JCI The Journal of Clinical Investigation

Abstracts

J Clin Invest. 1968;47(6):1a-57a. https://doi.org/10.1172/JCI105839.

ABSTRACTS

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

1. Increased Susceptibility to Infection Associated with a Defect of Complement Metabolism. CHESTER A. ALPER,* NEIL ABRAMSON,* RICHARD B. JOHNSTON, JR.,* CHARLES E. MCCALL,* JAMES H. JANDL, AND FRED S. ROSEN, Boston, Mass.

Complement (C′) participates in many diseases, but its importance for health is uncertain. Observations were made in a young man with Klinefelter's syndrome who has had repeated pyogenic infections since infancy. No abnormalities were found in immunoglobulins, in delayed hypersensitivity, or in dye reduction by his granulocytes during phagocytosis of latex particles. Serum activities of C′1, C′4, and C′2 were normal. However, C′3 concentration was only 25% of normal, and since 65% of that was in the inactive ($C'3_I$) form, the true level of C′3 was 9% of normal. The level of C′3 remained low during and between episodes of infection. The patient's red cells were strongly agglutinated by anti-C′3, and by "nongamma" Coombs sera, but red cell survival was normal. When purified C′3, labeled with ^{125}I, was injected into the patient, it was catabolized rapidly, with 35% of the ^{125}I appearing in the $C'3_I$ fraction within 2 hr. The calculated rate of C′3 synthesis was normal. The patient's serum was very deficient in hemolytic C′ activity, in bactericidal activity, and in C′-mediated leukotaxis. Granulocytes from the patient or from normals failed to ingest and kill pneumococci that were exposed to antiserum in the presence of the patient's serum, whereas granulocytes from either source did so with normal serum present. Although the addition of purified C′3 to the patient's serum produced some improvement in C′-mediated functions, much greater improvement resulted from the addition of a heat-labile 5–6S beta pseudoglobulin. These studies indicate that increased susceptibility to infection may be associated with deficient C′ activity. Although manifested by a continuously increased catabolism and depletion of C′3 and by the absorption of C′3 on the patient's red cells, the underlying abnormality is unknown. It may represent a deficiency of a protein involved in the activation of C′3.

2. The Effects of Polyene Antibiotics on Electrical and Osmotic Properties of Thin Lipid Membranes. THOMAS E. ANDREOLI,* VINCENT W. DENNIS,* AND MARCIA MONAHAN,* Durham, N. C. (introduced by James R. Clapp).

Optically black, thin lipid membranes separating two aqueous phases were formed from sheep erythrocyte lipid-decane solutions. Membrane DC resistance (R_m) was $\cong 10^8$ ohm cm², and the transference numbers (T_i) indicate that the membranes were cation selective ($T_{Na} \cong T_K \cong 0.85$; $T_{Cl} \cong 0.15$). The membrane osmotic permeability coefficient ($P_f = \sigma L_p$; P_f = permeability coefficient, σ = reflection coefficient, L_p = filtration coefficient), estimated from net water flux when the aqueous phases contained unequal solute concentrations (range = 0.01–0.15 M), was $\approx 25\ \mu$ sec^{-1}, in the presence of NaCl, urea, glucose, sucrose, and raffinose. Hence σ is 1 for these solutes. These properties were unaffected by extraction of cholesterol from the lipid solutions. Nystatin (10^{-5} M) or amphotericin B (2×10^{-7} M) reduced R_m to 10^2 ohm cm², and T_{Cl} became 0.92. The slope of the relation between log R_m and log antibiotic concentration was ≈ 4.5, and the slope of the relation between log R_m and salt concentration (at $\approx 10^{-5}$ M nystatin) was -1. In the presence of amphotericin B, P_f varied with the aqueous solute, and was either zero (NaCl and urea), ≈ 250 (glucose), ≈ 350 (sucrose), or ≈ 450 (raffinose). Thus, σ varied directly with solute size, and L_p increased more than 10-fold. The polyene effects required the presence of cholesterol in the membrane solutions. The data imply that in these membranes, polyene-cholesterol interactions are multimolecular and may promote pore formation.

3. A Physiologic Differentiation of Delayed and Immediate Hypersensitivity. MICHAEL A. APICELLA * AND JAMES C. ALLEN,* Baltimore, Md. (introduced by Benjamin M. Baker**).

A clear differentiation of physiologic effects of different types of hypersensitivity reactions in vivo has been made possible by studies of macromolecular kinetics. A quantitative comparison of outflow and resorption of isotopically labeled guinea pig albumin, γ-globulin, and PVP during the delayed hypersensitivity reaction (DHR), an immediate hypersensitivity reaction (IHR), and a turpentine-induced acute inflammatory reaction (AIR) was made over a 24 hr period in the pleural space of guinea pigs. The DHR was characterized by a normal movement of macromolecules into the pleural space, but a significant impairment of outflow was detectable at 12 hr after the injection of antigen. The IHR featured a significantly increased rate of inflow of macromolecules during the first 3 hr of reactions, but normal outflow throughout. The AIR showed a combination of these effects, with an increased inflow into the chest for the first 18 hr and a delayed outflow detectable at 18 and 24 hr. Thus, the mean ^{125}I-γG blood:pleural effusion ratio during the first 3 hr was 1.97 (±0.34) in the AIR, 1.03 (±0.24) in the IHR, and 0.66 (±0.21) in the DHR. 19% (±6) of the γG injected with the stimulant remained in the DHR and 14% (±5) in the AIR, whereas only 1.4% (±1.7) remained in the IHR. Natural and artificial molecules behaved comparably. Outflow of water in DHR and AIR was similarly impeded, and significant

pleural effusions were noted only during these reactions. These data suggest that collection of protein and fluid in the pleural space in these reactions is related to impaired removal rather than to increased inflow as with a capillary leak. The data have indicated, in addition, that the different types of hypersensitivity reactions can be clearly distinguished in vivo by their physiologic effects.

4. A Platelet Membrane Defect in Paroxysmal Nocturnal Hemoglobinuria (PNH): Usefulness in Detecting Platelet Antibodies in Thrombocytopenic Purpuras. RICHARD H. ASTER,* Boston, Mass, (introduced by James H. Jandl).

Hypersensitivity of red cells to complement (C')-mediated lysis is characteristic of paroxysmal nocturnal hemoglobinuria (PNH). The C' sensitivity of other PNH cells was investigated by incubating ^{51}Cr-labeled platelets with C'-dependent isoantibodies and antibodies from patients with drug-induced thrombocytopenia (quinine, quinidine). Lysis was observed microscopically and quantitated by measuring ^{51}Cr release. At a given C' concentration, platelets from 6 PNH patients were 10- to 100-fold more sensitive than normal platelets to lysis by titered antibodies. With antibody constant, PNH platelets lysed with only one-twentieth the C' required for normal platelets. Sensitivity of both normal and PNH platelets was enhanced 10-fold by trypsin. Spontaneous lysis of PNH platelets in serum exceeded normal and was enhanced by low ionic strength but not by acidification. Granulocytes, but not lymphocytes, from the 2 PNH patients so studied were abnormally sensitive to lysis by leukocyte antibodies. Sera from 22 patients with idiopathic thrombocytopenic purpura (ITP) had no detectable immunological effect on normal platelets. However, 8 of these sera lysed PNH platelets. Sera from normals and from 10 patients with other thrombocytopenic disorders gave negative results. The platelet-lysing factor in these ITP sera was not a known coagulation factor, but was a warm-acting immunoglobulin which could be eluted from platelets and which reacted with autologous cells. In the 3 ITP patients followed serially, the concentration of antibody fell during corticosteroid therapy, disappearing entirely as platelet levels rose. These studies show that the membrane defect in PNH is shared by red cells, platelets, and granulocytes, indicating that the presumed mutation in marrow precursors must affect multipotential cells. PNH platelets are highly sensitive to antibodies. Their use permits the detection and quantitation of the plasma antiplatelet factor in at least some patients with ITP and may further an understanding of the pathogenesis of this and other thrombocytopenic disorders.

5. Familial Pheochromocytoma with Unusual Features. NUZHET O. ATUK,* THOMAS MCDONALD,* AND VIRGINIA WESTFALL,* Charlottesville, Va. (introduced by J. Edwin Wood, III**).

Pheochrome tumors have been identified in 10 of 35 members of a family studied. These patients showed unusual patterns of hypertension and of catecholamine and the mode of inheritance is probably autosomal dominant. VMA excretion. The study of the pedigree indicates that No other definite endocrine abnormality was demonstrated. Neurofibromatosis was not present. Of the 10 patients in this present study, 6 had hypertension and 4 had normal blood pressure. The patients seem to fit into three distinct groups with regard to age, severity of hypertension, and catecholamine metabolism. The mean blood pressure (systolic/diastolic, mm Hg), catecholamine excretion (μg/24 hr), and VMA excretion (mg/24 hr) in the three groups were as follows: group I (ages 11, 12, and 13 yr), 167/112, 1185, and 18.2; group II (ages 17, 19, and 25 yr), 153/103, 515, and 16.3; and group III (ages 30, 35, 36, and 40 yr), 123/82, 283, and 7.4, respectively. One patient in group III had von Hippel–Lindau's disease and adenocarcinoma of the kidney. In addition, serum calcium levels were elevated (range 10.8-12 mg/100 ml) with normal serum phosphorus, tubular reabsorption of phosphate, and phosphate clearance in 5 patients. These patients had successful removal of tumors, after which serum calcium levels returned to normal (range 9.4–10.7). Incompatibility of turnover rate of catecholamines and blood pressure levels at later age suggests development of tolerance to the pressor effects of catecholamines by adrenergic receptor or end organ. The explanation for this newly described defect in calcium metabolism associated with pheochromocytoma is not clear.

6. Regulation of Sodium Excretion during Acute and Chronic Extracellular Volume Expansion in Man. R. BREWER AULD,* RICHARD C. LALONE,* AND NORMAN G. LEVINSKY, Boston, Mass.

To investigate the mechanism of "escape" from mineralocorticoids, renal hemodynamics and sodium reabsorption were studied in 4 subjects, and the inhibitory hormone of Rector and coworkers was assayed in 6 subjects before and after acute infusion of 2–3 liters saline, both in the control state and during escape. In controls, GFR increased from 148 to 153 ml/min after saline. After escape, GFR was 175 and increased to 185 ml/min after saline. RPF changed proportionately with GFR. Sodium excretion increased from 198 to 430 μEq/min after saline in controls. After escape, excretion was 392 and increased to 929 μEq/min after saline. Free-water excretion (C_{H_2O}) increased from 9.3 to 12.8 ml/min per 100 ml GFR after saline in controls. After escape, C_{H_2O} was 12.3 and increased to 13.6 after saline. C_{H_2O}/V was 0.81 before saline, 0.76 after saline in controls; during escape, it was 0.80 before and 0.68 after saline. No inhibitory activity was found in plasma dialysates from controls (Gertz shrinking-drop half-time, $t_{\frac{1}{2}} = 9$ sec); after saline, activity appeared in 4 subjects ($t_{\frac{1}{2}} = 13.2$ sec) but not in 2 ($t_{\frac{1}{2}} = 8.9$). After escape, inhibitory activity was present in 3 subjects ($t_{\frac{1}{2}} = 13.9$), not in 3 others ($t_{\frac{1}{2}} = 9.4$); activity increased strikingly in all after saline ($t_{\frac{1}{2}} = 22$). Comparable C_{H_2O}, C_{H_2O}/V, sodium excretion, and inhibitory activity in escape and after acute saline suggest that acute and chronic expansion increase sodium excretion by similar mechanisms. The increase in V and C_{H_2O} after saline

or escape indicates that proximal sodium reabsorption may be decreased. Inhibitory hormone may contribute to this decrease, but absence of hormone in some subjects suggests other mechanisms as well. The marked rise in inhibitory activity during saline after escape suggests that the hormone is important in the exaggerated natriuretic response to saline characteristic of escape and primary aldosteronism.

7. Glucagon-Induced Hypocalcemia in Man and Dog. LOUIS V. AVIOLI,* STANLEY J. BIRGE,* HAROLD KANAGAWA,* AND WILLIAM SHIEBER,* St. Louis, Mo. (introduced by Stanford Wessler**).

Crystalline glucagon was administered intravenously in 100 ml of isotonic saline at a constant rate (0.01 mg/kg per hr) for a 4 hr period to each of 15 normal adult volunteers, to 15 patients with proved primary hyperparathyroidism, and to 9 patients with hypercalcemia associated with breast carcinoma or sarcoidosis. In comparison with controlled saline infusions, a significant fall in serum calcium was observed in each instance which could not be accounted for by enhanced renal calcium excretion. The magnitude of the serum calcium fall observed in the 39 subjects was found to be significantly correlated with the preinfusion serum calcium level ($r = 0.78$, $P < 0.001$). The hypocalcemic effect of intravenous glucagon was also observed in 20 intact dogs. Whereas, in the dog, thyroidectomy and thyroparathyroidectomy abolished the hypocalcemic response to glucagon, bilateral nephrectomy did not. Perfusion of the dog's thyroid artery with glucagon resulted in a rapid fall in systemic calcium levels and the liberation into a thyroid vein of a biologically active substance which itself induced hypocalcemia in intact rats. Experiments with ^{47}Ca in four intact dogs before and during glucagon infusions revealed a glucagon-induced rise in plasma calcium specific activity with insignificant changes in total plasma radioactivity. These findings suggest that the observed glucagon-induced hypocalcemia in man and dog results from the stimulated release of thyrocalcitonin or a thyrocalcitonin-like substance from the thyroid with resultant inhibition of bone resorption.

8. The Nitro Blue Tetrazolium Test and Defective Degranulation in Leukocytes of Chronic Granulomatous Disease. ROBERT L. BAEHNER,* DAVID G. NATHAN, JOHN M. CRAIG,* AND MANFRED L. KARNOVSKY,* Boston, Mass.

Granulocytes in chronic granulomatous disease (CGD) ingest bacteria normally but fail to kill them at a normal rate. These cells also have a deficient respiratory response and poor reduction of nitro blue tetrazolium (NBT) during phagocytosis. Good and his coworkers have proposed that the defect in CGD cells is one of faulty degranulation during phagocytosis. To evaluate the possible contribution of granules to NBT reduction, normal and CGD leukocytes were exposed to NBT. Normal granulocytes were not stained by NBT in 15 min, but after 1 hr intracellular punctate blue deposits were noted in a few cells. When the cells ingested zymosan, heavy deposits of blue formazan were observed around many phagosomes within 15 min. The ingested zymosan was itself lightly stained. In 1 hr extensive intracellular deposits of formazan were evident, particularly in periphagosomal areas. Normal monocytes behaved similarly. CGD granulocytes and monocytes ingested zymosan, but after 15 min the staining pattern described above had not appeared. After 1 hr a few punctate deposits of intracellular dye were seen and in some cells a thin halo of dye was observed around the phagosome. These data suggested that the granules of normal and CGD granulocytes and monocytes contain an NBT reductase system that is normally rapidly released around the phagosome but is very slowly released in CGD. To evaluate further the localization of the NBT reductase system, normal leukocytes were homogenized in alkaline KCl. The granular fraction, collected at 40,000 *g*, contained two-thirds of the total NADH or NADPH:NBT reductase system. Ascorbate, GSH, malate, succinate, and 3,3'-4,4'-diaminobenzidine did not serve as hydrogen donors for this system. Sonicated CGD and normal cells contained the reductase in equal amounts and specific activities. Finally, frozen sections of normal and CGD cells incubated with NBT and reduced pyridine nucleotide showed prominent granular staining. The localization of NBT reductase in the granules of normal and CGD cells and its delayed appearance in the phagosome of the latter may provide a means of assessing the defect in degranulation which characterizes CGD monocytes as well as granulocytes. The involvement of monocytes may explain some of the unusual infections observed in these patients.

9. The Interaction of Diabetes and Obesity on the Regulation of Basal Lipolysis in Man. JOHN D. BAGDADE,* DANIEL PORTE, JR.,* AND EDWIN L. BIERMAN, Seattle, Wash.

Past observations of elevated FFA levels in mildly diabetic subjects have led to the suggestion that increased basal lipolysis might be a fundamental defect in diabetes mellitus. However, in some studies, elevated FFA were also noted in obesity. Since carbohydrate intolerance and obesity frequently occur in the same patient, either one or both of these metabolic abnormalities could be primarily associated with elevated FFA. In order to analyze the relationship of obesity and diabetes to basal lipolysis, 37 male subjects, aged 40–60, with varying degrees of carbohydrate intolerance and obesity were studied after 3 days of weight-maintaining diets containing 300 g of carbohydrate per day. Basal FFA and glycerol were correlated with glucose intolerance (the area circumscribed by the blood glucose–time curve after 100 g oral glucose) and relative body weight. When the subjects were divided by weight, carbohydrate intolerance correlated significantly with FFA ($r = +0.63$, $P < 0.01$) in obese (>125% ideal body weight) but not in thin subjects (<115% ideal body weight; $r = -0.39$, $P < 0.1$). This lack of correlation between glucose intolerance and FFA in thin subjects suggested that FFA were elevated

only when diabetes and obesity coexisted. As this analysis would predict, when the group was divided by carbohydrate intolerance, FFA and weight were directly related only in the diabetic subjects (>2 SD from normal glucose response; $r = +0.83$, $P < 0.001$). Thus FFA were elevated only in obese carbohydrate-intolerant subjects, and appeared to rise progressively with increasing weight. The inverse relation between FFA and body weight ($r = -0.42$, $P > 0.1$) in nondiabetic subjects (within 1 SD of mean normal glucose response) indicates that obesity alone does not elevate FFA. Accordingly, elevated FFA were found only in obese diabetics (880 μEq/liter $\pm$ 314; mean $\pm$ SD). FFA of thin diabetics (555 μEq/liter $\pm$ 105) were normal—lower than levels observed in either nondiabetic group (thin, 725 μEq/liter $\pm$ 144; obese, 541 μEq/liter $\pm$ 95). Analysis of plasma glycerol led to identical conclusions. Thus elevated FFA, presumably a reflection of increased basal lipolysis, are found only in the mild diabetic who is also obese. This abnormality does not appear to characterize either uncomplicated obesity or mild diabetes in the nonobese.

10. Lipemic Hemolysis: Evidence for Altered Erythrocyte Membrane Composition and Hemolytic Anemia in Hypertriglyceridemic States in Man. JOHN D. BAGDADE* AND PETER WAYS, Seattle, Wash., and East Lansing, Mich.

Altered plasma lipids in patients with liver disease have been associated with elevated erythrocyte cholesterol, abnormal phospholipid content and composition, and hemolytic anemia. Erythrocyte cholesterol and phospholipid are known to exchange continuously with corresponding plasma lipids. Therefore, patients with lipemia (lactescent plasma) and no liver disease who have abnormally elevated plasma cholesterol and phospholipid levels in association with triglyceride-rich lipoprotein might also be expected to have red cell lipid and hematologic abnormalities. Three severely insulin-deficient diabetics with lipemia due to impaired chylomicron removal (mean plasma triglyceride (TG), 3877 mg/100 ml; cholesterol (Chol), 499 mg/100 ml; and phospholipid (PL), 622 mg/100 ml) were studied. Each had a low erythrocyte Chol/PL ratio (9.0, 8.6, and 7.5; normal, 9.6–10.2), low PL per erythrocyte (1.07, 1.11, and 1.15×10^{-11} mg; normal, $1.2–1.3 \times 10^{-11}$ mg), and a high percentage of erythrocyte lecithin (33.1, 32.3, and 31%; normal, 28.5%) and plasma lecithin (73.5, 73.0, and 79%; normal, 65–70%). When the lipemia was reversed by insulin, red cell lipids uniformly returned to normal. At no time was increased red cell production (reticulocytosis, plasma iron (^{59}Fe) turnover) or destruction (^{51}Cr survival) demonstrable. Six patients with endogenous lipemia (mean TG, 696 mg/100 ml; Chol, 290 mg/100 ml; PL, 297 mg/100 ml) resulting from excessive hepatic synthesis of lipoprotein containing relatively more cholesterol and phospholipid than chylomicrons were also studied. Erythrocyte percentage of lecithin (mean 30.2%) and Chol/PL ratio (mean 9.7) were normal in these patients, but PL per erythrocyte (1.11×10^{-11} mg) was low and percentage of plasma lecithin (75.1%) elevated as in the first group. These patients, however, demonstrated reticulocytosis and increased plasma iron turnovers (mean, 0.88 mg/100 ml per 24 hr; normal, 0.6 ± 0.2 mg/100 ml per 24 hr) consistent with hemolysis. ^{51}Cr-labeled red cells from six normal compatible donors had a shortened survival (mean, 21.6 days; normal, 26–35 days) in these patients, data consistent with hemolysis resulting from an extrinsic alteration in the erythrocyte. Thus, dietary lipid (chylomicron) accumulation in plasma appears to modify red cell membrane lipid composition without predisposing to hemolysis. Elevated endogenous lipoproteins, conversely, effect less striking compositional changes, but are associated with premature red cell destruction.

