltimore, Md.

Although phagocytosis is usually a protective mechanism that clears microorganisms, tissue debris, and effete blood cells from the circulation, we have found that phagocytosis can lead to a 40-fold increase in the toxicity of the heavy metal indium. Indium was administered to mice in two chemical forms: ionic indium, which is bound by plasma proteins, primarily transferrin, and particulate indium oxide, which is removed from the circulation by the reticuloendothelial system (RES) of the liver and spleen. The acute lethal dose (LD_{50}) of the particles was 40 times greater than that of the ionic indium. The livers of the mice injected with lethal doses of hydrated indium oxide particles were studded with multiple hemorrhagic and necrotic areas. Administration of lethal doses of the ionic indium chloride resulted in damage to the proximal portion of the proximal convoluted tubules of the kidney, but only with doses 40 times that of the indium particles. Sublethal amounts of the hydrated indium oxide particles resulted in similar hepatic damage, but the hemorrhagic and necrotic lesions were less marked. With lethal doses, there was a marked release of intracellular indium, presumably indicating a breaking down of RES cells. Blockade of the RES before the administration of indium particles resulted in a decrease in the LD_{50}, which suggested that phagocytosis had a positive deleterious effect. At the physiologic pH of the body, many toxic heavy metals exist as insoluble particles. This fact, together with the finding of enhanced toxicity resulting from phagocytosis, suggests the following hypotheses, which are currently under study: (1) Phagocytosis may not always be a protective mechanism. (2) Phagocytosis can enhance the hepatic toxicity of heavy metals. (3) Perhaps heavy metals other than arsenic may play a role in hepatic cirrhosis.

278. Stimulation of Sodium Transport by Stretch. MACKENZIE WALSER, Baltimore, Md.

In order to examine the role of geometric factors in transepithelial transport of sodium, toad hemibladders were mounted as sacs on glass cannulae. By changing sac volume, calculated surface area can be varied from 0.03 to 0.3 cm^2 per mg wet weight, with only small changes in pressure. 3-fold increase in sac volume is followed by a rise in short-circuit current (SCC) of 10.6 ±1.3 μa per mg, a change of 65 ±10% (SE). 3-fold decrease leads to a fall of 10.1 ±1.3 μa per mg. These responses begin about 1 min after volume change and attain final values in about 35 min. The change in net sodium flux (measured with ^{24}Na and ^{22}Na simultaneously) is well correlated with the change in SCC, thus excluding electrical artifacts. Evidently an intrinsic mechanism regulates sodium transport in this tissue in response to variations in stretch. Various mechanisms were explored. The response in sulfate Ringer's was identical, excluding variations in chloride permeability as the cause; sacs mounted in nylon nets showed no response to hydrostatic pressure change; increased access of substrate was excluded because the response persisted in glucose-free media; increased oxygen access was excluded because changing from air to 100% oxygen did not stimulate. Conductance did vary with stretch and may play a role. Sodium permeability (serosal-to-mucosal flux in short-circuited bladders) also varied with stretch, but not strikingly. Isotopic calcium permeability, however, changed greatly, averaging 6-fold for a 2-fold change in area. Furthermore, the magnitude and speed of the SCC response varied with medium calcium concentration. Thus calcium penetration through the cell membrane may exert a regulatory effect upon sodium transport.

279. The Disparate Effects of Acidosis on the Performance and Metabolism of the Anoxic Heart. ARNOLD M. WEISSLER, THEODORE R. GELET,* RUTH A. ALTSCHULD,* AND RICHARD F. LEIGHTON,* Columbus, Ohio.

Ischemia imposes on the myocardium a multiplicity of metabolic defects including oxygen and substrate deprivation and end product accumulation with intracellular acidosis. The present studies on the constantly perfused isolated rat heart focused on the effect of diminished pH on anaerobic performance and metabolism of the heart perfused with glucose (200 mg/100 ml) and 5% albumin KRB in 95% N_2, 4% CO_2. At pH 7.4, the anoxic heart performed spontaneously with an idioventricular pacemaker for 30 min, at a mean rate of 31 ±6 beats/min (SE), mean systolic pulse pressure of 15 ±3.3 mm Hg, and end-diastolic pressure of 14 ±5 mm Hg. Addition of HCl to lower perfusate pH to 7.0 resulted in a significant decrease in performance and pacemaker function with complete cardiac arrest at an average of 16 ±2.4 min. At pH 6.6 there was a more marked decrement in ventricular performance and pacemaker function, with complete arrest occurring at an average of 3.5 ±0.5 min (SE). During anoxic performance, lactate production at pH 7.4, 7.0, and 6.6 averaged 0.84 ±0.13, 0.84 ±0.08, and 0.64 ±0.07 mmole/g protein per 30 min. The pH-induced decrement in ventricular performance hence was not associated with a parallel decrease in anaerobic lactate generation. To control

variability in performance, anaerobic metabolism was studied in KCl-arrested hearts. During anoxia with K arrest, lactate generation at pH 7.4, 7.0, and 6.6 was 0.88 ±0.12, 0.85 ±0.11, and 0.77 ±0.07 mmole/g protein per 30 min, and myocardial ATP and total adenine nucleotide levels were depressed equivalently at each pH level. The data are consistent with the thesis that the marked decrement in function of the anoxic heart at lowered pH is not mediated by metabolic blockade, but rather by primary inhibition of energy utilization or electromechanical function. (Research supported by a grant from the NIH.)

280. Experimental Murine Latent-Leukemia Viral Infections. E. FREDERICK WHEELOCK AND NANCY L. CAROLINE,* Cleveland, Ohio.