11. A Hereditary Sodium Transport Defect in the Human Red Blood Cell. J. W. BALFE,* C. COLE,* E. K. M. SMITH,* J. B. GRAHAM,* AND L. G. WELT,** Chapel Hill, N. C.

A Negro family has been found with a hereditary abnormality of the red blood cell characterized by abnormally high concentration of sodium. The propositus was a 55 yr old Negro female who had had an epidermoid carcinoma of the skin resected 10 yr previously with no evidence of recurrence. Her mother, aunt, and five of six living siblings who were tested had abnormally elevated red cell sodium concentrations (mean = 14.4 ± 2 mmoles/liter of red cells) as compared with the normal of 7.6 ± 1.3 mmoles/liter of red cells. Their red cells had a sodium pump defect. The ratio of the ouabain-sensitive sodium efflux of the patients to that of the normal was $49.7 \pm 5.1\%$. The ratio of ouabain-sensitive ATPase of the patients to that of controls was $50.6 \pm 5.5\%$. The concentration of sodium for half maximum activation of the glycoside-sensitive ATPase was not different in the members of this family from that in controls. Members of this family have been extensively investigated for the cause of their red cell abnormality. There was no evidence of hemolytic anemia, renal disease, or overt neoplastic disease, all of which may be associated with an elevated cell sodium. Analysis of the data obtained thus far from this kindred (32 tested) would suggest a dominant mode of inheritance, but it is not clear whether or not this is sex linked. Additional studies are being made to specify the mode of inheritance and to separate this hereditary defect of the red cells from the acquired defect seen in sick uremic patients.

12. The Effect of Bile Salts on Hepatobiliary Lecithin Metabolism. JOHN A. BALINT,* DONALD A. BEELER,* AND DONALD H. TREBLE,* Albany, N. Y. (introduced by Stuart Bondurant).

Biliary lecithin, predominantly linoleoyl-palmitoyl in type, is derived from a separate intrahepatic pool. In order to study the role of bile salts in the secretion and synthesis of biliary lecithin, male rats were equipped with biliary and duodenal cannulae and infused via the latter with 0.62 ml/hr of either saline, normal bile, or bile containing 6 times normal concentration of taurocholate (6T). Bile volume and content of cholesterol, di- and

trihydroxy bile salts, and phospholipids were measured at timed intervals for 48 hr. Other rats, injected intravenously with a mixture of ^{3}H–choline-methyl, ^{14}C–L-methionine-methyl, and $^{32}PO_4$, were sacrificed at 1, 2, and 4 hr. The lipids of liver and bile were extracted and were separated by silicic acid column and thin-layer chromatography, and the radioactivities were determined. Bile flow was 10% greater in bile-infused and 20% greater in 6T-infused rats, whereas the output of phospholipids and bile salts was respectively 200% and 480% greater than in saline-infused controls. Cholesterol output increased at the same time to 160% and 360% respectively. The specific activity of linoleoyl lecithin of liver and bile increased almost 2-fold with respect to ^{3}H-choline and ^{32}P in bile-infused rats, and by a further 12% in 6T-infused animals as compared with saline-infused controls. No increase in the incorporation of ^{14}C-methyl groups from methionine was observed in either case. These findings indicate that the secretion of biliary lecithin is linked to that of bile salts, thus maintaining a constant molar ratio between these compounds, and that the increased bile salt load presented for excretion accelerates the synthesis of linoleoyl lecithin via the CDP-choline pathway without significant effect on the methylation of phosphatidyl ethanolamine.

13. The Response of the Hypertrophied Kidney to Saline Infusion. NORMAN BANK AND HAGOP S. AYNEDJIAN,* New York, N. Y.

When the total nephron population is reduced, the remaining nephrons develop an enhanced ability to excrete a sodium and water load. This might be due to greater natriuretic hormone activity, which would reduce the intrinsic reabsorptive capacity ($C/\pi r^2$) of the tubular epithelium, to a decrease in tubular volume relative to GFR ($\pi r^2 d/V_0$), or to renal hemodynamic factors. In order to study this problem, we measured TF/P_{In} ratios and lissamine green transit time (T) in the proximal tubule of normal rats and unilaterally nephrectomized rats (3–16 wk after surgery) under identical conditions of saline loading. Intrinsic reabsorptive capacity was calculated from $C/\pi r^2 = 2.3 \log TF/P_{In}$ per T, and the ratio of tubular volume to GFR was calculated from $\pi r^2 d/V_0 =$ per cent reabsorbed/$C/\pi r^2$. It was found that $C/\pi r^2$ was not significantly lower in the hypertrophied kidney than in the normal, either under nondiuretic control conditions (0.082/sec vs. 0.085/sec) or during saline diuresis (0.051/sec vs. 0.055/sec). The ratio between tubular volume and GFR was also found to be unchanged in the hypertrophied kidney, during both control periods (7.3 sec vs. 7.4 sec) and saline diuresis (6.6 sec vs. 6.8 sec). Thus, neither greater natriuretic hormone activity nor altered tubular geometry could account for the increased sodium and water excretion by the hypertrophied kidney. During saline diuresis, GFR per kidney was 23% higher in the hypertrophied kidney, and GFR per surface nephron was 29% higher. When the abdominal aorta was constricted above the renal artery of the hypertrophied kidnev and GFR reduced to that of a normal kidney undergoing saline diuresis, proximal sodium reabsorption was inhibited as before, but the diuresis was markedly diminished, suggesting enhanced distal reabsorption. We conclude that the ability of the hypertrophied kidney to excrete a saline load depends upon the higher GFR, and is not due to reduced tubular reabsorption.

14. Hereditary Iron-Deficiency Anemia Due to a Specific Defect of Intestinal Absorption. ROBIN M. BANNERMAN,* PETER H. PINKERTON,* AND JOHN A. EDWARDS,* Buffalo, N. Y. (introduced by David K. Miller).

Hereditary iron-deficiency anemia of mice, gene symbol *sla*, which is an X-linked recessive trait, shows the main features of iron deficiency. The anemia is hypochromic and microcytic. It tends to regress with increasing age, and can be cured by treatment with parenteral iron. Tracer ^{59}Fe given intravenously is rapidly cleared from the plasma and effectively utilized in red cell formation. Total body iron stores are depleted, as determined by carcass analysis and as seen histochemically. By contrast, the cells of the mucosal epithelium of the small intestine appear laden with stainable iron. Absorption studies carried out by whole-body counting have shown malabsorption of inorganic iron, as we have reported previously. Ferrous sulfate labeled with ^{59}Fe is significantly less well absorbed by anemic than by nonanemic mice. This paradoxical result in animals which are iron deficient, and the presence of heavy iron deposition in the mucosal epithelial cells, suggest that the defect is in the transfer of iron from the intestinal mucosa to the plasma. Further studies have confirmed and extended these findings. No reduction in Fe^{++} absorption has been found in heterozygous *sla* carriers, nor in chimeras of *sla* bone marrow in normal irradiated hosts. The absorption of other substances has been investigated, and *sla* anemic mice show no significant impairment in the absorption of ^{64}Cu, ^{57}Co, and ^{65}Zn, nor of fat as determined by ^{131}I-labeled triolein. Thus the defect appears to be specific for iron. Since it probably involves a single genetically determined step in iron absorption, the *sla* mutation may be a useful tool in elucidating the control mechanism.

15. Altered Glucosamine Transport in Rheumatoid Synovial Cells in Tissue Culture. P. BARLAND,* E. SCHROEDER,* C. SMITH,* AND D. HAMERMAN, New York, N. Y.

Transport of monosaccharides across cell membranes influences cellular metabolic activity. Carrier molecules within the cell membrane of bacteria are under genetic control. In mammalian cells the presence of carrier molecules has been shown by competitive inhibition between various sugars for transport across the cell membrane. Viral infections and mutagens may alter membrane transport and thereby induce cell damage. This report presents evidence for an altered glucosamine transport in rheumatoid synovial membrane cells in culture. Glucosamine is used by synovial cells for hyaluronate synthesis. Replicate monolayer cultures of normal and rheumatoid

cells were incubated in Krebs-Ringer phosphate buffer containing 20 mM pyruvate and 6-^{3}H-glucosamine 1–3 μc/ml. After incubation, the cells were collected on Millipore (0.45 μ) filters and counted in a liquid scintillation spectrometer. Rheumatoid cells consistently accumulated significantly more glucosamine than normal cells over the concentration range 0.5–5 mM. The effect on glucosamine uptake produced by the addition of different sugars to the incubation medium was studied. Glucose inhibited the uptake of the amino sugar equally in normal and rheumatoid cells. However, galactose, 3-*O*-methyl glucose, and fructose all failed to inhibit the uptake of glucosamine by rheumatoid cells but did inhibit this activity in normal cells. These differences in uptake of glucosamine could be mediated at the level of membrane transport or at a subsequent step involving phosphorylation. The former possibility would appear more likely, for 3-*O*-methyl glucose is not phosphorylated and yet effectively inhibits the uptake of glucosamine. Moreover, if the level of glucosamine-6-PO_4 determined glucosamine uptake, then 6-diazo-5-oxonorleucine (DON), which inhibits the endogenous synthesis of glucosamine-6-PO_4 from fructose-6-PO_4, should enhance the uptake. No increase in uptake of glucosamine was found in normal or rheumatoid cells incubated with DON. These results suggest a fundamental change in the cell membrane of the rheumatoid cells in culture.

16. Evidence for the Role of Insulin Secretion in the Production of Endogenous Hypertriglyceridemia. RICHARD J. BARSE,* J. DAVID SCHNATZ,* AND LAWRENCE A. FROHMAN,* Buffalo, N. Y. (introduced by Evan Calkins**).

Controversy exists as to whether hyperinsulinemia is responsible for, or the result of, endogenous hypertriglyceridemia. The studies described were designed to distinguish between these two hypotheses by pharmacologically altering insulin release. Four otherwise healthy subjects with endogenous hypertriglyceridemia were studied for three consecutive 5-day periods: (1) control, (2) Diazoxide treatment, and (3) recovery. While the subjects were hospitalized, their usual diet and activity were maintained. Triglycerides were determined daily. Glucose tolerance (one subject oral, three subjects intravenous) was determined during each period and determined as increments under the glucose curve (ΔG) or disappearance rate (K_G). Insulin response to glucose was determined as the increment under the insulin curve (ΔI). Three subjects received 1-^{14}C-palmitate injections during Diazoxide treatment and a control period. Endogenously labeled triglyceride was isolated by thin-layer chromatography for determination of ^{14}C-palmitate incorporation and plasma disappearance rate (K_{TG}). Since all subjects reacted similarly, mean values are reported. On Diazoxide, the following were observed: (1) a 51% decrease in ΔI, (2) a 147% increase in ΔG and a 19% decrease in K_G, (3) a triglyceride decrease from a control of 292 to a nadir of 139 mg/100 ml (52%), (4) a 22% decrease in ^{14}C-palmitate incorporation into triglyceride, and (5) an increase in K_{TG} from 0.13 to 0.24%/hr (85%) in two patients and in the third an initial increase with subsequent curvilinear decay. For comparison, a juvenile diabetic with hypertriglyceridemia was given Diazoxide for 5 days with a minimal change in triglyceride. Thus, in four patients with endogenous hypertriglyceridemia, Diazoxide decreased insulin secretion, glucose tolerance, and triglyceride levels. These results were associated with decreased ^{14}C-palmitate incorporation into triglyceride and increased K_{TG}. The latter is compatible with a decreased triglyceride pool. We conclude that insulin secretion can contribute significantly to the pathogenesis of endogenous hypertriglyceridemia.

17. Localization of Production of Autoantibodies in Patients with Atrophic Gastritis and Adult Addisonian Pernicious Anemia. SONIA BAUR,* JUNE FISHER,* AND KEITH TAYLOR, Stanford, Calif.

Peroral gastric biopsies were obtained from several patients with superficial gastritis, atrophic gastritis, and adult Addisonian pernicious anemia. In this group of patients there were some with circulating antibody to parietal cells alone, some with antibodies to parietal cell and "blocking" antibody to intrinsic factor, some with antibodies to parietal cell and "blocking" and "binding" antibodies to intrinsic factor, and others with no circulating antibodies to either parietal cell or intrinsic factor. To localize the cells producing autoantibodies to parietal cell antigen, human "microsomal" stomach antigen was fluorescein conjugated and added to the biopsy section. Mononuclear cells in the lamina propria producing the antibody fluoresced. To localize the cells producing antibody to intrinsic factor, gastric juice and ^{57}Co-B_{12} were incubated together, the excess B_{12} was removed with charcoal, and the section was flooded with the resultant intrinsic factor-^{57}Co-B_{12}. Radioautographs were made of these sections, and cells producing binding antibody to intrinsic factor were localized. The same procedures were applied to sections of a coagulum of lymphocytes derived from peripheral blood of the same group of patients. Positive and negative results were correlated with serum titers of the respective antibodies.

18. Effect of Partial Hepatectomy on Protein Phosphorylation of Rat Liver Nuclei. WILLIAM BENJAMIN* AND ALFRED GELLHORN,** with the technical assistance of SANDRA BECK,* New York, N. Y.

The nucleoproteins are believed important in the control of DNA replication and RNA transcription. A phosphoprotein fraction may be directly involved in these processes. Therefore the in vivo and in vitro phosphorylation of both the nucleoproteins and the phosphorus-rich acidic protein fraction were studied in sham-operated control and partially hepatectomized rats. ^{32}P-orthophosphate injected i.p. was actively incorporated into the nucleoprotein fractions. The acidic protein fraction was found to have the highest specific activity. When ^{32}P was injected 18 hr after partial hepatectomy, we found both a 25% increase in the counts per mg of nucleoprotein and a significant increase in the specific activity of the protein

phosphorus as compared with controls. ^{32}P-orthophosphate is not a suitable substrate for the in vitro assay, as it is not incorporated into the nucleoproteins of isolated liver nuclei. This is presumably because nuclei prepared with hypertonic sucrose are deficient in an ATP generating system. Using AT^{32}Pγ as substrate, there was rapid phosphorylation of the nucleoproteins (0.7 mμmole phosphorus/mg protein per 10 min). Gel electrophoresis of the acidic proteins labeled in vitro demonstrated them to be heterogeneous and showed a radioactive profile similar to that obtained when ^{32}P was injected in vivo. Radioactivity was found associated with the phosphoserine of the protein. Therefore it is concluded that rat liver nuclei actively phosphorylate nucleoproteins in vitro. Nuclei from partially hepatectomized animals incorporate ATP phosphorus into nucleoproteins at a higher rate than controls.

19. Transmission Patterns of Coagulase-Negative Staphylococci. DAVID W. BENTLEY, RIAZ-UL HAQUE,* AND MARK H. LEPPER,** Chicago, Ill.

Recent studies of staphylococcal postcardiotomy endocarditis, methicillin resistance, and bacterial interference have documented the importance of coagulase-negative staphylococci, and the need to evaluate their transmission patterns and carrier states. Using a recently described 'biotyping" method, 108 subjects in a closed population had nose and skin cultures performed before, during, and after 15 had received a 2 wk course of chloramphenicol (CM). Antibiotic resistance patterns were detected by CM-containing plates plus multidisc concentrations of penicillin (PC), tetracycline (TC), streptomycin (SM), erythromycin (EM), and CM. 74 had a positive nasal culture before treatment, with 9% CM resistant including seven different biotypes. After 36 hr of treatment, 74 cultures were positive, with 26 (35%) resistant. 63 of the 93 untreated were positive, with 21 (33%) resistant, and 5 (45%) of the 11 treated. After 14 days, 82 were positive, with 64 (78%) CM resistant. 46 (67%) of the 68 untreated were resistant, with 18 (4 had two different resistant strains each) of the 14 treated. Approximately 50% changed biotype in each period, with a total of seven new biotypes noted. 14 days after treatment, 19 CM-resistant isolates remained—11 (19%) from the untreated and 8 (80%) from the treated group, with one new biotype noted. By contrast, *S. aureus,* cultured identically and typed by phage method, showed no CM resistance until the 14th day of treatment with infrequent transmission of only a few strains. Antibiotic disc sensitivities revealed marked multiple cross-resistance. Before treatment, 5% had CM-resistant triple patterns (PC-TC-CM and PC-EM-CM) and 13% had triple patterns without CM resistance (PC-TC-SM and PC-TC-EM). After 36 hr of treatment, 27% and 24% of the treated and untreated groups respectively had triple, quadruple, and quintuple CM-resistant patterns. By 14 days of treatment the multiple resistance patterns in the two groups had increased to 90% and 40% respectively. Such a picture appears most easily explained by an extrachromosomal transfer of CM resistance with the spread of multiple-resistant strains by means of whole-organism implantation.

20. Plasma Clearance of ^{14}C–Unconjugated Bilirubin (^{14}C-UCB) in Normal Volunteers. PAUL D. BERK,* ROBERT B. HOWE,* AND N. I. BERLIN,** Bethesda, Md.

Tracer quantities of ^{14}C-UCB were administered intravenously to four normal volunteers and blood samples were obtained for 24–30 hr. Over this period the plasma disappearance curve of ^{14}C-UCB took the form of the sum of three exponentials, with mean half-times of 16.2, 66.9, and 714 min. Using a Univac 1108 digital computer, data were fitted to a four-compartment model, in which plasma bilirubin (A) is in equilibrium with an extravascular pool (B) and a hepatic pool of unconjugated bilirubin (C). Irreversible removal from the system occurs from pool C to the hepatic conjugated bilirubin pool (D). Initially 13.6% of the isotope entered the extravascular pool, refluxing to plasma after a mean sojourn of 13.3 hr. Mean values for the parameters of the model are: pool A, 8.6 mg; pool B, 17.1 mg; pool C, 12.1 mg; bilirubin concentration in pool C, 1.0 mg/100 ml; concentration ratio between liver and plasma, 3.1; efficiency of net hepatic bilirubin uptake, 6.4%; bilirubin production rate (BRP), 213 mg/24 hr. Deducting 15% for nonerythrocytic sources of bilirubin, this corresponds to a mean red cell life span (RBCLS) of 104 days. BRP was also calculated from data restricted to the first 6 hr of the experiment, during which only two exponentials could be detected. The results of these calculations exceeded the values obtained with 24 hr data by 18%, and values of the RBCLS were correspondingly less. Ferrokinetic studies were carried out for 14–21 days in the same volunteers, using ^{59}Fe; the RBC-iron turnover was used to estimate the RBCLS. The average RBCLS estimated from the ferrokinetic data was 112 days. 24 hr studies of plasma clearance of ^{14}C-UCB may provide a rapid means of determining the total BRP, RBCLS, and several parameters of hepatic function.

21. The Active Transport of GSSG from Human Erythrocytes. ERNEST BEUTLER AND SATISH K. SRIVASTAVA,* Duarte, Calif.

The level of oxidized glutathione (GSSG) appears to be one of the important regulators of metabolism by way of the hexose monophosphate pathway. Heretofore it has been believed that the level of GSSG in the erythrocyte depends upon the relative rates of oxidation of reduced glutathione (GSH) and reduction of GSSG, and perhaps on binding of GSSG by red cell proteins. We have found that when human red cell GSH is oxidized, by exposure to low levels of hydrogen peroxide, to methyl phenylazoformate, an efflux of GSSG occurs. Estimations of GSH and GSSG levels in normal and G-6-PD–deficient erythrocytes and in the suspending medium were carried out for a period of several hours

during and after exposure to H_2O_2 or methyl phenylazoformate. Linear efflux of GSSG from the erythrocytes was observed throughout the period of study. The following further findings establish the active nature of the GSSG efflux: (1) It is strictly unidirectional; GSSG does not enter the erythrocyte from the exterior; (2) it proceeds against a concentration gradient; (3) it is strongly temperature dependent; (4) it depends upon the presence of substrate; and (5) it is completely inhibited by fluoride. GSSG has been shown to inhibit the activity of red cell enzymes and to complex with hemoglobin. Our findings indicate that the level of GSSG in the erythrocyte can be regulated by active transport of GSSG from the cell. This transport system may represent a second line of defense against the potentially toxic effects of high levels of GSSG within the erythrocyte.

22. ACTH Modulation of Mineralocorticoids. EDWARD G. BIGLIERI, PAUL E. SLATON, JR.,* AND MORRIS SCHAMBELAN,* San Francisco, Calif.

Continued administration of ACTH produces transient increases in aldosterone secretion. To study factors involved, ACTH (25 units) was given intravenously daily for 3 days to four normal subjects and eight patients with aldosterone-producing adenomas (APA) during fixed electrolyte intakes. Urinary tetrahydrodeoxycorticosterone (THDOC), tetrahydrocorticosterone (THB), and aldosterone were measured by double isotope techniques, and plasma renin activity (PRA) by the Boucher method. In normal subjects THDOC and THB increased progresssively to 500 and 3000 μg/24 hr, respectively, but aldosterone showed only an initial transient rise. On cessation of ACTH, aldosterone fell to $< 50\%$ of control values and remained reduced for 5 days, whereas THDOC and THB promptly returned to control levels. In another study, when ACTH was administered for 17 days both aldosterone and PRA were blunted and failed to rise during sodium restriction and potassium loading. In patients with APA the mineralocorticoid responses were the same as in normal subjects, but PRA was virtually absent. Cumulative sodium (+22 mEq) and potassium (−22 mEq) balances, changes in serum sodium (0) and potassium (−0.3 mEq/liter) concentrations, and weight changes (−0.5 kg) were minimal. In addition, THDOC and THB were elevated in seven patients with endogenous ACTH production: three had ectopic ACTH tumors and four had 17-hydroxylation deficiency. THDOC was 600–5400 and 1400–5200 μg/24 hr (N 50–250) in the two groups respectively, and THB was 34–72 and 44–114 mg/24 hr (N 0.9–4.4) respectively. Aldosterone secretion was reduced to 23–64 and 0–29 μg/24 hr (N 60–168) respectively. A possible mechanism suggested for the reduction of aldosterone excretion by ACTH is the reduction of PRA by increased DOC production. However, the similar mineralocorticoid responses when PRA was absent suggest a more dominant role for intra-adrenal modulation of aldosterone synthesis.

23. Pharmacologic Retardation of Oxygen Toxicity. G. DOUGLAS BLENKARN,* SAUL M. SCHANBERG,* AND HERBERT A. SALTZMAN,* Durham, N. C. (introduced by Herbert O. Sieker).

Central nervous system (CNS) toxicity has impeded therapeutic application of hyperbaric oxygen (OHP). Recent observations that hyperoxia lowers cerebral amine levels and increases intracellular monoamine oxidase (MAO) activity led to this study of relationships between cerebral amines, MAO metabolism, and OHP-induced CNS toxicity in rats after the intraperitoneal injection of selected drugs. When pargyline (40–60 mg/kg), an MAO inhibitor, was administered 30 min before exposure to 5 atmospheres of oxygen pressure for 2–2.5 hr, the mean frequency of convulsions fell from 81% to 31%, and the mean 72 hr survival increased from 21% to 71%. Signs of preconvulsive toxicity and morbidity were comparably reduced, and structural CNS lesions were absent 5 days later. Iproniazid (50–80 mg/kg) proved equally effective; isoniazid (50–100 mg/kg), an analogue lacking MAO activity, was ineffective; tranylcypromine (10 mg/kg), another MAO inhibitor, increased overt toxicity. Pargyline or iproniazid in larger doses, or given more than 2 hr before OHP, exacerbated the manifestations of oxygen toxicity. Pargyline and iproniazid were equipotent to large doses of glutathione (1.2 g/kg) and more effective than other drugs (L-cysteine (740 mg/kg), THAM (1.5 g/kg), pentobarbital (15 mg/kg), chlorpromazine (5 mg/kg)) previously reported to retard the onset of oxygen toxicity. An inhibitor of catecholamine synthesis, α-methyl-p-tyrosine (100 mg/kg), or an inhibitor of serotonin synthesis, p-chlorophenylalanine (200 mg/kg), exerted no protective action, but when these were followed by pargyline the protection was greater than from pargyline alone. Cerebral amine levels associated with these pharmacologic studies will be reported. Our findings indicate that premedication with pargyline or iproniazid protects rats from OHP-induced CNS toxicity; this effect does not appear to be mediated through increased CNS levels of noradrenaline or serotonin.

24. Heavy Chain (Fc Fragment) Disease: Report of an Additional Case with Further Evidence for the Synthesis of a Fragment of γG Globulin. KURT J. BLOCH* AND LEONARD L. ELLMAN,* Boston, Mass. (introduced by Marian W. Ropes**).

The term heavy chain (Fc fragment) disease denotes a malignant lymphoma, without skeletal involvement, accompanied by the presence in serum and urine of large amounts of a fragment of γG globulin. This fragment consists of portions of the two heavy polypeptide chains of the γG molecule and closely resembles the Fc fragment obtained by papain treatment. In the seventh patient with this disorder, immunoelectrophoresis of serum disclosed normal concentrations of γG, γA, and γM globulins and an additional precipitin arc outlined by antiserum to Fc, but not Fab, Fd, κ, or λ antigenic determinants. An identical protein was present in urine in concentration of about 15–

30 mg/24 hr. In gel diffusion, the isolated serum and urine M component showed a reaction of complete identity with Fc fragment and a reaction of nonidentity with the Fab fragment of γG globulin. Rabbit antiserum to the isolated serum M component of this patient precipitated in agar gel in a reaction of identity with the urinary M component from the fifth patient and in a reaction of partial identity with the urinary M component from the first patient with heavy chain disease. The isolated serum M component had a sedimentation coefficient of 3.0–3.1S, and possessed γG3 antigenic determinants, as well as a Gm marker characteristic of this subclass of γG globulins. Immunofluorescence studies of washed bone marrow cells showed a preponderance of cells with Fc determinants. Anti-Fc antiserum yielded precipitin lines in agar gel with saline extracts of neoplastic lymph nodes diluted 2- to 300-fold, whereas anti-Fab, -κ, -λ, and -α antisera yielded precipitin lines with extracts diluted only up to 10-fold. Together with evidence provided by other investigators, these findings suggest that the immunoglobulin fragment in heavy chain disease is a product of aberrant synthesis rather than a breakdown product of an unusually fragile γ-globulin molecule.

25. Phagocytosis Independent of Glycolysis in Human Blood Leukocytes. PHYLLIS T. BODEL* AND STEPHEN E. MALAWISTA,* New Haven, Conn. (introduced by Elisha Atkins).

Phagocytosis by polymorphonuclear leukocytes has been thought to be dependent on glycolysis, the major metabolic pathway for energy in these cells. During studies of energy requirements for phagocytosis by leukocytes of human blood, however, we have found that ingestion of particles occurs normally at a time when glycolysis is suppressed. Leukocytes, prepared from heparinized blood by dextran sedimentation, were suspended in a 12% serum-phosphate buffer, and incubated at 37°C in Warburg or Erlenmeyer flasks. Glycolytic inhibitors—iodoacetate (IAA, 2 or 4×10^{-4} M) or sodium fluoride (NaF, 2×10^{-2} M)—were added to some flasks initially. 40 min later, bacteria (alpha hemolytic streptococci, or *Staphylococcus albus* or *aureus*) were added. Phagocytosis was measured by the disappearance of live bacteria from flask supernatants. Samples were taken 20 and 40 min after addition of bacteria. Although IAA completely inhibited the increased oxygen uptake that normally accompanies phagocytosis, diminished the production of lactate, and decreased the degranulation of leukocytes (both morphologically and by acid phosphatase measurement), IAA consistently had no effect on phagocytosis. In 10 experiments, $P > 0.6$. In contrast, NaF inhibited both phagocytosis and increased oxygen consumption. However, even in the absence of bacteria, NaF caused swelling, vacuolization, increased fragility, and eosin staining of neutrophils. Thus, IAA suppressed glycolysis without altering phagocytosis. NaF, at the high concentration conventionally employed, interfered not only with glycolysis but with the structural integrity of neutrophils. These results indicate that active glycolysis is not essential for phagocytic function, and suggest that some preformed energy source may be available to the leukocyte for this activity. For this reason, changes in cellular ATP after phagocytosis were measured. As compared with levels in incubated control leukocytes, acid-soluble ATP decreased in 20 min as follows: (*a*) with phagocytosis, 35%, (*b*) with IAA alone, 52%, (*c*) with IAA and phagocytosis, 78%. Thus, stored ATP may provide immediate energy for phagocytosis.

26. Physiological Significance of Adrenal Cholesterol Fractions. A. J. BORKOWSKI,* S. LEVIN,* C. DELCROIX,* AND J. KLASTERSKY,* Brussels, Belgium (introduced by H. J. Tagnon**).

In order to study the functional distribution of cholesterol in the human adrenal gland, ^{14}C-cholesterol was administered to 25 patients with breast carcinoma at various intervals during the week preceding adrenalectomy. The specific activities (SA) of plasma cholesterol were compared in time with those of adrenal cholesterol in the surgical specimens. The distribution of the tracer was found to be of the same order of magnitude in the zonae reticularis and fasciculata, although the SA of adrenal cholesterol were almost always somewhat higher in the former. On fractionation of adrenal cholesterol by ultracentrifugation and thin-layer chromatography, the various adrenal cholesterol esters were found essentially in the supernatant; their SA were similar to one another and increased progressively with time. On the other hand, most of the free adrenal cholesterol was located in the nuclei, mitochondria, and microsomes; it was freely exchangeable between these organelles; its SA were much higher than in the adrenal esters but were lower than in total plasma cholesterol. The SA of free adrenal cholesterol, particularly in the zona reticularis, evolved in time very much like those of total plasma cholesterol: they increased rapidly during the first 24 hr, reached a peak, and thereafter fell progressively. In conclusion, adrenal cholesterol appears to comprise only two major compartments: (1) Free adrenal cholesterol, amounting to 5% of total adrenal cholesterol, represents a small compartment which appears to be in rapid exchange with plasma; it results in part from local synthesis and is probably in isotopic equilibrium with the immediate precursor of hydrocortisone. (2) The adrenal esters represent the bulk of adrenal cholesterol; they appear to be formed by in situ esterification and their turnover is relatively slow.

27. A Possible Regulator of the Thyroid System. C. Y. BOWERS,* K. L. LEE,* AND A. V. SCHALLY,* New Orleans, La. (introduced by G. E. Burch**).

Major regulators of the thyroid system have been well established to be T_3, T_4, and thet hypothalamic neuroregulator, thyrotropin-releasing factor (TRF). Since we have found that human serum will inactivate TRF, studies were performed to determine whether the serum may also be a regulator of the thyroid system. TRF when incubated for 15 min at 37°C in mouse, rabbit, rat, and guinea pig serum was found to be inactivated, and when the latter three animals were treated with T_3 the capacity

of the serum to inactivate TRF increased. The activity of this serum factor is temperature dependent and nondialyzable, and preliminary evidence indicates that the increase of this factor after T_3 administration in rats is prevented by actinomycin D and cycloheximide. It was found that TRF was not inactivated when incubated in homogenates of the anterior pituitary gland of T_3-treated mice. Although the T_3 inhibition of the TRF-TSH release response has been found to require normal protein and RNA synthesis, it is probable that this T_3 effect is not mediated by stimulating a protein which destroys TRF within the anterior pituitary gland. Besides the direct action of T_3 and T_4 on the pituitary gland, it may be that T_3 and T_4 regulate the thyroid system by controlling the factor in serum which destroys TRF. The level of TRF-inactivating capacity of serum must be considered when one is attempting to measure the TRF level in blood and possibly in hypothalamic tissue.

28. Hemodynamic Effects and Disappearance of ADP. RICHARD E. BRASHEAR* AND JOSEPH C. ROSS, Indianapolis, Ind.

Adenosine diphosphate (ADP) is an integral part of energy metabolism and a potent vasoactive compound. This study was undertaken to determine the effects of ADP on the pulmonary circulation. Single injections of ADP (12 mg/kg) into the pulmonary artery of six dogs produced significant decreases in aortic and pulmonary artery pressures persisting for 30 min ($P < 0.005$). However, no increase in blood or plasma ADP could be detected 1 to 2 min after injection. Subsequent studies demonstrated that ADP passed through the pulmonary vascular bed in large concentrations. The in vivo disappearance of injected ADP was very rapid as compared with the in vitro disappearance. Injected ADP seems to be altered or fixed in less than 1 min. ADP (12 mg/kg in 10 cc) was injected rapidly into the pulmonary artery of another seven dogs. Mean cardiac output before injection of ADP was 145 ± 28 cc/min per kg, but decreased to 86 ± 33 ($P < 0.005$) 5 min after injection and 120 ± 26 ($P < 0.02$) 30 min after injection. Aortic and pulmonary artery pressure was decreased significantly for 30 min, but wedge pressure and heart rate showed no consistently significant changes. A similar ADP injection was performed in seven dogs after atropine (1 mg/kg). Mean cardiac output after atropine and before ADP injection was 146 ± 29 cc/min per kg and again decreased to 75 ± 21 ($P < 0.005$) 5 min after injection and 122 ± 18 ($P < 0.02$) 30 min after injection. Heart rate, aortic pressure, and pulmonary artery pressure were significantly decreased for 20 min, but right atrial and wedge pressures demonstrated no consistently significant changes. It appears that injected ADP in the dose used produced sustained decreases in cardiac output, aortic pressure, and pulmonary artery pressure despite the disappearance of ADP from the blood or plasma in less than 1 min. The exact mechanism for this is not completely clear. The coronary and pulmonary depressor chemoreflex may participate to some degree.

29. A Mechanism of Renal Injury Based on Newer Aspects of the Clotting System. WILLIAM E. BRAUN,* CHARLES H. ASPLEN,* AND JOHN P. MERRILL,** Boston, Mass. (introduced by Kendall Emerson, Jr.**).

Fibrinogen fragments, corresponding to fragments D and E produced by plasmin digestion of fibrinogen or fibrin, were detected in the urine concentrates of human renal transplants within 2 wk of transplantation (number positive/number tested = 16/16), with rejection beyond 2 wk after transplantation (7/7), and with recurrent glomerulonephritis (1/1). Beyond 2 wk after transplantation when no rejections were occurring the fragments were absent (0/62). The same fragments were also detected in patients with progressive glomerulonephritis (2/2), lupus nephritis (1/1), acute tubular necrosis (1/1), and renal vein thrombosis (1/1), but not in the idiopathic nephrotic syndrome (0/8), multiple myeloma (0/2), pyelonephritis (0/1), other renal surgery (0/3), nonrenal surgery (0/2), normals (0/2), normal and transplant urine without the fragments to which fibrinogen was added before processing (0/2). The site of production of the fragments may be in the kidney for the following reasons: No fragments were detected in corresponding sera; urine collected directly from the renal pelvis of a transplanted kidney into a sterile receptacle with soybean trypsin inhibitor also had both fragments; 121 urine protein selectivity determinations in transplants failed to show any association between poor selectivity and fragment excretion except with rejection. In collaboration with Drs. G. Busch, P. Schur, and C. B. Carpenter these fragments have been eluted from a rejected renal allograft. Also, fragment-containing urine blocks positive fibrinogen fluorescence of allograft and glomerulonephritic kidneys, whereas normal urine with equal protein concentration does not. Since plasmin can release a chemotactic factor from C'3 and activate C'1, and thrombin can release a sustained-action vasoconstrictor peptide from fibrinogen, these factors may contribute to renal damage.

30. Induction of Myxedema by Iodine in Patients with Treated Thyrotoxicosis of Graves' Disease. LEWIS E. BRAVERMAN* AND SIDNEY H. INGBAR, Boston, Mass.

In both normal patients and those with untreated thyrotoxicosis, chronic iodine administration induces myxedema only rarely. Here, the underlying abnormality is unknown, but inferential evidence suggests an antecedent defect in thyroidal organic-binding of iodide. Since thyrotoxic patients treated with ^{131}I may display a mild defect of organic-binding, this hypothesis was tested in nine patients studied 6 months to 3 yr after standard ^{131}I treatment (mean dose, 7.1 mc), two who had received low doses of ^{131}I (2.0 mc), and six treated surgically. During control periods, all patients were euthyroid by clinical and laboratory criteria and none displayed defective organic-binding as assessed by the perchlorate discharge test. Antithyroglobulin titers greater than

1:250 were present in five radioiodine-treated and two surgically treated patients. After institution of saturated solution of potassium iodide (SSKI, 5 drops daily), the nine patients treated with standard doses of ^{131}I all promptly developed clinical and chemical evidence of hypothyroidism, mean serum thyroxine concentration decreasing from 6.0 to 1.0 μg/100 ml within 18 to 41 days, while serum cholesterol increased significantly. A euthyroid state rapidly returned when iodine was withdrawn. Myxedema did not occur in the two patients given low doses of ^{131}I. In patients treated surgically, SSKI induced myxedema only in the two with positive antithyroglobulin titers. The difference in frequency of iodide-induced myxedema after standard ^{131}I and surgical therapy was highly significant ($P < 0.005$, chi square). Conclusions: Standard radioiodine treatment of thyrotoxicosis renders the thyroid inordinately sensitive to the myxedema-inducing effect of iodine. This may result from subtle defects in organic-binding undetectable by perchlorate discharge. This finding provides the first model for the consistent production and study of iodide myxedema in man. In surgically treated patients, iodide myxedema may be inducible when autoimmune damage impairs organic-binding, as is thought to occur in Hashimoto's disease.

31. Studies on Bile Acids in Man. GEORGE A. BRAY* AND THOMAS F. GALLAGHER, JR.,* Boston, Mass. (introduced by E. B. Astwood**).

Unconjugated bile acids were fed to 21 men and women to test whether steatorrhea would be induced. Each patient weighed over 300 lb. The doses ranged from 300 to 1800 mg/day. 7 patients were fed liquid formula diets containing 900–2500 cal/day (40% of calories as fat) on a metabolic research ward. Deoxycholic acid was given to 5 and the other 2 received chenodeoxycholic, hyodeoxycholic, lithocholic, or cholic acid separately and with varying doses of deoxycholic acid. The results showed: (1) Fecal fat was not altered in most collection periods. (2) Stool sodium always increased 20–50 mEq/day without concurrent increase in potassium or nitrogen. (3) *d*-Xylose and vitamin B_{12} absorption were not changed, but serum carotene was reduced. (4) No histologic changes from pretreatment were detected in peroral jejunal biopsies of 5 patients after 2–6 months of bile acid. When some patients claimed that bile acids reduced their appetite, we undertook a double blind comparison of deoxycholic acid and placebo for 1-wk intervals. 7 of 10 outpatients associated appetite suppression with the bile acid. A five-part Latin square double blind study was conducted with 5 obese patients on normal diets. Chenodeoxycholic acid was an appetite suppressant with respect to placebo and cholic acid (which were indistinguishable in this study). Hyodeoxycholic and lithocholic acids may have been anorexigenic in some patients. In conclusion: (1) Oral unconjugated bile acids did not produce steatorrhea. (2) Fecal sodium loss without potassium loss was observed consistently. (3) Chenodeoxycholic and deoxycholic acids in sufficient doses suppressed appetite in some patients. (4) No histologic changes in intestinal biopsies or abnormalities in blood chemistry were observed.

32. The Study of Evolution through Isozymes. GEORGE J. BREWER AND CHARLES F. SING,* Ann Arbor, Mich.

It has been estimated that the genetic material of man is present in 90-fold excess in relation to gene products. The role of excess DNA is one of the important issues in genetics. Our electrophoretic studies of 19 randomly selected human erythrocyte enzymes reveal that of the 16 showing at least one enzyme band, 10 revealed multiple isozymes unrelated to allelic variation. Much of this isozymic diversity probably has come about through gene duplication (including polyploid evolution). Since electrophoresis markedly underestimates protein multiplicity (assuming absence of extensive artifact formation), it appears that a significant amount of DNA is tied up in duplicate genes which diverge from one another through events such as mutation. The resulting protein diversity of nonsegregating isozyme type may facilitate increasing intraorganismal complexity both of ontogenetic and of tissue differentiation types. To test this hypothesis, isozyme studies of 13 enzymes have been performed with nine plant species of varying complexity (simple algae to complex flowering plants). A highly significant positive regression of the mean number of isozyme bands on taxonomically rated complexity indicates that complexity is characterized by an increase in enzyme diversity. The question has been raised whether most excess DNA is nonsense, i.e. is not expressed because it is not properly coded. Information on this has been obtained by study of a polyploid series of wheat. The mean number of isozyme bands for 10 enzyme systems was not significantly different in the polyploids as compared with each widely divergent diploid progenitor. This finding suggests that organisms can suppress, or at least not express, large quantities of operationally active and meaningful genetic material. Evolution may consist in large part of selective expression of small segments of this material, which itself, whether expressed or not, is gradually further evolved by mutation.

33. Diminished Baroreceptor Reflex Sensitivity in Hypertensive Patients. J. D. BRISTOW, P. SLEIGHT,* A. J. HONOUR,* H. S. SMYTH,* AND G. W. PICKERING,* Oxford, England, and Portland, Ore.

Aleration in arterial baroreceptor function has been proposed but not demonstrated in patients with raised pressures. In this investigation, baroreceptor function was tested in (*a*) 11 patients with hypertension whose average mean arterial pressure was 124 mm Hg (range 115–135); (*b*) 8 "normal" subjects, average mean pressure 87 mm Hg (range 72–100). Sudden intravenous injection of 0.2–1.5 μg of angiotensin produced transient rises of arterial pressure (AP). A linear relationship was found when the systolic pressures of successive

arterial pulses were plotted against the lengths of the succeeding cardiac cycles (RR intervals) during the pressor response. The slope of the line is a measure of baroreceptor sensitivity, expressed as milliseconds increase in cycle length per mm Hg rise in systolic AP. Steeper slopes indicate greater cardiac slowing per unit rise in pressure, hence greater reflex sensivity. The slope in those patients with elevated pressures was 2.4 ± 0.49 (mean $\pm$ SE) and in those with lower pressures was 12.60 ± 2.96 ($P < 0.01$). Three patients with high pressures had virtually no change in heart rate with increase in AP. The increment of AP produced by the angiotensin injections was comparable in the two groups, being 19% in the first and 15% in the second. When the results from the two groups were pooled, a significant negative correlation was seen between the control mean AP and the slope of the baroreceptor sensitivity line ($r = -0.64$, $P < 0.01$), demonstrating progressively less reflex reactivity with increasing levels of AP. The results show an abnormality of baroreceptor reflex function in essential hypertension, allowing further increases in AP with less than normal cardiac slowing.