Inoculation of DBA/2 mice with Friend leukemia virus (FLV) produces leukemia characterized by hepatosplenomegaly and death within 10 wk in 100% of mice. Two systems were developed in which FLV was rendered latent. In the first, FLV was diluted to a dose at which half of the inoculated mice developed leukemia (ID_{50}). The nonleukemic mice at this dose had normal-sized spleens and livers but were resistant to FLV challenge. Nevertheless, as few as 800 spleen cells from these mice inoculated into normal mice rendered them immune to FLV. Heated noninfective FLV inoculated at the ID_{50} dilution did not initiate immunity. In the second system, FLV was inoculated i.p., and viremia demonstrated 3 days later. Statolon, an extract of *Penicillium stoloniferum* known to induce interferon, was then administered, and suppressed the virulent disease in 40% of mice which remained alive and had no clinical signs of leukemia at a time when all FLV controls had died. However, most treated mice developed FV leukemia during the subsequent year. Though clinically normal, the treated mice were resistant to FLV rechallenge. Infective FLV could not be isolated from them, but inoculation of large numbers (10^6) of their spleen cells into normal mice produced FV leukemia from which FLV could be reisolated. Inoculation of smaller numbers (10^2) of cells produced immunity to FLV. Histopathologic examination of the normal-sized spleens revealed many plasma cells and clusters of leukemia-like cells beneath the capsules. In the first system, dilution of FLV to the ID_{50} represented an inoculum of infective virus which could be contained as a latent infection by the host's defense mechanisms. In the second system, statolon stimulated host defense mechanisms such as interferon and virus-specific antibody production, thereby suppressing established FLV into a latent infection. (Research supported by grants from the NIH.)

281. Glutamic Acid Decarboxylase in Kidney: Relevance to Vitamin B_6 Dependence. DONALD T. WHELAN,* CHARLES R. SCRIVER, AND FAZL MOHYUDDIN,* Montreal, Canada.

Human vitamin B_6 dependence with convulsions is an autosomal recessive trait identified in 13 pedigrees throughout the world. The phenotype comprises intrauterine and postnatal convulsions. Continuous high doses of vitamin B_6 are required to allay convulsions and to prevent retardation or death. Nutritional or conditioned deficiency of the vitamin does not account for the disorder. A mutation affecting the binding of pyridoxal phosphate by glutamic acid decarboxylase (GAD) in brain has been proposed. This enzyme catalyzes the formation of gamma aminobutyric acid (GABA) from glutamic acid, and, in mammals, it has been demonstrated only in brain. Recently GABA has been found in human tissues other than central nervous tissue; kidney has a concentration (0.3–4.5 mg/100 g wet weight) about one-quarter of that in brain. GABA is absent from plasma; thus synthesis in situ in kidney seems likely. Rat kidney was used as a model system; GABA was present (10 mg/100 g wet weight). Kidney homogenates at pH 6.6 convert 1-^{14}C-L-glutamic to $^{14}CO_2$ and GABA stoichiometrically at 28% efficiency as compared with rat brain at a similar pH. Incubation with U-^{14}C-L-glutamic produced labeled GABA. GAD activity was directly proportional to enzyme or substrate concentration in the ranges used. Omission of pyridoxal phosphate (0.377 mM) from the assay inhibited kidney GAD 22% and brain GAD 45%. The cellular localization of this enzyme and its kinetics are under investigation. By means of autopsy and biopsy material from normal and vitamin B_6-dependent human subjects, we may determine the cause of dependence in this trait. (Supported by grant MT-1085 from the Medical Research Council of Canada.)

282. A Direct Assessment of the Importance of Conjugation in Sulfobromophthalein Sodium (BSP) Transport in Adult and Neonatal Animals. GREGORY WHELAN,* JANE HOCH,* STEVEN SCHENKER, AND BURTON COMBES, Dallas, Texas.

Although it has been known for almost 10 years that BSP undergoes metabolism within the liver and that the majority of the dye excreted in bile in most species is conjugated, the functional importance of conjugation is still debated. Development of a method for harvesting large quantities of conjugated BSP has permitted a direct assessment of the importance of conjugation in the over-all transfer of BSP from blood to bile. Conjugated BSP was synthesized in vitro by incubation of free BSP with glutathione in an alkaline medium, and differential acetone precipitation to isolate the conjugate, which was dissolved in water and lyophilized to yield a powder which chromatographically was approximately 95% BSP-glutathione. The maximal rate of biliary excretion of dye was almost doubled in adult guinea pigs and rats when conjugated BSP was administered as compared with that found when free BSP was injected intravenously. Hepatic uptake of conjugated dye was less rapid than that of free BSP (rats). Neonatal guinea pigs on the 2nd, 6th, 11th, and 16th days of life excreted dye at 24, 46, 72, and 107% of maximal adult rates when free BSP was injected, and at 82, 114, 125, and 93% of adult levels when conjugated BSP was administered. Thus, conjugation facilitates biliary excretion and, morevore, is rate limiting in over-all maximal BSP transfer from blood to bile in adult and neonatal animals. Moreover, in the neonatal animal, conjugation is markedly impaired at birth and matures gradually over a 2 wk period, whereas the excretory step by which dye is transported from liver cells into bile is almost mature by the 2nd day of life. (Research supported by grants from the NIH.)

283. **Hypoxemia in Pulmonary Embolism.** JAMES E. WILSON, III,* W. ROSS HARRELL,* CHARLES B. MULLINS,* EDWARD R. WINGA,* ROBERT L. JOHNSON, JR., AND ALAN K. PIERCE,* Dallas, Texas.