34. Evaluation of the Fowler Single Breath Test and Two New Tests of the Distribution of Ventilation, the Time of Equilibrium and the Forced Equilibrating Expiration, in Normal Subjects and in Patients. ALFRED W. BRODY,* JAMES J. NAVIN,* AND RICHARD STOUGHTON,* Omaha, Nebr. (introduced by Robert P. Heaney).

In neurotic, weak, and paralyzed patients, when indemnity is involved, and when there is pain on respiration, there is a need for tests of pulmonary function independent of the degree of muscular effort exerted by the patient. Tests of diffusion and abnormalities of arterial gas tensions indicate dysfunction only in advanced obstructive dysfunction. This paper describes an evaluation of these tests of the distribution of ventilation as possible objective tests in early and moderate dysfunction. 115 normal subjects of both sexes and of age 6 to 76 were studied to obtain normal standards for the time to equilibrium (TTE) and the forced equilibrating expiration (FEE), and to explore norms for a modified Fowler Single Breath Test applicable when the 1400 to 1500 cc expiration required was more than the patient could furnish. The records were examined for all of the 125 patients receiving "complete" function tests immediately before June 1967 in our laboratory, and the tests of distribution were evaluated by comparison with the spirometric tests, other pulmonary function tests, and the clinical findings. The patients were classified clinically as primarily (1) obstructive lung disease, or (2) fibrotic, or (3) dysfunction due to space-occupying lesions, heart failure, or atalectesis, or (4) not lung disease. The Fowler test and its modifications are quite sensitive in detection of mild obstructive dysfunction; the new tests of distribution of ventilation (TTE and FEE) are helpful in moderate and more advanced obstructive dysfunction. In other types of parenchymal disease, the percentage of patients with distributional defects was much smaller and less dependent on degree of disability.

35. The Action of Thiopurines in Lymphocytes Lacking Hypoxanthine-Guanine Phosphoribosyl Transferase. ROBERT S. BROWN,* WILLIAM N. KELLEY,* J. EDWIN SEEGMILLER,** AND PAUL P. CARBONE,* Bethesda, Md. (introduced by A. V. N. Goodyer**).

The chemotherapeutic and immunosuppressive action of 6-mercaptopurine (6-MP) may be effected by feedback inhibition of purine biosynthesis by thioinosinic acid (TIMP). The conversion of 6-MP to the ribonucleotide TIMP requires the enzyme hypoxanthine-guanine phosphoribosyl transferase (HG-PRTase). 6-MP resistance in certain neoplastic cells has been associated with a decrease in this enzyme. Patients with the Lesch-Nyhan syndrome, a familial disorder characterized by choreoathetosis, self-mutilation, and excessive purine biosynthesis, lack HG-PRTase. Since their cells cannot convert 6-MP to TIMP, the effect of thiopurines upon their dividing lymphocytes was explored. Deoxynucleic acid synthesis in cultured lymphocytes transformed by phytohemagglutinin was assessed by incorporation of tritiated thymidine. Thymidine incorporation by normal lymphocytes was inhibited logarithmically by 6-MP at increasing concentrations from 0.05 to 100 μg/ml. Lymphocytes lacking HG-PRTase were resistant to any additional 6-MP inhibition at concentrations from 0.5 up to 100 μg/ml, with only partial inhibition of thymidine incorporation at lower concentrations of 6-MP. In contrast, these enzyme-deficient lymphocytes were exquisitely hypersensitive to inhibition by 6-methyl mercaptopurine riboside (6-MMPR), which does not require HG-PRTase for conversion to the ribonucleotide. 6-MMPR completely inhibited thymidine incorporation by enzyme-deficient lymphocytes at 0.05 μg/ml, whereas the effect of 6-MMPR on normal lymphocytes was no different from that of 6-MP. Both normal and enzyme-deficient lymphocytes were resistant to azathioprine inhibition at concentrations up to 50 μg/ml. None of the thiopurines affected the lymphoblastoid transformation morphologically, although fewer cells survived in the sensitive cultures. This model system suggests that conversion of 6-MP to the ribonucleotide TIMP by HG-PRTase is necessary for its action on normal lymphoblastoid cells at therapeutic concentrations. Tissue cultures of transformed lymphocytes provide a new means of investigating the action of immunosuppressive antimetabolites.

36. The Mechanism of Action of Testosterone in Rat Prostate. NICHOLAS BRUCHOVSKY * AND JEAN D. WILSON, Dallas, Texas.

Evidence from this and other laboratories indicates that testosterone is bound to the nuclear chromatin of target tissues, after which nuclear ribonucleic acid synthesis is accelerated. Moreover, we have recently reported that dihydrotestosterone is the only testosterone metabolite present in these nuclei, that dihydrotestosterone formation is present only in accessory sex tissue and

does not occur in nontarget tissue, and that the nuclear enzyme that performs this reduction is located in the chromatin. To characterize further the binding phenomenon, the intranuclear fate of 1,2^{3}H-testosterone has been studied both in prostates of intact rats and in in vitro preparations of rat prostate utilizing ultracentrifugation, gel filtration, and gas-liquid and thin-layer chromatographic techniques. Whereas less than 10% of the cytoplasmic hormone is bound to macromolecules, more than 50% of the intranuclear hormone is bound at all time intervals from 5 min to 2 hr. The intranuclear binding is predominantly to a protein associated with the chromatin and soluble in 0.6 M NaCl; it is stable to DNase and RNase incubation and destroyed by short-term incubation with Pronase. This binding is stable to Sephadex rechromatography at 4°C and to freezing Partial purification of the binding protein has been achieved on Sephadex G-200, indicating a large molecular weight. Furthermore, the bound form of the hormone is predominantly dihydrotestosterone, a more potent androgen than testosterone in some tissues. On the basis of these findings, the following hypothesis for testosterone action is suggested: Testosterone does not act directly on target tissues but requires reduction to dihydrotestosterone in order to induce its effect; the reduction is accomplished by intranuclear binding to a specific protein, after which the reductive step is carried out by a specific enzyme located in the chromatin.

37. Morphine Metabolism in Normal Subjects before and during Triiodothyronine-Induced Hypermetabolism. S. FRED BRUNK* AND WILLIAM R. WILSON, Iowa City, Iowa.

The effects of thyroid hormone on the metabolism of morphine are unclear. We studied the effects of triiodothyronine-induced hypermetabolism on the metabolism of morphine in man. Six normal subjects were studied during a control session (I) and during a hypermetabolic session (II) after receiving triiodothyronine, 400–500 μg/day for 2 wk. Triiodothyronine increased average oxygen consumption and sleeping pulse rate and decreased body weight and serum cholesterol significantly. Plasma and urine concentrations of free and conjugated morphine were determined in both sessions after intravenous morphine-(^{14}C-*N*-methyl) sulfate (5.75 mg/m^2). Plasma samples were obtained at 15, 30, 45, 60, 120, and 360 min after injection. Fractional urine samples were collected for 24 hr. The average plasma level of free morphine at 15 min was 62 mμg/ml in I and 42 in II ($P < 0.05$). An equilibrium plateau was reached at about 45 min, when the plasma concentration was 32 in I and 22 in II ($P < 0.05$). At 120 min the level was 19 in I and 12 in II ($P < 0.05$). The biological half-life of free morphine in plasma was 110 min in I and 85 min in II ($P < 0.05$). 24 hr urinary excretion of free morphine (percentage of administered dose) was 11.32 in I and 10.97 in II ($P > 0.05$). In both sessions 50% of the free morphine excreted within the 24 hr period was excreted within the first hour; 90% within 6 hr. The biological half-life of plasma conjugated morphine in the two sessions was not significantly different (210 min in I and 165 min in II). The concentration of conjugated morphine in the urine tended to be higher in the first hour in II than in I. The findings support the hypothesis that triiodothyronine accelerates the metabolism of morphine in man.

38. Acetoacetyl-CoA Deacylase: An Enzymatic Site of Action of the Hypocholesterolemic Agent Ethyl Chlorphenoxyisobutyrate (Clofibrate). ROBERT E. BURCH,* New York, N. Y. (introduced by George L. Curran**).

It is becoming evident that hydrolysis of acetoacetyl-CoA to free acetoacetate and coenzyme A is important in acetoacetate production. The reaction may also be important in cholesterol synthesis because any factor which enhances acetoacetyl-CoA deacylase activity can decrease the amount of acetoacetyl-CoA available for this biosynthetic process. The effect of ethyl chlorphenoxyisobutyrate (CPIB) on acetoacetyl-CoA deacylase was evaluated in the following manner: Mitochondria were isolated from the livers of male rats which had been fed a diet containing 0.2% CPIB for a minimum of 3 wk. Intact and sonicated mitochondria were preincubated with iodoacetamide to inhibit both β-ketothiolase and β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) condensing enzyme. The mitochondria were incubated with purified acetoacetyl-CoA. The amount of acetoacetyl-CoA disappearing, which corresponded almost exactly to the amount of free acetoacetate formed, was determined with β-hydroxyacyl-CoA dehydrogenase. All determinations were done in triplicate and the results averaged. Synthesis of cholesterol from 1-^{14}C-acetate was determined in liver slices obtained from the same livers in which deacylase activity was measured. Acetoacetyl-CoA deacylase activity was increased 22% in intact and 49% in sonicated mitochondria from CPIB-treated rats as compared with normal animals. The specific activity of cholesterol synthesized by liver slices from CPIB-treated animals was decreased as compared with normal. One site of action of CPIB in the control of cholesterol biosynthesis has been proposed in the series of reactions between acetate and mevalonate—HMG-CoA reduction to mevalonate is said to be decreased. The data presented here suggest another possible mechanism related to CPIB administration, namely, increased acetoacetyl-CoA deacylase activity which increases free acetoacetate production and simultaneously decreases cholesterol synthesis by removing acetoacetyl-CoA as an available substrate. Such a control mechanism in cholesterol biosynthesis is unique in that it is associated with increased rather than decreased activity of an enzyme.

39. Control of Fluid Absorption in the Renal Proximal Tubule. MAURICE B. BURG AND JACK ORLOFF,** Bethesda, Md.

Glomerulotubular balance was investigated in isolated perfused rabbit proximal tubules in vitro in order to evaluate some of the mechanisms proposed to account for the proportionate relationship between glomerular filtra-

tion rate and fluid absorption generally observed in vivo. The rate of fluid transport from lumen to bath in proximal convoluted tubules in vitro was approximately equal to the estimated normal rate in vivo. The absorption rate in proximal straight tubules, however, was approximately one-half as great. If the mechanism responsible for the maintenance of glomerulotubular balance is intrinsic to the proximal tubule, as has been proposed on the basis of micropuncture studies, the rate of fluid absorption in vitro should be directly related to the perfusion rate and/or tubule volume. In the present studies absorption rate was only minimally affected when perfusion rate was increased or the tubule distended. Thus, glomerulotubular balance is not mediated by changes in velocity of flow of the tubular fluid or tubular diameter and therefore is not an intrinsic property of the proximal tubule. It has also been proposed that glomerulotubular balance results from a humoral feedback mechanism in which angiotensin directly inhibits fluid absorption by the proximal convoluted tubule. In the present experiments, angiotensin was found to have no significant effect on absorption rate.

40. Thyroxine Biosynthesis. HANS BURGI,* KLAUS F. R. SCHILLER,* AND FRANK F. RICHARDS,* Boston, Mass. (introduced by Farahe Maloof**).

Thyroxine (T4) is thought to be synthesized on thyroglobulin by coupling of two molecules of the peptide-linked intermediate 3,5-diiodotyrosine (DIT). Previous in vitro isotope incorporation experiments to test this hypothesis have been inconclusive. Rat thyroids were incubated for 24 hr with ^{131}I and ^{3}H-tyrosine under conditions in which good incorporation of ^{3}H-tyrosine into thyroglobulin and of ^{131}I into T4 could be demonstrated. Monoiodotyrosine (MIT), DIT, and T4 were isolated from soluble thyroidal proteins freed from low molecular weight substances by dialysis. The usual chromatographic separation methods for iodoamino acids proved unsatisfactory owing to the persistence of radioactive contaminants. Consequently, crystallizations to constant specific radioactivity in the presence of carrier were subsequently performed for each iodoamino acid. The corrected $^{131}I/^{3}H$ ratios of the rigorously purified iodoamino acids were 0.05:1 for T4, 3.2:1 for MIT, and 3.6:1 for DIT. The 60-fold difference in ratio between T4 and both MIT and DIT means that under the conditions of the experiment the peptide-linked MIT and DIT residues from soluble thyroidal proteins could not have been the sole precursors of T4, and that the hypothesis that T4 is formed from two peptide-linked DIT residues in protein is unlikely. The evidence favors the alternate view that a peptide-linked DIT residue couples with a low molecular weight intermediate which is relatively richer in ^{3}H than in ^{131}I.

41. Membrane Control of Events during Erythroid Cell Maturation. EDWARD R. BURKA,* Philadelphia, Pa. (introduced by Franklin R. Miller**).

Maturation of the mammalian erythroid cell is associated with changes in cell RNA content and rate of protein synthesis. Erythroid cell RNA is distributed between two compartments, free (78%) and membrane-bound (22%), which differ in rates of turnover. In order to gain understanding of factors controlling cell maturation, RNA metabolism and nuclease activity in the compartments were studied in intact rabbit reticulocytes, whole lysates, and isolated cell components. During in vitro maturation the rate of total RNA degradation in intact cells was $3.9 \pm 2.5\%$/hr. Comparable rates ($3.4 \pm 1.7\%$/hr) were seen during incubation of native or reconstituted whole hemolysates. In intact cells the rate of disappearance of bound RNA ($8.9 \pm 1.0\%$/hr) was twice that of free RNA ($3.2 \pm 1.6\%$/hr), resulting in a decreasing proportion of bound RNA with maturation. In whole hemolysates net RNA disappearance was confined to the membrane compartment. Size of the free RNA compartment remained constant, although studies with ^{32}P-RNA indicated destruction of free RNA, suggesting conversion of bound to free RNA. Reconstituted hemolysates, containing ^{32}P-labeled and unlabeled components, confirmed net flux from bound to free RNA, with some exchange in both directions. Isolated membranes lost RNA at rates similar to those of membranes in whole cells ($8.9 \pm 6.5\%$/hr). The loss represented approximately equal proportions of degradation due to nuclease activity and detachment of RNA from the membrane. Membrane-free hemolysates in themselves had little or no nuclease activity. The data indicate that the predominant site of nuclease activity in the erythroid cell is the cell membrane. The mechanism of RNA degradation in the cell appears to include exchange between free and bound RNA, with net flux from bound to free compartments, and continual degradation of free RNA as it becomes membrane bound. The cell membrane plays a major role in moderating RNA degradation, and thus capacity for protein synthesis, in the maturing erythroid element.

42. Effects of Cyclic AMP and Dibutyryl-Cyclic AMP on Basal and Stimulated Glucose Oxidation and Phospholipogenesis in Thyroid Slices. GERALD BURKE,* Chicago, Ill. (introduced by Eric Reiss).

Evidence has been presented that the effects of thyrotropin (TSH) and long-acting thyroid stimulator (LATS) on thyroid iodine uptake and discharge may be mediated by cyclic adenosine-3′,5′-monophosphate (C-AMP). Since both TSH and LATS stimulate thyroidal glucose oxidation and phospholipogenesis, we studied the effects of C-AMP and N^6-2′-*O*-dibutyryl-adenosine-3′,5′-monophosphate (DBC) on $^{14}CO_2$ production from 1-^{14}C-glucose and ^{32}P incorporation into phospholipids in sheep and dog thyroid slices. C-AMP (100–500 μg/ml) failed to increase 1-^{14}C-glucose oxidation, but DBC at 150–250 μg/ml was active (mean increase = 20%). C-AMP at 250–500 μg/ml had a variable stimulatory effect on phospholipogenesis (5 of 8 experiments, mean increase = 16%); DBC at 50–150 μg/ml consistently stimulated ^{32}P incorporation into phospholipids (8 of 8 experiments, mean = 55%); lower or higher DBC concentrations were inactive, and the maximal response was obtained at 50

μg/ml. The greater potency of DBC is presumed to be due to the greater facility with which it enters the thyroid cell and its resistance to enzymatic hydrolysis. However, neither C-AMP nor DBC was shown to potentiate TSH or LATS effects on glucose oxidation and phospholipogenesis in sheep thyroid slices. Indeed, DBC at 50 μg/ml lowered TSM- and LATS-stimulated ^{32}P incorporation into phospholipids (mean 15% and 19% reduction, respectively), although the doses of TSH (10 mU/ml) and LATS (0.5 MRC mU/ml) employed were well below maximal. Measurements of intrathyroidal pyridine nucleotide cofactors under these circumstances reveal increased NADP and NADPH levels and suggest that the failure of C-AMP and DBC to potentiate TSH or LATS in vitro may reflect a cofactor-inhibition phenomenon.

43. Deterioration of Ventricular Function during Angiocardiography. RICHARD A. CARLETON* AND JAMES G. CLARK,* Chicago, Ill. (introduced by John S. Graettinger**).

Measurements of left ventricular (LV) dimensions for clinical investigation must be both precise and innocuous. Angiocardiography has been commonly used to measure LV volume. This report presents measurements of LV function during angiocardiography. For these, a catheter-tip ultrasonic transducer, advanced through a femoral vein into the cleft between the right ventricular wall and the ventricular septum, was used to measure LV diameter. We have shown that such measurements agree within ± 1.6 mm (SD) with simultaneous radiographic measurements. 10 anesthetized dogs (15–24 kg) had constant atrial pacing (162 ± 3.9 beats/min) during aortic balloon catheter inflation and deflation, and 1 ml/kg LV injections (1.5 sec; 37°C) of 80% sodium iothalamate, of 70% sodium-methylglucamine diatrizoate, and, to establish the effects of injection alone, of the isotonic saline. Tension was calculated from LV systolic pressure and radius; end diastolic (EDD) minus end systolic diameter (ESD) ÷ ejection time provided mean shortening velocity. Measurements before, during, and after balloon inflation established each tension-velocity curve. EDD changed from a mean of 66.8 by 1.6 ± SE 0.2 mm, ESD from 61.6 by 2.1 ± 0.2 mm, wall tension from 36.1 by $7.9 \pm 0.8 \times 10^5$ dynes/cm, and velocity from 40.7 by −7.8 ± 2.5 mm/sec during inflation. Deflation produced opposite changes. All injections produced comparable changes through 1.1 sec: EDD 1.6 ± 0.5 mm, ESD 2.0 ± 0.8 mm, LV pressure 12.2 ± 1.8 mm Hg, wall tension $3.5 \pm 0.4 \times 10^5$ dynes/cm, and velocity −6.6 ± 1.6 mm/sec. Control values were reached 2.6 ± 0.13 sec after saline. Progressive deterioration occurred after contrast injections. At 1.8, 2.6, 3.3, and 4.1 sec respectively, LV pressure fell by 3.5 ± 1.6, −3.8 ± 1.6, −10.5 ± 0.9, and −16.5 ± 0.9 mm Hg; EDD remained large by 1.1 ± 0.2, 0.8 ± 0.3, 1.3 ± 0.4, and 1.4 ± 0.4 mm; velocity fell by −8.9 ± 1.7, −9.2 ± 2.1, −10.6 ± 2.6, and −10.4 ± 2.8 mm/sec; and tension fell by 1.6 ± 0.3, −0.1 ± 0.2, −1.2 ± 0.1, and $-2.4 \pm 0.3 \times 10^5$ dynes/cm. Injection of hyperosmolal angiocardiographic agents immediately alters LV diameter, as measured with a catheter-tip ultrasonic transducer. Depression of LV contractility follows within 2 sec.

44. Mechanisms of Staphylococcal Enterotoxin Fever. FRANK A. CAROZZA, JR.,* Baltimore, Md. (introduced by Richard B. Hornick).

Staphylococcal enterotoxin B is a highly purified protein possessing pyrogenic and other physiologic properties similar to those of bacterial endotoxin. The present studies were initiated to determine whether the basic mechanisms of action of enterotoxin and endotoxin might be similar. After intravenous injection into rabbits, enterotoxin produced fever peaking at 6–7 hr associated with a circulating endogenous pyrogen. Upon daily intravenous injection, increasing tolerance developed, which was transitory (lost after 9 days rest) and appeared specific, since enterotoxin-refractory animals reacted normally to endotoxin and endogenous pyrogen. Refractory animals exhibited no antibody to enterotoxin either by serologic testing or by passive protection with plasma. Reticuloendothelial system blockade rendered both normal and tolerant animals more sensitive to enterotoxin, but the major portion of tolerance was retained, indicating that early tolerance was not dependent upon generalized reticuloendothelial system hyperactivity. Intradermal enterotoxin in normal rabbits produced delayed erythematous lesions histologically similar to classical delayed hypersensitivity reactions. After four single daily intravenous injections of enterotoxin, when pyrogenic tolerance was maximal, skin reactivity diminished 10-fold, suggesting that early pyrogenic tolerance was associated with a desensitization to this antigen. Moreover, pretreatment of pyrogenically tolerant animals with 100 ml normal rabbit plasma did not restore reactivity, indicating that pyrogenic sensitivity to this antigen is not mediated by circulating natural antibody. Finally, a second probably late mechanism of enterotoxin tolerance could be demonstrated, since hyperimmune serum containing high levels of antibody transferred strong pyrogenic tolerance. These data are compatible with the thesis that staphylococcal enterotoxin pyrogenicity, like that of bacterial endotoxin, is mediated by hypersensitivity reactions, and that two mechanisms are concerned with tolerance, i.e. an early transient desensitization at the cellular level and a later antibody-dependent process.