In order to define the cause and duration of hypoxemia after pulmonary embolism, 10 patients without left heart failure were studied after acute pulmonary embolism. Right heart catheterization, pulmonary angiograms, and serial arterial blood gas measurements breathing room air and breathing 100% oxygen were performed. Virtually all of the hypoxemia measured while breathing room air could be accounted for by intrapulmonic shunts, as estimated by the alveolar-arterial Po_2 difference (ΔPo_2) breathing 100% oxygen. The amount of shunting did not correlate with the pulmonary artery pressure, pulmonary vascular resistance, or extent of vascular bed occlusion estimated from angiograms. Serial measurements of ΔPo_2 breathing 100% oxygen averaged 205 mm Hg the first 2 wk, 153 mm Hg the 3rd–4th week, and 82 mm Hg the 5th–6th week after embolism. Thus, right-to-left shunting can persist at least 4 wk after acute pulmonary emboli. During the first 2 wk the increase in ΔPo_2 breathing 100% oxygen could be reduced 32–70% (mean 54%) by IPPB with pressures of 20–40 cm H_2O, causing tidal volumes of 1500–4000 ml, a fact which suggests that at least part of the shunting is due to reversible atelectasis. Reduction in ΔPo_2 was maintained for 2 min but returned to the previous level within 15 min. Thus, after embolization the lung appears mechanically unstable with a tendency to atelectasis even in areas still perfused. (Supported by NIH grant HE-5812 and the Dallas Heart Association.)

284. **Measurement of the Miscible Cholesterol Pools in the Intact Animal.** JEAN D. WILSON, Dallas, Texas.

Recently Goodman and Noble have demonstrated that the exponential curve describing the disappearance of radioactive cholesterol from the blood of man best fits a model consisting of two miscible pools (a rapidly exchangeable pool [A] and a slowly miscible pool [B]) and a totally immiscible pool (C). Analysis of this curve allows for quantification of pool A, and, in addition, the size of pool B can be measured provided that entry into this pool occurs exclusively through pool A (Gurpide et al.). However, validation of this important assumption is not possible in man. Since it has also been recently shown that identical kinetics applies for the disappearance of cholesterol from the blood of the baboon, this species was used to test whether the size of the miscible pools as calculated indirectly from the die-away curves actually equals the size of these pools as determined by direct chemical analysis. Four male baboons were fed 1α-^{3}H-cholesterol until an isotopic steady state was attained, and they were then injected with 4-^{14}C-cholesterol. Blood samples were collected for 90 days and assayed for both cholesterol isotopes. The animals were then killed, saponified, and assayed directly for total carcass and total exchangeable cholesterol pools. The total miscible pool as measured directly (total body ^{3}H-cholesterol/serum ^{3}H-cholesterol specific activity in the isotopic steady state) differed from the sum of pools A and B as determined by die-away curve analysis by an average of only 7%, a value well within analytic error of the methods used. It is concluded that both miscible pools of cholesterol can be quantitated in the intact animal by analysis of the die-away curve of radioactive cholesterol in plasma.

285. **Components of Renal Vein Plasma in Renal Artery Stenosis.** BERTRAM M. WINER* AND MIRJANA KOSTICH,* Boston, Mass. (introduced by A. Stone Freedberg**).

Studies were carried out in 15 dogs with chronic unilateral renal artery stenosis to determine whether analyses of constituents of plasma from the two renal veins reflect functional differences between the kidneys. Total renal plasma flow and glomerular filtration rate were significantly decreased on the stenotic side; filtration fraction was reduced ($P < 0.001$). Renin activity, para-aminohippurate (PAH), ^{131}I-iodohippurate, and creatinine concentrations during clearance studies were significantly increased in renal vein plasma of the stenotic side. Plasma proteins, sodium, osmolarity, and oxygen content were equal on the two sides. Reductions in renal plasma flow and glomerular filtration rate on the stenotic side varied over a wide range, allowing separation of dogs into groups with functionally slight (group I), substantial (II), and severe (III) stenosis. The degree of increase in renal vein plasma concentrations of PAH, ^{131}I-iodohippurate, and creatinine correlated well with the degree of hemodynamic change. Reduced filtration in group I and reduced filtration and secretion in groups II and III accounted for the reduced extraction of PAH and ^{131}I-iodohippurate by the kidney with renal artery stenosis. Tm PAH was reduced on the stenotic side in groups II and III. In group II renin activity in renal vein plasma of the stenotic side was approximately twice that of the opposite kidney; renin secretion calculated as the product of the arteriovenous difference in renin activity and renal plasma flow was 5-fold greater on the stenotic than on the opposite side ($P < 0.001$). In groups I and III renal vein plasma renin activity and renin secretion were only slightly increased on the stenotic side. These studies indicate that analyses of components of renal vein plasma can be used to assess the degree of dysfunction attending renal artery stenosis. (Research supported by grants HE-11026 and HE-11414 from the NIH.)

286. **Heme Deficiency of Beta Chains: A Cause of Hemoglobin Precipitation in Congenital Heinz Body Hemolytic Anemia (CHBHA).** KASPAR WINTERHALTER* AND HARRY JACOB, Zurich, Switzerland, and Minneapolis, Minn.

Lifelong dipyrroluria and hemolytic anemia, which frequently worsen with oxidant drug ingestion, jeopardize patients harboring unstable, mutant hemoglobins (e.g. Köln). We discovered from Perutz's model that these hemoglobins uniformly involve mutations near the heme groups of β-chains. Heme binding suffers thereby. At 50°C hemoglobins Hammersmith, Köln, and Zurich from patients with severe, moderate, and mild CHBHA, respectively, progressively decreased in heme:globin (540/280 mμ) ratio and precipitated; hemoglobin A remained unchanged. Rates of heme loss correlated directly with clinical hemolytic severity. 540/280 mμ activity ultimately halved, suggesting that β-chains only lost hemes. Half stoichiometric amounts of crystalline hemin

when mixed with globin produced this suspected intermediate compound (IC), whose structure ($\alpha^h\beta^o\alpha^h\beta^o$) was spectrally, chromatographically, and ultracentrifugally verified; β-chains proved naked, labeling solely with ^{59}Fe-hemin. IC and CHBHA hemoglobins behaved identically. At 50°C both precipitated into coccoid Heinz bodies. Supernatants contained α^h-chains; precipitates, naked β^o-chains. Both also precipitated when oxidized to ferri- forms: ferrihemes detached from IC α-chains, then reattached to other IC molecules generating hemoglobin ($\alpha^h\beta^h\alpha^h\beta^h$). Heinz body weight doubled, both naked α^o- and β^o-chains now precipitating. As with CHBHA hemoglobins, IC's β-chain sulfhydryls formed mixed disulfides with glutathione, binding G^{35}SH 35 times more than hemoglobin A. Heinz bodies attach to erythrocyte membranes through disulfide bonds between precipitate and membrane thiols. Similarly, erythrocyte ghosts bound ^{59}Fe-IC (not hemoglobin A), unless membrane thiols were blockaded by paramercuribenzoate. These findings indicate that heme is crucial to globin stability. Mutations near heme-binding sites of β-chains may diminish heme avidity, perhaps causing dipyrroluria while generating IC. This compound readily cleaves into precipitable naked β^o-chains (Heinz bodies) and soluble α^h-chains (reported in CHBHA hemolysates). Heightening oxidant stress (e.g., sulfonamide ingestion) loosens ferrihemes from α-chains, which, now naked, also precipitate, binding more membrane thiols. This, shown previously, potentiates hyperpermeability, rigidity, splenic entrapment, and osmotic destruction of CHBHA erythrocytes.

287. Observations on the Origin of Ascites from Experimental Extrahepatic Portal Congestion. CHARLES L. WITTE,* YOUNG C. CHUNG,* MARLYS H. WITTE,* OSCAR F. STERLE,* AND WILLIAM R. COLE,* St. Louis, Mo. (introduced by John R. Smith**).

Since Hyatt and Smith described their observations on ascites in dogs with experimental hepatic venous outflow obstruction, the model of intrahepatic portal hypertension (IHPH) has become the prototype for studying ascites. Although the protein content of ascitic fluid (A) and excess thoracic duct lymph (TDL) in some patients with hepatic cirrhosis is indicative of an origin in the liver, in others it is more suggestive of an origin from the extrahepatic portal bed. Yet previous attempts to produce ascites by constriction of the portal vein alone have been unsuccessful. Therefore, in 15 dogs an aorta–portal vein shunt was constructed using a reversed segment of autologous external jugular vein, and the proximal portal vein was subsequently narrowed in stages until extrahepatic portal hypertension (EHPH) > 30 cm saline was sustained. Five animals survived, and each had visible ascites, numerous and dilated intestinal lacteals, and a greatly enlarged thoracic duct. TDL flow was markedly accelerated, but, in contrast to dogs with IHPH, protein content and electrophoretic patterns of TDL and A closely resembled low-protein intestinal lymph (IL) rather than high-protein liver lymph (LL). Results (mean $\pm$SD): control (14 dogs): TDL flow 2.7 $\pm$0.5 ml/10 min; total protein (% plasma) LL 90 $\pm$8, TDL 70 $\pm$5, IL 71 $\pm$8. IHPH (5 dogs): TDL flow 12.8 $\pm$8.3 ml/10 min; total protein LL 89 $\pm$8, TDL 72 $\pm$13, IL 55 $\pm$5, A 61 $\pm$5. EHPH (5 dogs): TDL flow 21.0 $\pm$8.1 ml/10 min; total protein LL 83 $\pm$3, TDL 20 $\pm$14, IL 13 $\pm$9, A 16 $\pm$10. Arteriograms, portograms, and lymphangiograms further elucidated the preparation. The findings in experimental EHPH resemble those in patients with cirrhosis in whom EHPH rather than IHPH predominates, thus further supporting the concept that ascites and excess TDL have a dual origin in this disease. (Supported by grants from the NIH, the American Heart Association, and the Institute of Medical Education and Research.)

288. Adenyl Cyclase Activity and Calcium Depletion in the Rat Submaxillary Gland. SIDNEY M. WOLFE* AND JOSEPH MUENZER,* Bethesda, Md. (introduced by Robert S. Gordon, Jr.).

Subcutaneous administration of isoproterenol to rats causes profuse salivation. Within 1 hr, the calcium content of the submaxillary gland decreases from 24 $\pm$5 mEq/kg wet weight (mean $\pm$SD) to 5.9 $\pm$0.9. Intraperitoneal administration of dibutyryl cyclic AMP also causes significant calcium depletion (15.5 $\pm$3.3 mEq/kg). There is no significant decrease in the calcium content of the sublingual gland with either isoproterenol or dibutyryl cyclic AMP. Reserpine, which causes a 2-fold increase in submaxillary calcium levels, does not interfere with the effect of isoproterenol on either salivation or calcium depletion. Washed membrane preparations from both submaxillary and sublingual glands exhibit adenyl cyclase activity in vitro. Isoproterenol and norepinephrine (10^{-6} M) cause a 3- to 5-fold increase in cyclase activity in submaxillary gland membranes but have no effect on activity in the sublingual gland. The stimulatory effect of each drug is completely inhibited by propranolol. Sodium fluoride (4×10^{-3}) causes a 6- to 8-fold stimulation of cyclase activity. Parathyroid hormone, methacholine, histamine, and serotonin have no effect on cyclase activity in either gland. These studies suggest that the beta adrenergic stimulatory effects of isoproterenol and norepinephrine on the submaxillary gland are mediated through cyclic AMP.

289. Measurement of Maximum Velocity in Auxotonic Systoles from the Rate of Relative Increase of Isovolumic Pressure (dP/dt)/kP. MICHAEL J. WOLK,* JOHN F. KEEFE,* OSCAR H. L. BING,* LAWRENCE J. FINKELSTEIN,* AND HERBERT J. LEVINE, Boston, Mass.