45. The Effect of Blood Flow and Metabolic Inhibitors on Electrolyte Loss in Canine Cholera. C. C. J. CARPENTER* AND W. B. GREENOUGH,* Baltimore, Md. (introduced by P. S. Norman).

In experiments designed to investigate the mechanism of electrolyte loss in cholera, the electrolyte loss induced by cholera exotoxin was studied in 64 chronic canine Thiry-Vella loops. After intraluminal challenge with exotoxin adequate to elicit a maximum response, fluid output by duodenum was 16 ± 3 ml/cm length (mean ± SD), by jejunum 13 ± 2, and by ileum 6 ± 2. Bicarbonate concentration of loop fluid was 14 ± 6 mEq/liter (mean ± SD) in duodenum, 27 ± 4 in jejunum, and 75 ± 6 in

ileum. Mean time intervals between exotoxin administration and detectable fluid loss were <15 min for duodenum, 88 min for jejunum, and 150 min for ileum. In loops perfused by isotonic electrolyte solution at 15 ml/kg per hr, enhancement of sodium absorption by intraluminal glucose was not altered by administration of exotoxin. In 11 chronic studies in dogs with electromagnetic flowmeters around the base of the superior mesenteric arteries (SMA), SMA blood flow uniformly decreased coincidentally with exotoxin-induced fluid loss. In 6 acute studies, exotoxin-induced gut fluid output was not decreased when SMA pressure was reduced to <40% of control levels by tightening a snare placed around the base of the SMA; constant fluid output was demonstrated at mean SMA perfusion pressures of 20–30 mm Hg for 90 min. In separate experiments, intravenous acetazolamide (500 mg/kg, 3 hr after challenge), actinomycin D (0.3 mg/kg, 3 hr before challenge), and ouabain (0.3 mg/kg, 3 hr after challenge) caused no decrease in fluid production by jejunal loops. These studies suggest that the mechanism of electrolyte loss is an active movement of solute from blood to gut lumen; this movement cannot be explained by filtration, since SMA blood flow and pressure are not critical variables. The process is not inhibited by relatively large quantities of the metabolic inhibitors studied.

46. A Mechanism for Inhibition of Solute-Free Water Reabsorption (T^cH_2O) by a Prostaglandin. ALBERT A. CARR* AND ARTHUR L. HUMPHRIES,* Augusta, Ga. (introduced by Alfred Jay Bollet).

Peripheral vein infusion of a prostaglandin (PGA_1, Upjohn) 0.95–1.5 μg/kg per min in hydropenic dogs during mannitol diuresis inhibited T^cH_2O. Sodium-retaining steroid and antidiuretic hormone secretion variables were controlled by supramaximal infusions of deoxycorticosterone and vasopressin. The significant inhibition of T^cH_2O was in association with a significant decrease in femoral artery blood pressure and a significant rise in renal plasma flow (RPF) as measured by clearance of sodium aminohippurate. There was an insignificant drop in glomerular filtration rate (GFR) as measured by inulin clearance. There was a small drop in sodium and potassium excretion. Direct renal artery infusion of PGA_1, 0.7–16 μg/min, which did not lower femoral artery blood pressure, resulted in an inhibition of T^cH_2O but insignificant changes in GFR and sodium excretion. There was a rise in RPF in each experiment, but over all, the changes were insignificant. At the highest renal artery infusion rate of PGA_1, 16 μg/min, there was a dramatic increase in RPF and a rise in sodium excretion also. The renal papillary sodium concentration was used as a measure of medullary-papillary tonicity. It was found to be decreased in the PGA_1-infused kidney as compared with the contralateral kidney. Thus PGA_1 caused a decrease in medullary-papillary hypertonicity and thus inhibition of T^cH_2O. There is no evidence that PGA_1 in these concentrations can inhibit supramaximal amounts of antidiuretic hormone. The exact mechanism by which PGA_1 caused a decrease in medullary-papillary tonicity is unknown. One is tempted to conclude that changes in medullary-papillary blood flow resulted in either dilution or washout of the area. A decrease in delivery of sodium to the ascending loop of Henle or inhibition of sodium transport at that site could give the same results. Studies in progress relating to the effect of PGA_1 on free water clearance will help resolve the answer as to effect on the ascending loop of Henle.

47. Studies on the Mechanism of Glucose Transport and Insulin Effect in Adipose Cells Utilizing Sulfhydryl Blockade. JAMES R. CARTER, JR.,* AND DONALD B. MARTIN, Boston, Mass.

Both basal and insulin-stimulated glucose transport have been shown to be inhibited by sulfhydryl binding agents in a number of intact tissue preparations. As an approach to the mechanism of glucose transport, we have examined the inhibition by *N*-ethylmaleimide (NEM) of glucose transport in isolated adipose cells from rat epididymal pads. Fat cells were first exposed to NEM, then washed free of inhibitor and assayed for conversion of ^{14}C-glucose to $^{14}CO_2$ in the presence (insulin stimulated) and absence (basal) of 1 mU/ml insulin. Exposure to 10^{-4} M NEM for 15 min resulted in a decrease of 84% in basal CO_2 production and a complete loss of insulin stimulation. Homogenates prepared from inhibited cells and fortified with ATP and TPN showed no reduction in ability to metabolize ^{14}C-glucose, thus localizing the inhibition in the intact cell to the level of membrane transport. Kinetic studies utilizing either different concentrations of NEM for a given time, or one level of inhibitor for different times, showed three separate parts of the NEM effect: (1) an initial increase in basal transport with no change in insulin effect; (2) after this, a rapid decrease in insulin-stimulated CO_2 production with no further change in basal transport; (3) finally, steady decrease in basal transport occurring after complete inhibition of the insulin effect. We interpret this to mean that at least two distinct sulfhydryl groups are involved. The first, readily accessible to NEM, is directly associated with the effect of insulin on membrane, and blockade prevents insulin action without inhibiting glucose transport per se. The second, less "available" to inhibitor, is directly involved in the transport process itself and may be part of a "carrier" molecule. The observation of a transient increase in basal glucose transport before inhibition remains unexplained.

48. Serum Arginase Activity as a Sensitive and Specific Test for Liver Disease. NICOLA CARULLI,* SHIGEKOTO KAIHARA,* AND HENRY N. WAGNER, JR., Baltimore, Md.

Assay of serum enzymes is useful in the diagnosis of various diseases, but the lack of organ specificity of these enzymes has been a disadvantage. Arginase is known to be present almost exclusively in the liver, a fact which suggested that it might be useful for the specific diagnosis

of liver disease. Therefore, we developed a new, sensitive method of serum arginase assay and evaluated its role as a test of liver disease. Arginine-guanido-^{14}C (0.227 M, SA 0.625/mmole; pH 9.5) was incubated with Mn^{++}-activated serum for 1 hr at 37°C; the ^{14}C-urea produced was hydrolyzed by urease into ammonia and $^{14}CO_2$, which was measured by a liquid scintillation counter. Arginase activity was expressed as μmoles of urea produced in 1 hr by 1 ml of serum. This method is very sensitive (lower limit of detection, 0.05 μmole) and is not affected by the urea present in serum. Serum arginase activity was 0.24 ± 0.07 (1 SD) μmole/hr in 25 normal subjects. In 14 patients with infectious hepatitis, serum arginase ranged from 0.6 to 6.3 μmole/hr, and the values correlated well with transaminase values. In 23 patients with cirrhosis, serum arginase ranged from 0.1 to 1.05 μmole/hr, with poor correlation with SGOT. In 7 patients with metastatic cancer of the liver, serum arginase levels ranged from 0.6 to 6.5 μmole/hr, but SGOT was less affected. In 10 patients with myocardial infarction, serum arginase was only slightly elevated in spite of increased SGOT. In 5 patients with typhoid fever without signs of liver involvement, serum arginase was elevated although SGOT levels were not. From these results, we conclude that serum arginase is a sensitive as well as a specific test for liver disease.

49. Response of Plasma Renin Activity to Furosemide in Hypertension. BERTRAM J. CHANNICK,* E. VICTOR ADLIN,* AND ALAN D. MARKS,* Philadelphia, Pa. (introduced by Alan J. Johnson**).

A significant proportion of hypertensive patients do not respond with an increase in plasma renin activity to measures designed to diminish extracellular fluid volume. Plasma renin activity was determined in 100 hypertensive patients 4 hr after a single oral dose of 80 mg of furosemide. 38% of this group did not have a rise in plasma renin activity above 100 ng/100 ml, the lowest level observed in 20 normals after furosemide. 88% of the patients with suppressed plasma renin activity after furosemide also had low renin levels on a low sodium diet. The plasma renin activity after furosemide correctly predicted the results of salt restriction in 80% of all the patients tested by both maneuvers. The aldosterone excretion rate was elevated in 11% of the patients with suppressed plasma renin activity. In three hypertensive patients with hypokalemia, suppressed plasma renin activity, and normal aldosterone excretion rates, adrenal adenomas were removed. Postoperatively, plasma renin activity responded to furosemide, serum potassium became normal, and there was improvement in the hypertension. It appears that a proportion of hypertensive patients with suppressed plasma renin activity have excess mineralocorticoid secretion from an adrenocortical adenoma despite normal aldosterone excretion rates. Furosemide seems to be a satisfactory alternative to a low sodium intake for the screening of hypertensive patients for suppressed plasma renin activity.

50. Red Cell Aldolase Deficiency in Hereditary Spherocytosis. ROBERT G. CHAPMAN,* Denver, Colo. (introduced by Matthew Block**).

Erythrocyte aldolase activity decreases with cell aging, a process approximately described by equation (1): $A(t) = 23.7\ e^{-0.036t}$, where A = aldolase activity in nanounits per cell, t = time in days. Aldolase activity was measured by Beck's modification of the method of Sibley and Lehninger in 18 members of seven unrelated families with typical hereditary spherocytosis. 16 of these showed excellent clinical response to splenectomy 2 or more years earlier. The remaining 2 were untreated. Red cell survival was measured with intravenous $DF^{32}P$ in the 2 nonsplenectomized and 11 of the splenectomized patients, six families being represented. Erythrocyte aldolase activity was 4.60 ± 0.39 nanounits per cell in the 16 splenectomized patients, significantly less than the 5.38 ± 0.71 value in 23 normal persons ($P < 0.005$). In the 2 nonsplenectomized patients, values were 4.69 and 5.63. Red cell survival was 40 and 60 days in the nonsplenectomized and 96 ± 13 days (range 76–118) in the splenectomized patients, as compared with 123 ± 14 days in 12 subjects with normal red cells. The normal erythrocyte aldolase activity, $\bar{A}(T)$, for a person with a uniform red cell life span of T days is given by equation (2): $\bar{A}(T) = 23.7/T\int_D^T e^{-0.036t}dt$. Erythrocyte aldolase activity was 37–72% of the activity predicted by equation (2). The magnitude of this reduction is comparable to those for deficiencies observed in heterozygotes for inherited red cell abnormalities such as sickle hemoglobinopathy and pyruvate kinase deficiency. This suggests that deficiency in aldolase activity is the basic biochemical lesion in hereditary spherocytosis, a disease appearing in the presumed heterozygote. In addition, the results show that red cell survival remains significantly subnormal in hereditary spherocytosis after splenectomy ($P < 0.0005$), contrary to the usual assumption that it becomes normal.

51. "Hereditary Persistence of Fetal" Red Cells. SAMUEL CHARACHE,* JOHN J. SCHRUEFER,* AND WILMA B. BIAS,* Baltimore, Md. (introduced by C. Lockard Conley**).

An apparently healthy 8 yr old boy whose red cells contain exclusively Hb F (fetal hemoglobin) is homozygous for "hereditary persistence of fetal hemoglobin." The anomaly, previously thought to involve only hemoglobin synthesis, has been attributed to deletion of structural genes for the β and δ polypeptide chains of hemoglobin, or to suppression of these genes by mutation of an operator gene controlling both loci. The child has maintained a hematocrit value of 44–46%, above the normal range for his age. Oxygen affinity of his blood was increased (P_{50O_2} 16 mm Hg; normal adult 26 mm Hg, pH 7.40, 37°C) to a degree similar to that of normal umbilical cord blood. The high O_2 affinity cannot be ascribed to the high level of Hb F, since the oxygen affinity of solutions of Hb F is the same as that of Hb A. The blood of the patient's mother, containing 70% Hb F, had a normal adult oxygen affinity. The child's red cells,

and those of his heterozygous mother and sister, contained almost as much "i" antigen as fetal red cells, and approximately 5 times as much as normal adult cells. Red cells of these individuals previously have been shown to contain increased amounts of the fetal type 2 isozyme of hexokinase. Normal adult values for red cell carbonic anhydrase and for serum ceruloplasmin and γA and γM globulin were obtained in the child and his mother. The concentration of 2,3-diphosphoglycerate in their red cells was normal. Erythrocytes of persons with "hereditary persistence of Hb F" appear to have constituents, apart from the hemoglobin, which are similar to those of the fetus. The pleiotropic effects of this mutation, which appears to limit the acquisition of certain adult characteristics by fetal red cells, cannot be explained by either of the genetic hypotheses considered previously.

52. Changing Spectrum of Immune Pathology in Man. PATRICIA CHARACHE,* JERRY A. WINKLESTEIN,* DIANNE N. ABUELO,* JOHN D. JOHNSON,* AND JOHN M. NEFF,* Baltimore, Md. (introduced by Robert E. Cooke**).

Immune deficiency diseases have been classified, and a specific cellular defect has been hypothesized for each. New patterns which fail to conform to defined categories have been observed. A 4 yr old white male had type I dsygammaglobulinemia: hepatosplenomegaly, elevated γM globulin, depressed γG globulin, plasma cells in bone marrow and lymph node. Over 4 yr, γM titers gradually fell to zero, and anti-*E. coli* antibody and plasma cells disappeared, shifting his classification to "agammaglobulinemia." Thus classification at a single point in time can be misleading; dysgammaglobulinemia and agammaglobulinemia may be etiologically interrelated. A 1 yr old white male developed type 9 pneumococcal septicemia and meningitis, followed by two separate episodes of type 9 septicemia. Immunoglobulins were quantitatively within normal limits for his age (γG 750 mg/100 ml), diphtheria and anti-blood group A antibodies were present, plasma cells were numerous in bone marrow, node structure was normal. No type 9 antibodies were produced in over 3 months. Thus a pattern resembling pneumococcal immune paralysis was seen clinically, suggesting either impending agammaglobulinemia or a complication of overwhelming sepsis. Excluding age, a 5 yr old white male (without telangectasia) fulfilled criteria for athymic (Swiss type) agammaglobulinemia: lymphopenia, γG <16 mg/100 ml, absent delayed sensitivity to standard antigens, DNFB, and transfer factor. Rectal, marrow, and node biopsies were depleted of plasma cells and small lymphocytes. Explosively progressive reticulum cell sarcoma developed 6 months after presentation. Limited postmortem examination showed no thymus in the lower neck, chest, or pericardium. The association between reticulum cell sarcoma, ataxic telangectasia, and lymphopenic agammaglobulinemia must be reassessed; lymphopenic agammaglobulinemic pattern can be present in acquired form. New patterns of immune deficiency states which fail to conform to defined categories are of clinical and theoretical importance, and suggest that rigid classification of these disorders is premature.

53. Metabolic Abnormality in Pseudohypoparathyroidism: Defective Renal Excretion of Cyclic 3′,5′-AMP in Response to Parathyroid Hormone. LEWIS R. CHASE,* G. LELAND MELSON,* AND G. D. AURBACH, Bethesda, Md.

Our earlier reports showed that parathyroid hormone (PTH) activates adenyl cyclase in the renal cortex, thereby causing a marked increase in the urinary excretion of 3′,5′-AMP. In this study, we measured changes in excretion of 3′,5′-AMP after rapidly infusing 300 units of purified PTH into normal volunteers (NV) and idiopathic hypoparathyroid (IHP), surgical hypoparathyroid (SHP), and pseudohypoparathyroid (PHP) patients. Basal excretion of 3′,5′-AMP in eight NV's was 2.84 ± 0.24 (mean ± SE) nmoles/mg creatinine per min (range 0.75–8.25); results were not significantly different in five patients with SHP, one patient with IHP, and five patients with PHP. In the 30 min period after PTH infusion, excretion of 3′,5′-AMP increased to 68.0 ± 10.2, 56.8 ± 12.9, and 149.0 nmoles/mg creatinine per min in the NV, SHP, and IHP patients, respectively. An infusion of vehicle without PTH was without effect on excretion of 3′,5′-AMP. PTH caused no increase in 3′,5′-AMP excretion in four of the patients with PHP; excretion in one increased from 4.4 to 13.1 nmoles/mg creatinine per min. This deficient response to PTH clearly separated the PHP patients from all others tested, in contrast to the gross overlap of results with measurements of phosphate clearance. Infusion of calcium into PHP patients in order to suppress endogenous secretion of PTH did not alter the defective response to exogenous hormone. These results show that (1) PTH causes a marked increase in 3′,5′-AMP excretion in NV, SHP, and IHP patients; (2) the test for urinary 3′,5′-AMP after PTH is a better diagnostic index of PHP than measurements of phosphate clearance; (3) the apparent renal defect in PHP is better explained by deficient or unresponsive adenyl cyclase than by secretion of an aberrant form of the hormone that blocks the renal receptor sites.

54. The Role of the Spleen in Oxygen Storage during Asphyxia. N. S. CHERNIACK,* N. H. EDELMAN,* G. S. LONGOBARDO,* AND A. P. FISHMAN,** Chicago, Ill.

When breathing stops, the body uses its stores of oxygen to meet its metabolic needs. The known stores are in the form of gas in the lungs, oxyhemoglobin in the blood, and dissolved oxygen in the tissues. Theoretically, the extent and availability of these stores during arrested breathing is reflected in serial changes in blood gas tensions. But experimental evaluation in nine asphyxiated dogs failed to account for the observed change in blood gas tensions. In looking for an additional source of oxygen, splenic arterial and venous blood flow were continuously monitored with electromagnetic flowmeters in six dogs during asphyxia; gas contents and tensions in splenic arterial and venous blood were determined peri-

odically. It was observed that during asphyxia contraction of the spleen on the average doubled the splenic venous outflow, whereas splenic arterial inflow decreased by 10%. Accompanying the reduction in the blood volume of the spleen was a reversal of the O_2 difference across the spleen, so that by 3 min of asphyxia, the average splenic venous O_2 content exceeded the arterial by 10.5%; the splenic venous hematocrit increased simultaneously by 25%; and the splenic venous Po_2 exceeded the arterial by 15 mm Hg. CO_2 gradients also reversed, and by 3 min of asphyxia, the splenic venous Pco_2 was 14 mm Hg less, and the CO_2 content 8 volumes per cent less, than in the artery. The experimental results were tested in a multicompartment model of gas stores. When the splenic contribution to the gas stores was taken into account, the model accounted for the serial changes in arterial oxygen tensions observed during asphyxia.

55. The Effect of Altered Rates of Erythropoiesis upon Granulocytopoiesis. P. A. CHERVENICK* AND D. R. BOGGS, New Brunswick, N. J.

Different opinions have been expressed regarding the kinetics of pluripotential stem cell differentiation into granulocytes, erythrocytes, and megakaryocytes. Till and coworkers suggested that differentiation is a random process, whereas studies by Hellman suggested that selective differentiation may occur. Erythropoiesis was altered by bleeding or hypertransfusing mice before exposing them to whole-body irradiation. 10 days later the number of granulocytes in the humerus was calculated from the total number of nucleated cells and the percentage staining with peroxidase. Bleeding before irradiation resulted in a marked increase in erythropoiesis at 10 days, measured by an elevated hematocrit, increase in number of spleen colonies, and radioactive iron uptake. Although erythropoiesis was increased, the number of granulocytes in the humerus was decreased (1.43×10^6) as compared with irradiated controls (2.04×10^6) ($P < 0.001$). Hypertransfused mice showed no evidence of erythropoiesis in 10 days after irradiation. However, in these animals, marrow granulocytes were increased to 4.6×10^6 as compared with 2.8×10^6 in controls ($P < 0.05$). The injection of nonspecific stimuli may alter the marrow granulocyte reserve. The possibility that hypertransfusion was acting through such a nonspecific mechanism was studied in nonirradiated mice. 6 hr after hypertransfusion, a slight but statistically insignificant decrease was noted in marrow granulocytes. 1 wk later there was a slight but significant increase to 4.29×10^6 as compared with 3.69×10^6 in normal controls ($P < 0.05$). These data suggest that selective differentiation of hematopoietic stem cells may occur. It seems quite likely that under normal conditions, adequate numbers of stem cells are present to permit an accelerated rate of differentiation into various cell lines simultaneously. In situations where stem cells are reduced, a preferred pathway of differentiation determined by demand for a specific cell type may be in operation. These observations suggest a possible means of protecting hematopoietic tissue from the undesirable effects of cytotoxic agents.