The contractile state of the left ventricle (LV), measured as the maximum velocity (Vmax) of the unloaded contractile element (CE), has been determined by extrapolation of a plot of CE velocity and LV pressure (P) during the isovolumic period. The accuracy of this technique, however, has not been validated. Furthermore, since the segment of the inverse curve analyzed is generally only 15–30 msec in duration, conventional slow recording speeds (100–200 mm/sec) preclude accurate analysis of these pressure transients. Using oscilloscopic recordings (up to 4000 mm/sec) of LVP (SF-1 micromanometer) and its time derivative (high-fidelity differentiator), CE velocity in muscle lengths/sec was calculated at 0.5–2.0 msec intervals of isovolumic systole as (dP/dt)kP, where $k = 24$ cm^{-1} (normalized modulus of series elasticity of the intact dog LV). In 35 studies in seven dogs, plots of CE velocity versus LVP yielded inverse curves from peak CE velocity to aortic valve opening

averaging 22 msec in controls and ranging from 3 msec (norepinephrine, NE) to 86 msec (barbiturate). In lengths/sec, Vmax averaged 3.8 (controls), 3.8 (volume load), 5.6 (low dose NE), and 6.6 (high dose NE). In each instance, Vmax was also determined from force-velocity curves of isovolumic beats produced by abrupt aortic occlusion (analyzed at 10 msec intervals). Vmax by the two methods correlated well ($r = 0.89$) and means differed by only 7%. Correlation coefficients between Vmax and (maximum dP)/dt, (maximum dP/dt)/kP, and (maximum dP/dt)/(peak isovolumic pressure) were 0.88, 0.88, and 0.87, respectively. With changes in preload, however, maximum dP/dt rose while Vmax changed little. Thus, the contractile state of the LV may be simply and accurately determined over a wide range utilizing a single high-fidelity catheter system and high-speed recordings of isovolumic pressure during auxotonic systoles.

290. Thyroxine Effect on Lipoxidase-Catalyzed Peroxidation. JAMES WYNN, Little Rock, Ark.

The effect of thyroxine on lipoxidase-catalyzed peroxidation of linoleic acid has been examined because thyroxine has been shown to function as an antioxidant in nonenzymatic lipid peroxidation. The products of thyroxine formed during such peroxidation, the influence of thyroxine on the kinetics of oxidation, and a preliminary survey of the nature of the hydroperoxide products formed in the presence of thyroxine have been carried out. Reactants included thyroxine, 1–32 $\times 10^{-6}$ M; linoleate, 1.9–12 $\times 10^{-5}$ M; soybean lipoxidase, 0.008–0.040 mg/ml; phosphate buffer at pH 7.0; and ethanol in a final concentration of 2–6%. Measurements included rates of thyroxine degradation; rates and quantities of O_2 uptake; and polarographic studies of hydroperoxide products formed. Thyroxine is deiodinated and the diphenyl ether is cleaved during peroxidation. Thyroxine appears to form an enzyme substrate complex. The apparent K_m is 4.0×10^{-7} M. Under optimum conditions, 1 mole of thyroxine is degraded for each 15 moles of linoleate peroxidized. Thyroxine does not react with linoleate, enzyme, or hydroperoxide end products. It reacts only with the enzyme-intermediate of peroxidation. Despite small amounts of thyroxine degraded relative to linoleate peroxidized, there is a large effect on the hydroperoxide product formed. Polarographic studies suggest that thyroxine permits the formation of the *cis-cis* conjugated hydroperoxide, whereas the enzyme without the presence of thyroxine forms the *cis-trans* conjugated hydroperoxide. These studies demonstrate the ability of thyroxine to participate in free radical reactions and to alter the reaction products of such reactions.

291. The Biologic Properties of Mitogenic Proteins Derived from *Phaseolus vulgaris*. STANLEY YACHNIN, ROBERT H. SVENSON,* LAWRENCE W. ALLEN,* AND JOSEPH M. BARON,* Chicago, Ill.

Highly purified mitogenic proteins which differ greatly in their hemagglutinating potency have been isolated from commercially available phytohemagglutinin (PHAP) preparations. Absorption of the high-titer hemagglutinin mitogen (H-PHAP) with erythrocytes abolishes its mitogenic activity, whereas similar treatment of low-titer hemagglutinin mitogen (L-PHAP) does not alter its mitogenic capacity; more than 90% of ^{125}I–H-PHAP can be removed from solution by red cell absorption. Analysis of H-PHAP and L-PHAP with specific antisera reveals marked immunologic cross-reactivity. However, the capacity for nonimmune precipitation with a variety of mammalian sera displayed by crude PHAP is possessed solely by the H-PHAP mitogen. H-PHAP and L-PHAP have similarly shaped dose-response curves for lymphocyte transformation; after attainment of peak transformation response at optimal concentrations of either, further increments are inhibitory. Pure lymphocytes devoid of erythrocytes and platelets were prepared by nylon column passage, osmotic shock lysis, and centrifugation through a saline-plasma gradient. Under these conditions it is possible to show that the addition of red cells markedly potentiates the mitogenic capacity of H-PHAP; L-PHAP mitogenicity is not enhanced. Potentiation is most marked at low and intermediate points on the ascending portion of the dose-response curve (3- to 10-fold increase in DNA synthesis), and diminishes as the transformation plateau is approached. Potentiation is evident at erythrocyte:lymphocyte ratios of 1:10 and reaches a maximum at 5–10:1. These findings indicate that hemagglutination is a permitted but not necessary property of mitogenic molecules, and that the ability of PHAP mitogens to form nonspecific precipitates with serum proteins is not required for lymphocyte transformation. They also suggest that lymphocyte transformation may be enhanced by increasing lymphocyte membrane contact with erythrocytes through mixed cell agglutination mediated by H-PHAP, but not L-PHAP.

292. Bile Salt Stimulation of Hepatic Lecithin Synthesis and Release into Bile and Plasma. DAVID L. YOUNG* AND KENNETH C. HANSON,* Durham, N. C. (introduced by Malcolm P. Tyor).

Liver is the primary source of plasma and bile lecithin. It has been suggested that in addition to the effect of osmotic choleresis associated with increased bile salt transport, macromolecular complexes involving bile salts and lecithin may facilitate lecithin secretion. To determine effects of micelle- and non-micelle-forming bile salts on lecithin synthesis and transport, the incorporation rate of 1,2-^{14}C-phosphorylcholine into lecithin and the release of total and ^{14}C-labeled lecithin into bile and plasma were measured in perfused rat livers. Single additions of taurocholate, a micelle former, to the perfusate caused a marked, brief increase in bile flow. Associated temporally was a proportionally greater bile lecithin secretion which persisted after flow returned to control levels. Specific radioactivity of stimulated lecithin increased. Equimolar addition of the glycine conjugate of dehydrocholate, a triketo non-micelle-forming bile salt, caused a similar increase in bile flow. Though lecithin secretion was slightly increased, the ratio of lecithin excretion to flow decreased. The specific activities of phosphorylcholine-cytidyl transferase and phosphorylcholine-glyceride transferase were not altered by taurocholate administration to acutely bile salt depleted rats. Taurocholate and glycodehydrocholate caused a slight delayed increase in release of ^{14}C-lecithin into the perfusate. Bile duct ligation in unstimulated preparations

caused no increase in perfusate ^{14}C-lecithin. Addition of taurocholate to bile duct ligated preparations caused an abrupt 7-fold increase in ^{14}C-lecithin release into the perfusate. In contrast to the effect on bile lecithin secretion, glycodehydrocholate was as effective as taurocholate in increasing ^{14}C-lecithin release into the perfusate. These data indicate that lecithin transport in bile is primarily mediated via micelle formation, presumably in canaliculi. The stimulated release of lecithin into the perfusate in duct ligated preparations, on the contrary, is not related to micelle-forming characteristics of administered bile salts. Increased 1,2-^{14}C-phosphorylcholine incorporation is unrelated to altered enzyme specific activity, but probably reflects increased turnover of a lecithin pool.

293. Effect of Insulin on the Metabolism of Circulating Maltose. Sr. John M. Young* and Elliot Weser,* San Antonio, Texas (introduced by S. J. Friedberg).

Circulating maltose and glucose are similarly metabolized in the rat. It has been suggested that circulating maltose is first hydrolyzed to glucose intravascularly. Nonfasting adult male rats were injected intravenously with 0.5 ml of 10% 1-^{14}C-maltose (0.5 μc) or 10% 1-^{14}C-glucose (0.5 μc) with or without 0.2 unit of crystalline insulin. Expired CO_2 was collected for 6 hr. In similar experiments on fasting rats, the epididymal fat pads were removed 1 hr after injection, total lipid was extracted, and incorporation of radioactivity was determined. The percentage of injected ^{14}C expired as $^{14}CO_2$ was the same for glucose, 49%, and for glucose plus insulin, 47%, and also for maltose, 59%, and for maltose plus insulin, 59%. Insulin caused a more rapid oxidation of both sugars. An earlier peak specific activity for expired $^{14}CO_2$ was observed for glucose plus insulin, 47 ±26 min (mean ±SD), than for glucose alone, 85 ±37 min ($P < 0.01$), and for maltose plus insulin, 70 ±20 min, than for maltose alone, 100 ±15 min ($P < 0.01$). The incorporation of ^{14}C from the carbohydrates into lipids (calculated as the fraction of injected ^{14}C per gram lipid $\times 10^5$) was similar for glucose, 29.5 ±4.3, and for maltose, 28.7 ±6.5, and was significantly enhanced by the presence of insulin with glucose, 45.2 ±11.1 ($P < 0.01$), and with maltose, 40.6 ±8.1 ($P < 0.01$). These findings confirm the theory that circulating glucose and maltose are similarly metabolized, and indicate that maltose metabolism proceeds via hydrolysis to glucose. (This work was supported by the Morrison Trust of San Antonio.)

294. Structural Gene Defects and Preponderance of γG3 Subgroup in Patients with Hypogammaglobulinemia. W. J. Yount,* R. Hong,* M. Seligmann,* and H. G. Kunkel,** New York, N. Y., Minneapolis, Minn., and Paris, France.

Analysis of γ-globulins from 45 hypogammaglobulinemic patients was undertaken to assess the function of the several cistrons concerned with synthesis of immunoglobulin classes, γG subgroups, and Gm genetic markers. 11 patients with non-sex-linked disease and two sibling pairs were found to have preponderance of the γG3 subgroup to levels of 65% of total γG (normal, 7.3 ±38%). Absence or marked diminution of other subgroups was frequently encountered. In most instances of γG3 preponderance it was the Gm(b) type of γG3 that was selectively elevated. This was particularly evident in two heterozygous siblings with molecules from the Gm(b) allele in 300-fold excess over molecules from the Gm(g) allele. A family with structural gene abnormalities in the autosomal Gm loci was seen. The propositus received abnormal gene complexes from each parent. The common gene complexes in Caucasians are Gm^n, Gm^b, Gm^f and Gm^{n-}, Gm^g, Gm^{ga}. The father and a sister both showed the abnormal complex Gm^{n-}, —, Gm^{ga}, and the mother showed Gm^n, Gm^b, —. These unusual gene complexes with presumed deletions at the γG3 locus and γG1 locus are known to occur in rare individuals in the screening of normal Caucasian populations. They have previously been seen only in the heterozygous state, however, and were not associated with clinically evident hypogammaglobulinemia. Two of several other families of patients with subgroup imbalance were informative in that structural gene defects could be excluded. We conclude that non-sex-linked primary immunoglobulin deficiencies occasionally may be associated with structural gene abnormalities.