56. Intracellular Calcium and Myocardial Contractility: Effects of Positive Inotropic Stimuli. C. A. CHIDSEY AND Y. UEBA,* Denver, Colo.

To examine the hypothesis that alterations in intracellular calcium (Ca) are involved in the regulation of myocardial contractility, we studied the effect of several positive inotropic stimuli on the Ca content of isolated rabbit hearts. When the contractile state was varied by altering perfusate Ca from 0.6 to 9.0 mM, total Ca (Ca_T) and mitochondrial Ca (Ca_{MT}) increased respectively from 0.9 ± 0.02 to 10.8 ± 3.2 μmoles/ ventricle and 10.0 ± 1.1 to 167.1 ± 37.9 μmoles/g protein, whereas at a perfusate Ca of 2.3 mM nearly maximal values were observed for heavy microsomal Ca (Ca_{MS}), 27.0 ± 1.4 μmoles/g protein, and for contractile force. Temperature reduction from 37° to 20°C markedly increased contractile force as well as total and fractional Ca contents of hearts perfused with 0.6 mM Ca. Ouabain administration (6 μg/min) significantly augmented contractile force ($85 \pm 22\%$) in hearts perfused with 0.6 mM Ca at 37°C and increased Ca_T (to 1.1 ± 0.1), Ca_{MT} (to 13.6 ± 0.07), and Ca_{MS} (16.6 ± 1.0 to 21.6 ± 1.2), $P < 0.05$. At higher levels of ouabain (12 and 18 μg/min) a fall in contractile force occurred, with a further elevation of Ca_T (1.4 ± 0.1 and 1.5 ± 0.1) and Ca_{MT} (18.3 ± 1.4 and 24.4 ± 1.9), but with a fall of Ca_{MS} (18.1 ± 0.7 and 18.7 ± 1.4), $P < 0.05$. In hearts perfused with 1.1 mM Ca, ouabain resulted in a smaller increase of contractility, no change in Ca_T and Ca_{MS}, and a small but dose-related increment in Ca_{MT}. Stimulation by norepinephrine (1 μg/min) of hearts perfused with 0.6 mM Ca also resulted in significant augmentation of Ca: Ca_T to 1.5 ± 0.17 μmoles/g; Ca_{MT} to 22.2 ± 2.8 μmoles/g protein; and Ca_{MS} to 29.9 ± 2.9 μmoles/g protein, $P < 0.01$. To eliminate oxygen deficiency resulting from stimulation as a cause for these changes, tachycardia, produced by electrical pacing, and hypoxia, produced by Po_2 reduction from 590 to 150 mm Hg, were shown to have only a minor effect on intracellular Ca. These observations indicate that a number of positive inotropic stimuli facilitate accumulation of intracellular calcium, resulting in a measurable increase of this cation when extracellular concentration is maintained at a low level. We conclude that alterations of the total calcium and/or its distribution within the myocardial cell may be a primary determinant of the contractile state of the heart.

57. Reversal of Renal Hypertension in Rats after Immunization against Angiotensin. A. RICHARD CHRISTLIEB,* THOMAS U. L. BIBER,* AND ROGER B. HICKLER,* Boston, Mass. (introduced by George W. Thorn **).

To assess the role of angiotensin in experimental hypertension in rats, the BP response, the degree of refractoriness to infused angiotensin, and the appearance of antibodies (AB) against angiotensin after immunization with angiotensin were studied. Stable hypertension with BP of 170–230 was recorded by the indirect caudal method in 14 rats with unilateral renal artery clipping (G rats), 8 rats with unilateral nephrectomy and contralateral renal

artery clipping (GN rats), and 8 rats made hypertensive by unilateral nephrectomy, DOCA, and 0.8% saline (DCA rats). Angiotensin complexed to rat albumin by carbodiimide and mixed with Freund's adjuvant was injected into the toe pads six to nine times at intervals of 7 to 100 days, the longer intervals permitting a study of booster responses. Caudal pressures were recorded up to 269 days from the onset of hypertension. A fall in BP from control levels after a series of immunizations of at least 30 mm Hg (2 SD of spontaneous mean pressure variation) lasting 8–120 days occurred in 6 G and 3 GN rats. In these rats BP returned to or near to control levels and again fell significantly on subsequent immunizations. No BP fall was seen in the DCA rats and in 5 mock-immunized G or GN rats. After immunization a significant increase in refractoriness to angiotensin acid and amide was observed in the GN rats but not in the G rats, the latter being markedly refractory even before immunization. Plasmas from 14 G and 6 GN immunized rats were assayed for AB by Dr. Lot B. Page using a radioimmune assay system with results expressed as bound/free ratios (B/F) after 18 hr incubation at 4°C. The rats with a B/F ratio of 0.2 or greater had a mean BP *fall* of 30 mm Hg at the time of AB determination, as compared with control BP ($P < 0.01$). In contrast, rats with a B/F ratio of <0.2 had a mean BP *rise* of 16 mm Hg. No rat with a low B/F ratio had a BP fall. These data suggest that angiotensin is an etiological factor in experimental renal hypertension.

58. The Metabolism of Sex Hormones in the Arterial Wall. A. V. CHOBANIAN,* P. I. BRECHER,* R. D. LILLE,* AND H. H. WOTIZ,* Boston, Mass. (introduced by Robert W. Wilkins**).

Sex hormones were recently found in this laboratory to influence arterial metabolism; therefore studies of their fate within the arterial wall were undertaken. The uptake, binding, and metabolic conversion of the hormones were studied in vitro using human and canine arteries incubated with labeled estrogens and testosterone, and the fate of ^{3}H-estradiol was studied after its injection into intact dogs and rats. The results indicated that: (1) Both human and canine intima in vitro metabolized (*a*) estradiol to estrone, and (*b*) estrone sulfate to estrone and estradiol. (2) The arterial intima in vitro converted testosterone to metabolites chromatographically identical with androsterone and androstenedione. (3) No evidence for specific arterial binding of estradiol or testosterone was apparent; (*a*) unlabeled hormone did not compete with the tracer for arterial uptake; (*b*) less than 30% of the total radioactivity was contained in the nonnuclear fraction; (*c*) in contrast to the uterus treated similarly, no specific arterial binding of estradiol was demonstrable by sucrose density gradient ultracentrifugation. (4) Arterial uptake of ^{3}H-estradiol was less than that in the uterus, adrenal gland, or liver, but comparable to that in other tissues examined. The disappearance rate of ^{3}H-estradiol from aorta was greater than that from uterine endometrium or myometrium but generally similar to that from other organs. (5) Unexpectedly, the uptake of ^{3}H-estradiol in the dog (but not rat) adrenal gland was greater than that in the uterus or other tissues, although the retention in the adrenal gland was much less than that in the uterus. As much as 40% of adrenal radioactivity was recovered as estrone, whereas almost all uterine radioactivity was present as estradiol. A metabolic effect of sex hormones on arterial metabolism may not be associated with prior specific binding. Metabolic conversion of these hormones can occur in the arterial wall.

59. The Lack of Effect of Free Bile Salts on the Rate of Fat Absorption In Vivo. MICHAEL L. CLARK,* VALERIE BURKE,* AND JOHN R. SENIOR,* Philadelphia, Pa. (introduced by Truman G. Schnabel, Jr.).

Although deoxycholate has been shown to inhibit fatty acid esterification by rat intestinal mucosa in vitro, this inhibitory effect of deoxycholate has been questioned as the predominant factor in production of bacterially induced steatorrhea. We have studied effects of deoxycholate-taurocholate mixtures in vivo in unanesthetized rats with bile and lymph duct fistulas. Clear micellar solutions containing 1–5 mM ^{14}C-palmitate in glucose-saline-phosphate buffer at pH 6.5 and varying concentrations of taurocholate were infused at 1.2 ml/hr with or without added deoxycholate. Lymph was collected over two consecutive 5 hr periods each day; on the following day the order of infusions to be compared was reversed. With 10 infusions of palmitate in 15 mM taurocholate, total fatty acid recovery in lymph lipid was 70–80%, of which 70–85% was triglyceride. Infusion of 10 mM taurocholate produced slight but not significant reduction in lipid absorption, but with 3 mM taurocholate recovery of fatty acid was markedly reduced to about 2%, without change in the lymph lipid triglyceride fraction. Addition or substitution of 1–5 mM deoxycholate for varying concentrations of taurocholate in 15 infusions produced no decrease in either the total fatty acid recovery or esterification to lymph triglycerides. Our observations suggest that in vivo as well as in vitro the concentration of conjugated bile salts is the major determinant in regulating the rate of fatty acid absorption. Deficiency of conjugated bile salts proved more important than the presence of free bile acids in depressing intestinal entry-esterification of fatty acids from the micellar state in vivo, the difference from in vitro results perhaps being due to the selective proximal gut reabsorption of free bile acids known to occur. The often reported toxic effect of unconjugated bile salts in vitro thus appears to be overcome or circumvented by mechanisms operating in vivo.

60. Implant of Fetal Thymus in an Infant with the III-IV Pharyngeal Pouch Syndrome. W. W. CLEVELAND,* B. J. FOGEL,* AND H. E. KAY,* Miami, Fla. (introduced by A. A. Yunis).

An infant, age 4 wk, was found to have hypoparathyroidism responsive to parathormone. He also had an anomaly of the aortic arch. Absence of the thymus was suspected because this triad of abnormalities occurs in the

III-IV pharyngeal pouch syndrome. No thymus was visible in the usual chest X-rays or after instillation of air into the mediastinum. The infant had recurrent diarrhea, persistent rhinorrhea, and a diminished rate of growth. Lymphocyte counts declined during the first 6 months of life to 1200–1500/cu. mm. Immunoglobulin concentrations were normal, however, and the patient responded to stimulation with diphtheria antigen. Dinitrochlorobenzene, thioglycerol, and diethylfumarate applied to the skin on four occasions produced no hypersensitivity reaction. At age 6 wk skin was grafted in the inguinal area but was lost, probably owing to mechanical factors. A second skin graft from a female donor placed in the thoracic area was probably rejected; cultures of skin from the area yielded only male karyotypes. A lymph node obtained at age 6 wk showed definite diminution of small lymphocytes in the mid-cortical area, a pattern consistent with that found in thymectomized animals. At age 7 months thymic tissue from a female fetus, age 13 wk, was implanted in the rectus muscle. Within 24 hr lymphocyte counts rose to 4000–5000/cu. mm. and have remained within normal limits. Transient eosinophilia to levels of 2500–3000/cu. mm. also developed. After operation, diarrhea and rhinorrhea ceased. The first postoperative application of the previously used chemicals produced a severe hypersensitivity reaction. An autograft was accepted and a homograft rejected after operation. At 14 months of age the infant is growing normally and has had no unusual infections. These observations indicate that thymic activity has been successfully produced by transplantation of fetal thymus. They provide support in man for current concepts of thymic function.

61. Adaptation of Skeletal Muscle to Acute Arterial Hypoxemia. RONALD F. COBURN AND LESTER MAYERS,* Philadelphia, Pa.

We have applied the carbon monoxide indicator method of estimating intracellular oxygen tensions in muscle to the study of effects of arterial blood hypoxemia on this tissue. The method is based on the competitive reaction of CO and O_2 with myoglobin. It can be shown that in an equilibrium state there is a unique myoglobin P_{O_2} (P_{MO_2}) for a given relationship of myoglobin Pco and carboxymyoglobin % sat. ([COMg]). [COMg] is measured in biopsy specimens; intracellular Pco is assumed to equal mean capillary Pco, and this was calculated from measurements of arterial and venous P_{O_2}, [O_2Hb], and [COHb]. Seven mongrel dogs were anesthetized with pentobarbital and connected via a tracheal cannula to a respirator-rebreathing system. Catheters were placed in the carotid artery and deep femoral vein (DFV), which was ligated just above the knee. DFV blood was assumed to represent efferent blood from muscle. Baseline blood samples were taken and the P_{O_2} in inspired gas was lowered by adding N_2 to the rebreathing system. After 20 min additional blood samples and a muscle biopsy were taken. In five experiments where P_{aO_2} was 30–50 mm Hg, P_{MO_2} averaged 5.1 ± SD 0.04 mm Hg, a value that was only 1 mm Hg less than that found previously with P_{aO_2} 78–94 mm Hg. The rate of blood flow in muscle, assessed from measurements of arterial and venous oxygen content using the Fick equation, did not increase significantly as compared with base line. In two experiments with P_{aO_2} lowered to 28 and 25 mm Hg, P_{MO_2} was 1.4 and 1.0 mm Hg. Calculations using the Krogh equations suggest that the average diffusion distance between capillary and myoglobin decreased progressively with hypoxemia. The major adaptation to hypoxemia appears to be a 4- to 5-fold opening of capillaries. Intracellular P_{O_2} was preserved until P_{aO_2} decreased below approximately 30 mm Hg.

62. Capacity of a Cobra Venom Protein to Inactivate the Third Component of Complement (C'3) and to Inhibit Immunologic Reactions. CHARLES G. COCHRANE,* HANS J. MÜLLER-EBERHARD, AND KARL-ERIK FJELLSTRÖM,* La Jolla, Calif.

Inactivation of C'3 by cobra venom factor (CoF) requires combination of CoF with a heretofore unrecorded serum protein called C'3 serum proinactivator (C'3-SPI). CoF was isolated from crude venom in a high degree of molecular homogeneity by current protein separation techniques. C'3-SPI was purified from human serum and identified by disc electrophoresis as a distinct protein. The molecular weights of CoF, C'3-SPI, and the C'3 inactivator complex (C'3-IC) were 140,000, 80,000, and 220,000, respectively. Formation of C'3-IC from CoF and C'3-SPI required bivalent cations. C'3-IC was found to act as an enzyme on C'3, cleaving it into at least two fragments, F(a)C'3, which has anaphylatoxin activity, and F(b)C'3. Administration of isolated CoF to rabbits, rats, and guinea pigs caused a rapid fall in hemolytic C' levels and a complete loss of the C'3 protein from the circulation. Using ^{125}I-CoF, it was shown that CoF circulated in rabbits in the form of the C'3 inactivator complex with a half-life of 32 hr. After 5 days the CoF underwent immune elimination. The procedure caused no apparent untoward effects. Cellular elements in the blood were unaffected except for polymorphs, which were increased 1.5- to 2-fold in number. Clotting times and kininogen levels were unaffected. Tests of the effect of CoF treatment on immunologic reactions showed that the Arthus reactions in rats and acute glomerulonephritis in rabbits and rats were markedly inhibited. Polymorph accumulation, essential for the development of these lesions, was greatly diminished. Homocytotropic PCA reactions in guinea pigs were unaffected.

63. Effect of Saline Infusion on Bile Formation. JAIME B. COELHO* AND VERONIKA MACAROL,* New York, N. Y. (introduced by Henry O. Wheeler).

Saline infusion promotes a reduction in renal tubular sodium reabsorption. In view of the controversy as between proposed intra- and extrarenal mechanisms, it was considered of interest to study the response of bile formation to saline infusion. Seven nonanesthetized dogs with chronically implanted Thomas cannulae and three anesthetized dogs were studied. Four nonanesthetized dogs received DOCA, 10 mg i.m. A constant bile acid infusion

was maintained throughout. After control bile samples were collected, 100 ml/kg of 155 mM NaCl were infused intravenously at 28 ml/min. Bile samples were analyzed for Na^+, K^+, Cl^-, HCO_3^-, and osmolal concentrations. Bile acid concentration was calculated as the difference between cations and anions. The following parameters increased significantly after saline infusion: bile flow ($P < 0.01$), Na^+ output ($P < 0.05$), Cl^- output ($P < 0.01$), HCO_3^- output ($P < 0.05$), osmolal output ($P < 0.05$), and Na^+ output per unit of bile acid secreted ($P < 0.01$). Bile acid output did not change significantly. The results show that saline infusion brings about changes in bile formation qualitatively similar to the urinary changes and suggest that the latter may in part be due to extrarenal factors.

64. Skin Blood Flow in Scleroderma. JAY D. COFFMAN AND ALAN S. COHEN, Boston, Mass.

Blood flow in various involved skin areas in patients with scleroderma has not been systematically studied and values have not been determined in normal subjects for some skin areas (e.g., forehead). Since circulatory factors have been implicated in the pathogenesis of scleroderma, capillary blood flow was measured in the forehead, forearm, and digital skin and forearm subcutaneous tissue. Normal subjects and 7 patients with scleroderma were studied. The determinations were performed by measuring the disappearance rate of $Na^{131}I$ (0.01 ml) from injections in the tissues in a 78°F room. Forehead cutaneous flow averaged 28.4 ± SD 72 ml/100 g per min in 34 normal subjects and 18.2 ± 8.6 ml in the 7 patients ($P < 0.01$). Fingertip flow averaged 10.4 ± 1.9 ml in 11 normal subjects; 2 patients showed no clearance of $Na^{131}I$, whereas 4 other patients had an average fingertip flow of 7.9 ± 5.5 ml ($P < 0.01$). Forearm cutaneous flow averaged 6.8 ± 2.0 ml in 29 normal subjects and 6.8 ± 2.5 ml in the 7 patients ($P > 0.5$). Forearm subcutaneous flow averaged 5.5 ± 1.9 ml in 20 normal subjects and 4.3 ± 1.6 ml in the 7 patients ($P < 0.2$). Although the averages for skin flow in the foreheads and fingertips of the patients were significantly different from those in normal subjects, normal disappearance rates were sometimes obtained from obviously involved scleroderma tous skin in these areas. Forearm skin was clinically involved in all but one patient; however, there was no significant difference in flow as between patients and normal subjects. Therefore, it seems that sclerodermatous skin does not necessarily have a decreased flow and that decreased capillary flow is probably not a primary fault in this disease. It is also concluded that in normal subjects forehead flow is almost 3 times greater than digital capillary flow and 5 times greater than forearm skin flow.

65. Isolation, Purification, and Physicochemical Properties of the Plasma Kallikreins. ROBERT W. COLMAN* AND SOL SHERRY,** St. Louis, Mo.

Human plasma kallikrein, a proteolytic enzyme distinct from glandular kallikreins, releases the vasodepressor peptide bradykinin from an α_2-globulin precursor. Plasma kallikrein has been purified from fresh frozen plasma by alcohol fractionation, isoelectric precipitation, ion exchange chromatography, and gel filtration. Three enzymatically active fractions were isolated and termed plasma kallikreins I, II, and III, representing purifications of 970-, 323-, and 590-fold as compared with activated plasma. All three kallikreins were active biologically; they increased vascular permeability in the guinea pig and released a kinin from human plasma as measured in the rat uterus bioassay and by radioimmunoassay. Kallikreins I, II, and III have similar ratios of hydrolytic activity toward a variety of arginine and lysine esters, and share a common antigenic determinant, but differ in physicochemical characteristics. The kallikrein I preparation is homogeneous; it has a molecular weight of 99,800 and migrates as a single band in the slow gamma globulin region on polyacrylamide gel electrophoresis. Kallikrein II, also homogeneous, has a molecular weight of 163,000, migrates as a fast gamma globulin, and has enzymatic properties identical with those of kallikrein I. Kallikrein III, an alpha globulin, reacted quite differently with polypeptide inhibitors. Activated Hageman factor determines the rate of activation of plasma kallikreinogen, and the latter exists in plasma in amount comparable to prothrombin and plasminogen. Simple biochemical techniques, based on the measurement of arginine esterase activity under controlled conditions, have been developed for assaying the components of the kallikreinogen-kallikrein enzyme system and studying their variations in disease states.

66. Mechanism of Action of an Immunoregulatory Human Alpha Globulin (IRA). SIDNEY R. COOPERBAND,* ROBERT C. DAVIS,* KARL SCHMID,* AND JOHN A. MANNICK, Boston, Mass.

Recent studies by Mowbray and from these laboratories have suggested the presence of a natural immunoregulatory agent (IRA) in plasma which can suppress both humoral and cellular immunity. In order to understand the mechanism of action of this agent, we have studied its effect upon in vitro lymphocyte proliferation and antigen recognition by macrophage immobilization. In human lymphocyte cultures the homologous IRA inhibited both PHA- and antigen (tuberculin, diphtheria, and tetanus toxoids)-induced DNA and protein synthesis, and cell transformation. It had little effect on unstimulated cells and was not toxic as determined by trypan blue dye exclusion. IRA activity was removed by absorption with washed fresh lymphocytes for 2 hr. The activity per mg of isolated protein varied somewhat in six active preparations. The range of IRA dose yielding graded inhibition of PHA stimulation was very narrow (0.1–0.6 mg/ml of the most active preparation). The inhibitory effect could be overcome with increased dosage of PHA. Cells activated by prior exposure to PHA for 24 hr could not be suppressed. In addition, IRA inhibited

the antigen-induced immobilization of peritoneal macrophages from specifically sensitized guinea pigs. This assay system depends upon the interaction of macrophages with a lymphocyte-produced factor found in the supernatant of peritoneal cell cultures. IRA also blocked the effect of this supernatant factor, "macrophage immobilization factor," on nonsensitized macrophages. These observations suggest that IRA acts to prevent lymphoid cell proliferation and macrophage immobilization by competitively blocking antigen (and PHA) recognition sites on the surface of these cells.

67. Sodium Transport and CO_2 Production by the Toad Bladder: An Unexpected Effect of Malonate. NORMAN S. COPLON* AND ROY H. MAFFLY, Palo Alto, Calif.

Several years ago it was observed that glucose-stimulated sodium transport by the toad bladder was greatly inhibited by malonate, whereas pyruvate-stimulated sodium transport was minimally affected. Further studies have now been performed on this action of malonate. CO_2 production was determined by a conductometric method which permitted the short-circuit current (SCC) to be simultaneously measured. The effect of malonate was quantitated by expressing the per cent decrease of SCC and CO_2 production 60 min after addition of malonate in relation to that at the time of addition. The results, expressed as the mean ± SE, were: (*a*) with no exogenous substrate (n = 5): CO_2 −38 ± 4%, SCC −61 ± 8%, ratio 0.63 ± 0.02; (*b*) after stimulation by glucose (n = 9): CO_2 −7 ± 2%, SCC −48 ± 7%, ratio 0.16 ± 0.04; (*c*) after stimulation by pyruvate (n = 5): CO_2 −4 ± 2%, SCC −5 ± 6%, ratio 0.58 ± 0.15; (*d*) after stimulation by glucose and pyruvate combined (n = 5): CO_2 −12 ± 4%, SCC −21 ± 3%, ratio 0.54 ± 0.18; (*e*) controls without malonate (n = 12): CO_2 −5 ± 2%, SCC −17 ± 2%, ratio 0.34 ± 0.07. For further comparison sodium transport was reduced by (*f*) removing sodium from the mucosal bath (n = 5): CO_2 −32 ± 4%, SCC −84 ± 4%, ratio 0.38 ± 0.03; (*g*) adding ouabain (n = 11): CO_2 −39 ± 2%, SCC −80 ± 3%, ratio 0.48 ± 0.02; (*h*) adding fluoropyruvate (n = 4): CO_2 −34 ± 3%, SCC −81 ± 3%, ratio 0.42 ± 0.04. Therefore malonate uniquely inhibits glucose-stimulated sodium transport without comparable inhibition of CO_2 production, and has little effect on pyruvate-stimulated sodium transport. We postulate that malonate reduces sodium transport in the absence of exogenous substrate by its classical inhibition of the Krebs cycle at succinic dehydrogenase; in the presence of exogenous pyruvate, sufficient succinate accumulates to overcome the inhibition by malonate; exogenous glucose is diverted by a direct or indirect action of malonate from a pathway leading to pyruvate to a pathway producing CO_2 but not coupled to sodium transport. Our studies appear relevant to understanding how metabolic energy is distributed within the cell between transport and nontransport processes.

68. Effect of Adrenal Glucocorticoid Hormones on Zinc Metabolism in Mammalian Cell Cultures. RODY P. COX,* New York, N. Y. (introduced by Colin M. MacLeod**).

While investigating the mechanism of induction of increased alkaline phosphatase activity by prednisolone in tissue culture, we found that adrenal glucocorticoid hormones increase the uptake of zinc in certain mammalian cells. Whereas in the absence of prednisolone the zinc content of HeLa S_3 cells is approximately 10- to 20-fold greater than that of an equivalent volume of medium, after growth in the presence of prednisolone the cellular zinc content doubles or triples. Kinetic studies suggest that cells must grow from 10 to 15 hr in the presence of prednisolone before an increased zinc uptake becomes apparent. Cells pregrown in medium with prednisolone retain their capacity for increased uptake of zinc for many hours after removal of the hormone. Uptake of zinc is markedly depressed at low temperatures and in the presence of sulfhydryl blocking agents. Inhibitors of glycolysis and oxidative metabolism have little effect on ^{65}Zn uptake. Since ^{65}Zn taken up by mammalian cells in culture is nearly all soluble in TCA, it is unlikely that it is incorporated into macromolecules. Hormonal effects on zinc uptake are selective in that (1) only those steroid hormones with potent glucocorticoid effects stimulate zinc uptake, and the degree of effectiveness of each hormone parallels its glucocorticoid activity; (2) susceptibility to hormone effects on zinc uptake was confined to certain established "epithelial-like" cell lines, and in each instance paralleled the hormone-induced increased alkaline phosphatase activity; (3) the uptake of several other cations, for example calcium and rubidium, was not significantly enhanced by the steroid hormone, and a number of monovalent and divalent cations did not compete with zinc. Certain effects of hydrocortisone on cells may be explained by an increase in the zinc content of cells.

69. The Importance of Sulfhydryl Groups in the Binding of Insulin to Isolated Fat Cells. O. B. CROFFORD, D. S. CRUMBO,* AND T. MINEMURA,* Nashville, Tenn.

The binding of native insulin to the plasma membrane of isolated fat cells can be prevented by a brief exposure of the cells to the sulfhydryl-blocking agent maleimide. Experiments were performed to test the specificity of the brief maleimide exposure by investigating two biological indices of insulin action: (1) acceleration of glucose transport, and (2) inhibition of lipolysis. A suspension of isolated fat cells was prepared from rat epididymal adipose tissue and exposed to maleimide (5×10^{-4} M) for 10 sec. The exposure was stopped by adding glutathione (10^{-3} M), followed by washing the cell suspension four times. Aliquots of the cell suspension were then incubated with and without insulin. Glucose utilization by cells briefly exposed to maleimide was not stimulated by insulin. The failure to respond to insulin was not due to inactivation of the glycolytic enzymes, since (1) basal

glucose utilization was not decreased, (2) increasing glucose transport by raising the glucose concentration of the incubation medium from 1 to 80 mM produced a 5-fold increase in the rate of glucose utilization, and (3) insulin added to the cell suspension before the maleimide exposure protected the cells against maleimide, and their capacity to respond to insulin was retained. Maleimide-exposed cells had an accelerated rate of lipolysis which was not inhibited by insulin. The results indicate that the effect of a brief maleimide exposure is to block two of the known actions of insulin rather than to inactivate the glycolytic and lipolytic enzymes of the cell. These observations plus the known specificity of maleimide for sulfhydryl groups support the theory that one or more disulfide linkages are involved in the binding of insulin to its receptor on the fat cell membrane.

70. Role of Arterial O_2 Tension in the Regulation of Coronary Vascular Resistance. R. F. P. CRONIN* AND M. EDELSTEIN,* Montreal, Canada (introduced by Carl Goresky).

Although arterial hypoxemia is a potent stimulus to relaxation of the coronary resistance vessels, it is not known whether this results indirectly from myocardial hypoxia or directly from the effect of lowered P_{O_2} on vascular smooth muscle tone. To elucidate this question, pressure-flow experiments were performed in 13 open-chest dogs anesthetized with morphine-chloralose. The left circumflex coronary artery was cannulated and perfused via a peristaltic pump with blood from the femoral artery. A small disc oxygenator was incorporated into the circuit so that the circumflex artery could be perfused with blood from the oxygenator for brief periods during which left circumflex coronary perfusion was reduced stepwise at 30 sec intervals while perfusion pressure was recorded. Vascular resistance for the perfused myocardium at each flow was calculated and plotted against flow. From resistance-flow curves so obtained, the coronary flow at maximum coronary vasodilation (CFMV) was readily determined. In 53 paired experiments, the perfused myocardial segment was rendered hypoxic by perfusion with desaturated blood either (*a*) with a reduced P_{O_2} (< 70 mm Hg) obtained by increasing the concentration of N_2 in the gas phase of the oxygenator or (*b*) with a normal arterial P_{O_2} (> 70 mm Hg) obtained by admitting 3% CO to the gas phase for varying intervals. The pH and P_{CO_2} of the perfusing blood were kept constant in each group by including 5% CO_2 in the gas phase. In both groups (*a*) and (*b*), the results were similar. CFMV increased semilogarithmically with decreasing O_2 saturation of the perfusing blood. A comparison of the regression coefficients for the two groups of data showed no significant difference ($P > 0.9$). These data support the concept that autoregulation of the coronary circulation is determined by myocardial O_2 supply and demand; arterial P_{O_2} per se exerts no measurable effect on coronary vascular resistance.

71. A Consistent Abnormality in Fibroblasts of Patients with Cystic Fibrosis and in Heterozygous Carriers. B. SHANNON DANES* AND ALEXANDER G. BEARN, New York, N. Y.

Cystic fibrosis of the pancreas (mucoviscidosis) is a common autosomal recessively inherited condition in which there is a generalized dysfunction of the exocrine glands. Skin fibroblasts from 7 patients (1½ to 11 yr) derived from six families have been studied to determine whether a morphologic or metabolic abnormality could be detected in tissue culture. Cytological evaluation of all cell cultures was based on the examination of 1000 cells per culture. In all 7 patients, 28–100% of the fibroblasts exhibited large numbers of sharply demarcated vesicles or vacuoles containing metachromatic staining material. 9 presumptive heterozygotes (parents of affected individuals) were studied, and in all instances the fibroblasts (18–78%) showed similar metachromatic staining vesicles. It was not possible to distinguish the homozygous from the heterozygous state under the present conditions of cell culture, although the degree of metachromasia was, on the average, greater in the 7 affected individuals. Fibroblasts derived from 26 unrelated individuals (6 months to 74 yr) and 11 patients with a variety of other diseases were studied. Metachromatic staining vesicles were observed in only 0–1% of the fibroblasts examined. This technique appears to provide a simple and reliable method of identifying healthy heterozygous carriers of this common inherited disease, besides affording a tool for determining directly the heterozygous frequency of this highly lethal gene in the normal population. Preliminary chemical studies on the nature of the metachromatic staining material present in the fibroblasts of affected individuals and their carrier parents will be discussed.

72. Reversal of Phenformin-Induced Mitochondrial Inhibition by Long-Chain Free Fatty Acids. FRANK DAVIDOFF,* Boston, Mass. (introduced by Samuel Gargill**).

The relationship between respiratory inhibition and hypoglycemia induced by guanidine derivatives remains unclear. The current studies suggest that the mitochondrial action of these compounds relevant to hypoglycemia may be unrelated to respiratory inhibition. Phenformin, 3×10^{-5} M, a level which approximates that achieved by hypoglycemic doses in vivo, inhibits respiration of tightly coupled guinea pig heart mitochondria by 50% in the presence of fatty acid–free serum albumin. Addition of long-chain free fatty acid as the fatty acid/albumin complex diminishes mitochondrial sensitivity to inhibition 7-fold without uncoupling oxidative phosphorylation or decreasing respiratory control. Increasing fatty acid chain length and saturation increases effectiveness of antagonism to phenformin-induced respiratory inhibition. When serum albumin and fatty acid are absent from the medium, 50% inhibition of respiration is achieved only at 1.5×10^{-4} M phenformin; this decrease in sensitivity is probably due to the presence of endogenous

long-chain free fatty acids released within the mitochondria. Binding studies with radioactive long-chain free fatty acids indicate the presence of a limited number of mitochondrial binding sites with a high affinity for fatty acids. Bound fatty acids are not esterified, and fatty acid binding is unaltered by the presence of phenformin. Binding of radioactive phenformin to mitochondria has also been demonstrated; the binding decreases in the presence of long-chain fatty acid and in the absence of serum albumin. Free fatty acids may serve to modulate the effectiveness of respiratory inhibition by phenformin in vivo. Conversely, free fatty acids bound to mitochondria may play a physiological role as regulators of mitochondrial function in a manner not yet defined, and phenformin may interfere with these effects of free fatty acids.

73. Studies on the Action of ACTH on the Rate-Determining Step of Steroidogenesis in the Organized Adrenal Cell. W. W. DAVIS,* H. L. MOSES,* A. S. ROSENTHAL,* AND L. D. GARREN, Hershey, Pa., Bethesda, Md., and New Haven, Conn.

Current evidence indicates that the rate-limiting step in the pathway of steroidogenesis and the site of action of ACTH is the conversion of cholesterol to pregnenolone. Cycloheximide, an inhibitor of protein synthesis, blocks the stimulation of steroidogenesis by ACTH by acting at this step in the pathway. The enzyme system responsible for this reaction is situated within the mitochondrion; however, the localization of the substrate of this reaction, cholesterol, has not been established. The present study was performed to further elucidate the mechanism of stimulation of corticosterone biosynthesis by ACTH within the complexities of the organized adrenal cell. To perform these studies a technique was developed for electron microscopy–radioautography that enable preparations of adrenal tissue to retain over 90% of their cholesterol content previously rendered radioactive by exchange with ^{3}H-cholesterol. In agreement with concomitantly performed chemical determinations on subcellular fractions of the adrenal gland, the electron microscope radioautographs revealed that over 90% of the cholesterol was situated in the extramitochondrial cytoplasm, predominantly within the lipid droplets, rather than in association with the mitochondrion as previously suggested. The administration of ACTH caused a depletion of cholesterol in all cell fractions, but most markedly in the lipid droplets; cycloheximide prevented this action of ACTH. Studies performed in vivo and in vitro revealed similar findings. These data indicate that the components of the rate-determining step of steroidogenesis are anatomically separate and that ACTH is involved in stimulating the translocation of cholesterol from its storage site within the lipid droplet into the mitochondrion, where it is further metabolized along the pathway to corticosterone. In addition, these studies illuminate the subcellular inhibitory site of cycloheximide.

74. Patterns of Renal Response to Graded Saline Challenge. B. B. DAVIS,* M. J. WALTER,* AND H. V. MURDAUGH, Pittsburgh, Pa. (introduced by Jessica H. Lewis**).

Inhibition of sodium reabsorption by the proximal tubule has been indicated as the major factor which increases sodium excretion ($U_{Na}V$) after saline infusions. The response of sodium reabsorption by the proximal tubule and of $U_{Na}V$ to graded isotonic saline infusions was tested in dogs. Control clearances and ratios of proximal tubule fluid to plasma inulin, using re-collection micropuncture, were measured during hydropenia, and after infusion of 0.0, 1.0, 2.0, 3.0, 4.0, or 10.0% body weight (BW) isotonic saline. Half the saline was given in 15 min, the remainder in 30. Infusion rate and urine flow were then balanced, 15 min allowed for equilibration, and measurements repeated. Mean $U_{Na}V$ increments with loads of 0.0, 1.0, and 2.0% BW were 1.97, 20.63, and 51.81 μEq/min, respectively. There was no measurable change in fractional sodium reabsorption by the proximal tubule. A dose of 3.0% BW saline was required to decrease fractional sodium reabsorption. There was no measurable difference in per cent depression of fractional sodium reabsorption by the proximal tubule among 3.0, 4.0, and 10.0% BW groups. The depression was 17.0, 17.0, and 16.0%, respectively. After depression of fractional sodium reabsorption, the mean rate of increase of $U_{Na}V$ relative to dose was greater (increments for 3.0, 4.0, and 10.0% BW were 88.7, 124.7, and 380.4 μEq/min). $U_{Na}V$ therefore continued to increase despite relatively constant fractional sodium reabsorption by the proximal tubule. These results indicate that a large dose (3.0% BW) of intravenous saline is required to depress fractional sodium reabsorption in the proximal tubule. Additionally, the major factor responsible for saline-induced natriuresis is inhibition of sodium reabsorption distal to the proximal tubule. This is true even after inhibition of sodium reabsorption by the proximal tubule.

75. Ventricular Receptors for Acetylcholine (ACh): Evidence for Nicotinic and Muscarinic Sites. PETER J. DEMPSEY * AND THEODORE COOPER, Bethesda, Md.

It has been reported that the positive inotropic effects of ACh on slowly beating papillary muscles at 30°C may be independent of catecholamine (CA)-mediated mechanisms. Others, however, have demonstrated that "nicotinic" doses of ACh will release norepinephrine from the isolated perfused heart. In an attempt to demonstrate dual positive inotropic (CA-dependent and -independent) mechanisms, the ACh responses of 18 normal and 10 CA-depleted feline hearts were compared. The hearts were perfused with Krebs solution at 34° ± 0.5°C and electrically paced at 132 per min. Maximum tension developed in the isovolumetrically beating left ventricle was used as an index of contractility. In normal hearts ACh depressed ventricular contractility in doses of 10^{-6} to 10^{-5} g; higher doses produced a positive inotropic effect, the magnitude of which was dose independent.

Administration of atropine ($4–12 \times 10^{-6}$ g) eliminated the negative inotropic effect and shifted the upper part of the dose-response curve to the left. Although the dose-response curve was not affected by hexamethonium ($3–20 \times 10^{-6}$ g), all positive inotropic effects were competitively blocked by *d*-tubocurarine ($6–12 \times 10^{-6}$ g) and by propranolol (10^{-6} g). In hearts depleted of CA stores by complete extrinsic denervation (5 cats) or by prior administration of 5 mg/kg of reserpine (5 cats) only negative inotropic effects were seen, and these were blocked by atropine. These studies demonstrated that ACh has both negative and positive inotropic effects on the intact ventricle. The negative effects appear to be mediated by muscarinic receptors (blocked by atropine) which are located on the muscle cells. The positive effects appear to be CA dependent (blocked by propranolol and CA depletion) and mediated by "nicotinic" receptors (blocked by *d*-tubocurarine). The nicotinic receptors are presumably located along intramyocardial postganglionic sympathetic axons.

76. The Effect of Controlled Interruptions of the Enterohepatic Circulation on the Composition of Bile in the Rhesus Monkey. R. HERMON DOWLING* AND DONALD M. SMALL,* Boston, Mass. (introduced by Robert M. Donaldson, Jr.).

The extent to which increased hepatic synthesis may compensate for loss of bile salts (BS) after interruptions of the enterohepatic circulation (EHC) has not been clearly defined. To study this and other changes in biliary composition which result from graded interruptions of the EHC, we designed an experimental model for use in rhesus monkeys. Bile from a chronic bile fistula was returned to the intestine through an electronic stream-splitter which diverted different percentages of bile to a collecting system, thus providing controlled interruption of the EHC. Bile samples were analyzed for BS, phospholipid, and cholesterol, and at each level of interruption, observations were continued for 5–7 days after steady-state conditions were reached. During each period, fat balance studies were performed, fecal BS were estimated, and *de novo* BS synthesis was calculated. After recovery from surgery (14 days), "base-line" data were obtained in 12 monkeys by collecting 5% of the bile. Base-line secretory values were: BS, 4.60 ± 0.26 (mean $\pm$ SEM); phospholipid, 0.51 ± 0.04; cholesterol, 0.32 ± 0.03 mM/12 hr. At 10 or 20% interruption, BS secretion remained unchanged, since increased synthesis compensated for BS loss. Beyond this, however, synthesis was maximal but inadequate, and BS secretion fell progressively from 2.67 ± 0.29 (33%), through 1.52 ± 0.22 (66%), to 1.00 ± 0.07 mM/12 hr (100%). As BS secretion decreased, there was a corresponding increase in fecal fat. Although the secretion pattern of cholesterol and phospholipid was similar to that of BS, phospholipid secretion fell proportionally less. After complete ileal resection, BS secretion (2.01 ± 0.07 mM/12 hr) was approximately equivalent to 50% interruption of the EHC. These studies demonstrate that (1) the adaptive increase in hepatic BS synthesis is limited and becomes maximal at 20% interruption of the EHC; (2) diversion of 33% or more of the EHC produces BS deficiency and steatorrhea; (3) ileal resection causes only partial interruption of the EHC.

77. Persistence of Group A Streptococcal Carbohydrate Antibodies in Patients with Chronic Inactive Rheumatic Heart Disease. BURTON A. DUDDING* AND ELIA M. AYOUB,* Minneapolis, Minn. (introduced by Lewis W. Wannamaker).

Antibody levels for group A and A-variant streptococcal carbohydrate antigens were determined on 40 patients with chronic rheumatic heart disease (CRHD), by a modification of the radioimmune precipitin technique. The mean "A" antibody level was significantly higher in patients with CRHD than in normal controls. It is not likely that recent streptococcal infections were responsible for this difference, since (1) no significant differences for the ASO, anti-DNase B, and A-variant antibody levels were found, and (2) the 25 patients with CRHD on penicillin prophylaxis showed similar findings when compared with 25 age-matched controls. Because previous studies have shown elevated levels for all the antibodies in acute rheumatic fever as well as acute streptococcal glomerulonephritis, the possibility that the "A" antibody persists in patients with chronic rheumatic carditis was explored. Titers for the above antibodies were determined on 21 sera from 9 patients with streptococcal glomerulonephritis during 5 yr after the acute illness, and compared with matched acute and/or convalescent sera from 16 patients with rheumatic carditis. The results suggest that although the mean "A" antibody levels were similar during the first 6 months after both acute illnesses, patients with nephritis showed a definite decline in "A" antibody levels during the subsequent 4½ yr, whereas a significant drop did not occur during that interval in patients with rheumatic carditis. These studies indicate that group A streptococcal carbohydrate antibody levels are higher in patients with CRHD than in normal individuals and persist longer in patients with rheumatic carditis than in patients with acute streptococcal glomerulonephritis. The persistence of this antibody might be ascribed to a prolonged antigenic stimulus due either to a slower degradation of the streptococcal "A" antigen or to the presence of a similar antigen in host tissue.