295. New Bronchographic Technique in Humans Using Powdered Tantalum. N. Zamel,* W. Wolfe,* J. Youker,* J. Austin,* W. Hinchcliffe,* R. Greenspan,* and J. Nadel, San Francisco, Calif.

Previous studies in dogs and rats have demonstrated that powdered tantalum provides a superior contrast medium for bronchography because of its inertness, radiopacity, and firm adherence to airway mucosa. In the present study, we introduced powdered tantalum into airways of 20 individuals who had serious lung disease and in whom we anticipated that tissue exposed to the tantalum would be removed. Therefore, we limited the areas of study to the region of a suspected lesion. We anesthetized the airways, introduced an appropriately shaped catheter into the suspected area, and insufflated powdered tantalum (average mass diameter, 2.5 microns) into selected airways. The technique produced excellent outlining of the airways, with sharp contrast and fine mucosal detail. Most airways were outlined with double contrast. Manipulation of the catheter occasionally caused cough, but no other symptoms attributable to tantalum insufflation occurred. Coughing did not affect the adherence of tantalum to the airways. Roentgenographic clearance of tantalum occurred within 48 hr, except for very peripheral airways. In each of eight patients, microscopic sections showed no inflammation or other deleterious response to tantalum. None of the patients showed a significant increase in temperature in the 24 hr after bronchography. Pulmonary function studies performed in 12 patients showed no significant change in maximal expiratory flow rate, flow-volume curve, airway resistance, or single-breath CO diffusing capacity, although eight of the patients had physiologic evidence of obstructive airway disease before bronchography. (Supported in part by NIH program project grant HE-06285 from the National Heart Institute, and NIH training grant GM-01272 from the National Institute of General Medical Sciences.)

296. Linkage of Lactate Dehydrogenase *B* and *C* Loci in Pigeons. William H. Zinkham, Harriet Isensee,* and J. H. Renwick,* Baltimore, Md.

Synthesis of lactate dehydrogenase (LDH) in somatic and gametic tissues of certain avian and mammalian species is controlled by alleles at three loci: LDH_A, LDH_B, and LDH_C. The activities of the *A* and *B* loci are manifest in both sexes and in all tissues throughout life, whereas the activity of the *C* locus is restricted to a particular sex (male), a particular tissue (testis), and a particular stage of maturation (puberty). A survey of somatic tissues and testes from approximately 1000 wild pigeons revealed polymorphisms at the *B* and *C* loci, thus making it possible to establish matings of the appropriate genotypes ($B^1B^2/C^1C^2 \times B^1B^2/C^1C^2$) to determine whether the *B* and *C* loci are linked. The distribution of phenotypes in the male offspring of these matings conclusively demonstrates linkage between the *B* and *C* loci. The most probable recombination fraction is 0, and contiguity is not excluded. The upper 95% probability limit is 4.5%. Such linkage of two loci coding for similar polypeptides suggests that one may have arisen from the other or that they both stem from a common origin. Also the fact that B and C polypeptides occur homologously in birds and in mammals suggests that the *B* and *C* loci had separate identities before the evolutionary separation of birds and mammals. If so, then linkage has persisted for a very long time. Despite the close linkage, there are wide differences in the biological behavior of the two loci. Thus, as is frequently the case in microorganisms, factors controlling the activity of the *C* locus may be specific to a short DNA segment constituting one or more loci.

297. Separate Autoimmune Mechanisms for 7S and 19S Globulins in Laënnec's Cirrhosis. Horace H. Zinneman* and Donald F. Levi,* Minneapolis, Minn. (introduced by Wendell H. Hall**).

Starch block electrophoresis and gel filtration on columns of Sephadex G-200 were employed to isolate the immunoglobulins of three cirrhotic patients and to separate them into 19S (IgM) and 7S (IgG and IgA) components. The purity of these fractions was ascertained by immunoelectrophoresis and ultracentrifugal determination of the sedimentation constants. These fractions as well as 19S (IgM) and 7S (IgG and IgA) from normal human sera and 19S (IgM) from two patients with Waldenström's macroglobulinemia were conjugated with fluorescein isothiocyanate (FITC) and incubated with tissue slices of liver, kidney, thyroid, and adrenal glands from normal humans as well as from patients with alcoholic cirrhosis and several nonrelated diseases involving the liver. Neither 19S nor 7S globulins of normal human sera, nor the 19S of the patients with macroglobulinemia, were bound to any of the tissues examined. The 19S from the sera of all three cirrhotic patients were bound to the nuclei of cells of cirrhotic or noncirrhotic livers as well as to the nuclei of adrenal, thyroid gland, and pancreas. The 7S (IgG and IgA) from all three cirrhotic patients were bound specifically to necrotic liver tissue, particularly Mallory bodies. They were not bound by normal liver tissue or cells of other human organs. These preliminary results suggest that the increase of immunoglobulins which is found in most patients with alcoholic cirrhosis is a secondary antibody response to constituents of hepatic cells which have been changed antigenically by a specific toxic agent.

298. Stimulation of Anterior Pituitary (AP) Adenyl Cyclase Activity (ACA), Cyclic 3',5'-Adenosine Monophosphate (cAMP), and LH Release by Hypothalamic Extract (HE). Uriel Zor,* Toshio Kaneko,* H. P. G. Schneider,* S. M. McCann,* and James B. Field, Pittsburgh, Pa., and Dallas, Texas.

The role of ACA and cAMP in release of AP hormones mediated by HE was studied in rat AP in vitro. Ovine HE added to whole AP or homogenates stimulated ACA 80% within 1 min. HE increased cAMP in whole AP from 14.7 to 40 mμmoles/g within 3 min and from 5 to 158 mμmoles/g during 60 min incubations. LH release (bioassay) was increased 3-fold by HE. Stimulation of ACA and cAMP was obtained with HE equivalent to one-fifteenth of an ovine hypothalamus, and maximum effects with the equivalent of one-half fragment. HE did not increase thyroid or posterior pituitary ACA or cAMP, and cerebral cortex extract did not change ACA or cAMP in AP. Prostaglandin E_1 (0.6 μg/ml) augmented ACA (150%) and increased cAMP from 15.5 to 169 mμmoles/g in AP. Thyroxine (10 μg/ml), dexamethasone (1 μg/ml), and estradiol (0.05 μg/ml) added together to the incubation medium did not modify HE stimulation of ACA or cAMP. Although Ca^{++} is required for release of AP hormones by HE, stimulation of ACA and cAMP by HE was undiminished in AP incubated without Ca^{++}. 56 mM K^+ stimulates AP hormone release but did not increase cAMP. Although monoamines have been implicated in AP hormone release, $1–5 \times 10^{-4}$ M epinephrine, norepinephrine, histamine, serotonin, dopamine, and vasopressin (0.002–2 U/ml) did not increase ACA or cAMP. NaF augmented AP ACA but not cAMP. The specificity and rapidity of HE stimulation of ACA and cAMP provides strong support for their role in hypothalamic control of AP hormone release. The effects of Ca^{++}, thyroxine, dexamethasone, and estradiol on AP hormone release appear to depend upon reactions beyond cAMP generation. (These studies were supported by grants AM-06865 and AM-10073 from the NIH.)

299. Studies of Human Amyloid. Dorothea Zucker-Franklin,* Mordechai Pras,* and Edward C. Franklin, New York, N. Y.

The major constituent of amyloid is a fibrillar protein. Once deposited, amyloid rarely undergoes resorption. To study its origin, tissues from five patients were subjected to electron microscopy. Cells in intimate contact with amyloid were either plasma cells, reticuloendothelial cells, or fibroblasts. In each instance, the cells had abundant rough ER, and a distorted cell surface with innumerable processes extending into the adjacent amyloid. Typical amyloid fibrils were also seen in the cytoplasm of many cells. Often these fibrils were continuous with extracellular amyloid in areas where no plasma membrane could be resolved. To compare amyloid from different subjects, fibrils from nine individuals were solubilized in water. Seven of the proteins had sedi-

mentation coefficients of 45S or greater. After degradation to a 2S component (DAM) with 0.1 M NaOH, the material was immunogenic in rabbits. After absorption of the antisera with normal human serum and extracts of spleen, liver, and kidney, they gave a single precipitin line with DAM and did not react with serum or tissue extracts. Antisera conjugated with fluorescein or peroxidase reacted with amyloid deposits in tissues as detected by fluorescence and with fibrils as resolved by electron microscopy. Using five antisera, DAM from different individuals were not identical and showed varying degrees of cross-reaction by complement fixation and quantitative precipitation. Complement fixation and absorption studies revealed antigenic determinants in DAM which were not detectable in fibrils. Soluble amyloid was resistant to digestion with trypsin, papain, pepsin, and plasmin, and was degraded only by Pronase. The intact fibrils were not phagocytized by blood leukocytes which ingested particles and denatured proteins under identical conditions. This stability coupled with the unusual solubility properties of amyloid may explain its deposition in the proximity of cells and its long persistence in situ. (Research supported by grants from the NIH, the Health Research Council of the City of New York, and the P. H. Hiss Foundation.)

300. A Role for Antinuclear Factors in Chronic Articular Inflammation. NATHAN J. ZVAIFLER* AND MARIA M. MARTINEZ,* Washington, D. C. (introduced by Laurence H. Kyle).

Phagocytosis of antigen-antibody complexes by leukocytes is an important step in immune injury. Antinuclear factors (ANF), nuclear debris, and leukocytes containing immunoglobulins are present in effusions of some patients with rheumatoid arthritis. To investigate the role of ANF in chronic joint inflammation, 40 to 110 ml of joint fluid, containing 2 to 8×10^8 leukocytes, were obtained from 10 patients. Seven had classical rheumatoid arthritis, one juvenile rheumatoid arthritis, one spondylitis, and one Reiter's disease. Leukocytes were separated by centrifugation, washed, and disrupted. The remaining pellet was treated with citrate buffer and then DNase. Supernatants from each step were concentrated and analyzed for total protein, immunoglobulins, and ANF. ANF was measured and immunoglobulin classes were determined by immunofluorescence using human leukocytes as substrate. Four of the eight rheumatoid effusions showed ANF activity; two had a mixture of γG and non-γG ANF, two were predominantly non-γG. The two nonrheumatoid fluids had no ANF. ANF was demonstrated from the disrupted cells of all fluids containing ANF, in titers equal to, or only slightly less than, that of the original fluid. All but one was non-γG ANF. Protein concentrations of the cell lysates were one-fiftieth or less than the fluid. ANF was present in cells from three ANF-negative fluids. Supernatants of citrate-treated pellets were negative, but two DNase-treated pellets showed weak ANF reactions. A specificity for the ANF from one fluid was suggested by a positive reaction with leukocytes, but not with mouse liver. Antibodies (ANF) capable of reacting with leukocyte nuclear debris have been found concentrated in cell lysates from six of seven effusions of adults with rheumatoid arthritis, and in low titers from a patient with Reiter's syndrome. Such antibodies, stimulated by the inflammatory process, may play a role in the perpetuation of articular inflammation.