78. Effects of Glucagon on Hepatic Bile Flow in Man. WALTER P. DYCK* AND HENRY D. JANOWITZ,** New York, N. Y.

Glucagon resembles secretin in its amino acid sequence, insulinotropic effect, inhibitory influence on gastric acid secretion, and choleretic effect in the dog. The present study was designed to examine the effects of glucagon on hepatic bile flow in man. In patients with T tubes implanted in the common bile duct after cholecystectomy, rates of bile flow and electrolyte composition were mea-

sured before and after i.v. injection of glucagon (0.8–15.0 μg/kg). Basal rate of bile flow and electrolyte composition were unaltered during 4 hr of collection in three subjects, nor did this diversion alter the response to a subsequent dose of glucagon. Glucagon augmented the rate of bile flow in each subject (range 31–66%). Significant alterations in electrolyte concentration were not observed. Maximal augmentation of bile flow occurred between 10 and 20 min after glucagon injection, and the stimulatory effect lasted no longer than 30 min. Glucagon augments the flow of common duct bile in man without significantly altering its electrolyte composition.

79. Stimulation of Beta Lipoprotein-Protein Synthesis by Dietary Monosaccharide in the Rat. R. PHILIP EATON* AND DAVID M. KIPNIS, St. Louis, Mo.

The present study was undertaken to determine whether increased synthesis of carrier protein contributes to the development of carbohydrate-induced hyperlipemia. Rats maintained for 2–6 days on 40% glucose exhibited increased levels of beta and pre-beta lipoprotein-protein as measured qualitatively by paper electrophoresis and quantitatively by heparin precipitation (control, 1.5 ± 0.1 mg/ml; treated, 2.3 ± 0.1 mg/ml). The specific activity of the serum beta lipoprotein of the glucose-fed rat (Gl-R) 4 hr after the intravenous injection of ^{14}C-leucine was increased 100% over controls. In in vitro experiments with Gl-R liver slices incubated with ^{14}C-leucine, the specific activity of the medium beta lipoprotein-protein increased $140 \pm 20\%$ as compared with control incubations. "Beta lipoprotein-protein" isolated from the liver tissue showed similar increases in specific activity which correlated with the duration of glucose feeding (2 days, +30%; 4 days, +100%; 6 days, +300%). In contrast, in vitro hepatic beta lipoprotein synthesis was reduced 50–75% after 24 hr fasting and 70–80% in the 72 hr alloxan diabetic rat. Identical studies in small bowel slice preparations failed to demonstrate any change in lipoprotein-protein synthesis under the above conditions. Changes in plasma and liver amino acid pools determined by chromatography could not account for the changes in lipoprotein specific activity. These results indicate that (1) excessive glucose intake results in stimulation of amino acid incorporation into beta lipoprotein-protein; (2) this stimulation occurs within 2 days of glucose feeding, before a rise in plasma lipid concentration can be demonstrated; and (3) the earliest demonstrable effect in plasma is an increase in the concentration and specific activity of the beta and pre-beta lipoprotein-protein. Increased synthesis of carrier protein may, therefore, represent a significant causative factor in the development of carbohydrate-induced hyperlipemia.

80. The Dynamic Characterization of Ventricular Premature Beats in Man. LESLIE M. EBER,* JOHN M. COOKE,* AND RICHARD GORLIN, Boston, Mass.

The gross hemodynamic qualities of ventricular premature beats are well known. Wiggers and others have shown decreased ventricular pressure and ejection volume with ectopically stimulated beats even when the R-R intervals were equal to that of preceding control beats. Ventricular premature beats (VPB) and normally conducted beats were analyzed and compared for both general and segmental left ventricular motion and force—velocity—length characteristics, utilizing simultaneous cine ventriculography and pressure recording in 10 human subjects. In comparison with normally conducted beats, VPB exhibit decreased shortening velocity at peak tension and lower isolength points on a force-velocity plot. Motion analysis of sequential cine frames during VPB revealed a bizarre pattern of contraction resembling the hydraulically inefficient asynergy of contraction observed in some ventricular aneurysms. There is marked distortion of time and sequence and topography of contraction of particular segments of myocardium. Roughly, VPB fell into one of two over-all patterns, with outward bulging of wall and pooling of blood in either the periapical or the peribasal regions. Further studies of ventricular motion in the intact dog indicate that each pattern of contraction observed is related to the anatomical site chosen for electrical ectopic stimulation. Conduction may have undergone a series of delays: first, through muscle of varying thickness, and second, by retrograde entry into the Purkinje system. These observations of asynergy during a VPB indicate why ectopic beats may propel little or no blood despite apparently adequate diastolic filling time and Starling effect (preload). The marked degree of asynergy which can readily occur in normal hearts with ectopic pacemaker activity emphasizes the importance of orderly sequence of contraction to the proper mechanical function of the normal heart.

81. The In Vivo pH of the Extravascular Space of the Lung. RICHARD M. EFFROS* AND FRANCIS P. CHINARD,** New York, N. Y.

The distribution of nine amines between the pulmonary vascular and extravascular volumes was determined by the sudden-injection multiple-indicator dilution technique in anesthetized dogs. A mixture of T1824, a water label ("W":THO or ^{14}C-antipyrine), and an amine (N) was injected into the jugular vein; blood was sampled from the carotid artery. The ratio C_{EV}/C_B (extravascular to vascular amine concentration) that would prevail during constant infusion of amines was calculated from the ratio of observed mean transit time ($\bar{t}$) differences: $C_{EV}/C_B = (\bar{t}_N - \bar{t}_{T1824})/(\bar{t}_W - \bar{t}_{T1824})$. The ratio exceeded 1.0 for nicotine, quinine, and benzylamine (amines with a pK_a above 7.5), an indication of greater extravascular concentration. Furthermore, these ratios markedly increased at high arterial pH (pH_A) and diminished at low pH_A. Calculation of extravascular pH (pH_{EV}) was based on the assumptions that the distribution ratios of unbound indicator were determined by the pH gradient between blood and tissue and that only uncharged molecules diffused between these two compartments. At pH_A 7.38–

7.43, pH_{EV} determined with nicotine was 6.71 ± 0.09, and with quinine, 6.65 ± 0.10. The apparent extravascular concentration of benzylamine ($pK_a = 9.37$) was limited by low concentrations of the uncharged (permeant) species. Acute metabolic and respiratory pH changes elicited parallel but significantly smaller changes in pH_{EV}. Alterations in P_{CO_2} at constant pH_A consistently produced very small reciprocal changes in pH_{EV} (−0.027 unit pH/10 mm Hg P_{CO_2}); alterations in tissue P_{CO_2} appear to be rapidly buffered. As anticipated, extravascular concentrations of aniline and antipyrine, amines that are predominantly neutral at physiological pH, were uninfluenced by alterations of pH_A. Guanidine, atropine, morphine, and mescaline (amines with pK_a above 7.0) and DMO were essentially restricted to the vascular compartment during a single circulation. This technique provides a means of estimating organ pH in vivo.

82. Insulin Secretion in the Duct-Ligated Pancreas. RICHARD H. EGDAHL, JOHN M. HIEBERT,* AND EBERHARD MACK,* Boston, Mass.

The morphological and functional status of islet cells in the long-term duct-ligated pancreas is unknown, but of extreme importance with respect to the possibilities of permanently successful islet cell allografts. The present studies were designed to provide information on islet cell histology and ability to release insulin up to 15 months after transection of the pancreas and atrophy and fibrosis of exocrine tissue. 20 dogs were operated upon and the pancreas was transected in its midportion. The distal remnant of gland rapidly became atrophic, whereas the proximal gland remained normal in terms of exocrine activity. At intervals of 3 months the animals were operated upon and biopsies were taken of the remnant of both proximal and distal gland. Blood draining the remnant was obtained before and after an intravenous glucose tolerance test. Immunoreactive insulin was determined in peripheral and pancreatic venous blood samples by the method of Morgan and Lazarow. In addition to light microscopy, immunofluorescent studies were carried out on the pancreatic remnants as well as on the normal residual pancreas, at intervals of up to 15 months after pancreatic transection. In four animals, pancreatoduodenectomy was carried out 1 yr after transection of the pancreas in order to determine residual islet cell function of the remnant. All animals revealed normal peripheral insulin levels and demonstrated increases in peripheral blood and pancreatic venous blood insulin after intravenous glucose administration. The pancreatic remnant showed large numbers of islets, although the acini appeared to be absent. It is concluded that islet cells persist anatomically and function for long periods of time after pancreatic duct ligation, and that they do not become destroyed through progressive fibrosis and atrophy as has been suggested by others. These findings make possible the consideration of pancreas allografting or perhaps even pancreas xenografting between primates with islet cell remnants.

83. Serum Bacteriostasis of *Staphylococcus aureus*: Evidence for Action of IgM Antibody. N. JOEL EHRENKRANZ,* DAVID F. ELLIOTT,* AND ROMEO ZARCO,* Miami, Fla. (introduced by William J. Harrington).

Human serum can delay the onset of the logarithmic phase of growth of *S. aureus* for several hours. This bacteriostasis is attributed to nonimmune mechanisms, since growth of other micrococci is also affected and since both siderophilin and globulin fractions are found to be bacteriostatic. Measuring the bacteriostatic effect of serum diluted serially in trypticase soy broth permits quantitation and insures adequate nutrient for bacterial growth. The end point is the highest serum dilution, yielding at least 0.1 fewer colonies than appropriate controls which have increased 30 times or more. Inhibition of serum bacteriostasis by killed *S. aureus* reduced titers of 1/160 to <1/20, whereas *S. epidermidis* had no such effect. Absorption with *S. aureus* generally reduced titers as much; however, absorption with *S. epidermidis, Micrococcus rubens, Sarcina lutea,* and *Gaffkya tetragena* was without effect. Inactivated purified coagulase as well as active coagulase (a gift of Dr. Morris Tager) decreased titers, indicating that an enzymatic effect of coagulase is not responsible for inhibition of serum bacteriostasis. Bacteriostatic activity was not present in the colostrums of four postpartum women although *S. aureus* agglutinins were. Their serums were bacteriostatic in titers of 1/40 to 1/640. Umbilical cord infant blood with IgM levels of 2–22 mg/100 ml had bacteriostatic activity up to 1/80. Mercaptoethanol treatment of three serums with bacteriostatic titers of 1/160 to 1/320 abolished bacteriostasis but did not change agglutinin levels of 1/160 to 1/320. DEAE column chromatography also separated the bacteriostatic and agglutinating activities of serum; the bacteriostatic fractions reacted with IgM but not with IgA or IgG antisera. It is concluded that human serum bacteriostasis of *S. aureus* is due to IgM antibody.

84. A Genetic Basis for Beta Cell Adenomas. GEORGE J. ELLIS* AND HAROLD E. LEBOVITZ,* Durham, N. C. (introduced by William S. Lynn).

This study was designed to determine whether islet cell adenomas arise from a preexisting metabolic disorder of the β-cell and, if so, whether this abnormality exists in asymptomatic relatives of patients with the disease. An 18 yr old male who presented with hypoglycemia and coma had two discrete β-cell tumors removed. After surgery, the patient no longer had hypoglycemia, but fasting plasma insulins were 44 μU/ml; and 1 g of tolbutamide intravenously resulted in a striking output of insulin (area under the insulin curve for 60 min was 10,247 μU-min/ml) and a fall in blood glucose to 63 mg/100 ml. Insulin secretion estimated by the same method in a control population ranged from 500 to 4000 μU-min/ml. Six siblings (ages 14–24 yr) underwent studies to assess insulin secretion. Two with fasting plasma insulins less than 10 μU/ml had tolbutamide responses of 978 and 2155 μU-min/ml. Two with fasting

insulins of 14 and 22 μU/ml produced 4818 and 5145 μU-min/ml after tolbutamide. Two with elevated fasting plasma insulins (34 and 59 μU/ml) had exaggerated responses to tolbutamide (7945 and 10,048 μU-min/ml). Fasting blood glucose was 84–100 mg/100 ml for all the siblings; none had an abnormal fall after tolbutamide. The sibling with the highest insulin secretion could not be distinguished from patients with β-cell tumors by her insulin responses to L-leucine, glucose, or glucagon. However, she maintained a normal blood glucose and her plasma insulin suppressed normally during a 72 hr fast. These data are consistent with the thesis that a genetic abnormality predisposes to the development of some β-cell tumors and that this abnormality exists in asymptomatic members of the family.

85. Familial Renal Glycosuria: A Genetic Reappraisal of Hexose Transport in Kidney and Intestine. L. J. ELSAS* AND L. E. ROSENBERG,* New Haven, Conn. (introduced by P. K. Bondy**).

Two forms of renal glycosuria have been defined by previous glucose titration studies: type A, characterized by a low threshold and a low maximum tubular reabsorptive capacity for glucose (T_{m_G}); type B, characterized by a low threshold, an exaggerated splay, but a normal T_{m_G}. Such glucose titration studies have not been performed in pedigrees with familial renal glycosuria to determine whether these two types are genetically distinct and to test the accepted hypothesis of autosomal dominant inheritance. We have performed glucose titration studies in six normal volunteers and in two families with inherited renal glycosuria. The mean T_{m_G} of normal volunteers was 290 ± 30 mg/min. In one family, three of four affected brothers had titration curves indicative of type A renal glycosuria. The fourth had a normal T_{m_G} (261 mg/min) and a titration curve of type B renal glycosuria. Intestinal transport of the ^{14}C-labeled glucose analogue, 3-*O*-methyl-glucose, was studied in three of these brothers. Jejunal mucosa obtained by peroral biopsy accumulated this hexose against a concentration gradient throughout a 60 min incubation period in a manner identical with that noted in controls, and no differences in affinity or capacity were detected. In a second family in which a brother and sister had type A renal glycosuria, no abnormality in renal glucose transport was found in either parent, their titration studies demonstrating a normal threshold, splay, and T_{m_G}. These studies indicate that type A and type B renal glycosuria may represent different expressions of the same genetic lesion; that all familial renal glycosuria is not inherited as a simple autosomal dominant trait; and that the hexose transport system in the intestine is not identical with that in the kidney.

86. Transfer of Cellular Triglyceride Fatty Acid to Lecithin by Engulfing Leukocytes. PETER ELSBACH, New York, N. Y.

During phagocytosis in vitro granulocytes convert more medium lysolecithin (LPC) to cellular lecithin (PC) by direct acylation than they do at rest. This pathway of PC formation requires free fatty acids (FFA). Earlier studies indicate that incorporation of medium FFA into cellular complex lipids is not stimulated during phagocytosis. Therefore, the origin of FA for increased PC synthesis remained to be determined. Since granulocytes contain active lipase(s), our attention was directed to cellular triglyceride (TG) as a possible source of FA. Granulocyte lipids were labeled with ^{14}C-linoleic acid during preincubation for 1 hr. After washing in nonradioactive medium, containing albumin, more than 95% of cellular radioactivity was in esterified form, PC and TG each containing about 40%. Aliquots of the cell suspension were reincubated in a medium that contained LPC for periods of up to 2 hr, at various pH values, with and without polystyrene particles. At all levels of pH, TG radioactivity tended to fall and PC radioactivity to rise. At pH 6.5 or higher, TG radioactivity always fell more in the presence of particles. The radioactivity lost from TG during phagocytosis was largely recovered in PC, indicating increased synthesis of this phospholipid. At pH 5.0, however, a sharp drop in TG radioactivity was accompanied by an equivalent rise in FFA radioactivity, without increased labeling of PC in the presence of particles. The flux of radioactivity from TG to PC at higher pH represents an amount of FA that more than matches the increased conversion of medium LPC to PC previously observed during phagocytosis. $^{14}CO_2$ production from prelabeled lipid by resting and engulfing leukocytes is not appreciably different. We conclude that granulocyte TG, which constitutes about 20% of total cell lipid, serves as a reservoir of FA used during phagocytosis for increased phospholipid synthesis, rather than as an additional source of energy.

87. Stimulation of Amino Acid Incorporation into Rat Diaphragm by Nonsuppressible Insulin-like Activity (NSILA). JOHN W. ENSINCK* AND SOLOMON S. SOLOMON,* Seattle, Wash. (introduced by Albert E. Renold).

Recent evidence suggests that the terms "bound" insulin, "atypical" insulin, and NSILA refer to a single component of mammalian plasma that simulates some actions of insulin in vitro, yet is distinguished from insulin by its physical and immunologic properties. Because proteolytic enzymes enhance glucose transport and utilization and antilipolysis, as is observed with both insulin and NSILA, a specificity for NSILA may be questioned. Therefore, studies were undertaken to compare the effects of NSILA with those of insulin and proteolytic substances on glucose utilization and protein synthesis in the rat diaphragm. NSILA was partially purified from pork plasma by chromatography on Dowex 50 and extraction into 70% acetone (0.3–1.25 mU insulin equivalents per mg assayed in the isolated lipocyte). Glucose uptake, glycogen synthesis, and incorporation of ^{14}C-amino acids (L-arginine, L-methionine, L-histidine), $H^{14}CO_3^-$, and ^{14}C-pyruvate into protein were measured in the "cut" rat hemidiaphragm without and with insulin (0.1–50 mU/ml), NSILA (0.01–5 mg/ml) plus insulin antiserum, α-chymotrypsin (0.78–

780 μg/ml), trypsin (0.72–720 μg/ml), and phospholipase C (0.5–500 μg/ml). It was observed that the dose-response relationship of NSILA paralleled that of insulin, with the same maxima (0.8–1.0 mU/ml) for glucose uptake, glycogen deposition, and incorporation of ^{14}C-amino acids, $H^{14}CO_3^-$, and ^{14}C-pyruvate into protein. In contrast, the effect of the proteolytic enzymes on glucose transport and glycogen synthesis plateaued at a level equivalent to 0.3 mU of insulin per ml, and at all concentrations no incorporation of amino acids into protein was found. These studies demonstrate that the action of NSILA by these in vitro parameters is indistinguishable from that of insulin, and it is unlikely that either operates by a mechanism in common with proteolytic enzymes.

88. The Influence of Ouabain and 3-Pentene-1,4-olide on Cardiac Sarcoplasmic Reticulum. M. L. Entman,* J. W. Cook, Jr.,* and Rubin Bressler, Durham, N. C.

Calcium accumulation by cardiac sarcoplasmic reticulum has been postulated to play an important role in excitation-contraction coupling. We investigated the influence of ouabain and 3-pentene-1,4-olide (an unsaturated lactone with demonstrated positive inotropic activity in doses 600 times that of ouabain) on calcium binding, calcium turnover, and the additional ATPase activity stimulated by the presence of calcium ("calcium-stimulated ATPase") in a suspension of dog cardiac sarcoplasmic reticulum. Calcium binding (the accumulation of calcium in the absence of oxalate or added phosphate) was rapid, reaching maximal values in the first minute. Control experiments demonstrated calcium binding of 16.8 ± 3.4 mμmoles/mg per min (± SD). Addition of ouabain (10^{-6} M) or 3-pentene-1,4-olide (10^{-3} M) resulted in binding of 19.2 ± 1.8 and 23.1 ± 3.2, respectively. These values were significantly greater than controls ($P < 0.01$). In the absence of ATP, the binding in control was 6.4 ± 0.2, which was not affected by the presence of ouabain or 3-pentene-1,4-olide (6.2 ± 0.3 and 6.3 ± 0.4, $P > 0.5$). Calcium turnover was defined as the change in ^{45}Ca bound to sarcoplasmic reticulum after a change in the specific activity of calcium in the medium. It is considered to be a function of the rate constants of calcium binding at equilibrium. Calcium turnover was consistently increased by both ouabain and 3-pentene-1,4-olide both in the presence (50% increase) and in the absence (100% increase) of ATP. Both ouabain and 3-pentene-1,4-olide decreased base-line ATPase activity by 25%. "Calcium-stimulated ATPase" was 0.128 μmole P_i/mg per min and was stimulated by both ouabain (0.173) and 3-pentene-1,4-olide (0.253). The results demonstrate that ouabain and 3-pentene-1,4-olide alter the total binding and rate of turnover of calcium in cardiac sarcoplasmic reticulum; and that this stimulation of calcium movement is accompanied by augmented hydrolysis of ATP. It is proposed that these two unsaturated lactones, with known positive inotropic activity, exert effects on calcium binding and transport sites in cardiac sarcoplasmic reticulum which increase the calcium flux.

89. Study of the Influence of Ultraviolet Light (UVL) on the Mitotic Cycle and Macromolecule Synthesis in Hairless Mouse Epidermis. John H. Epstein,* Kimie Fukuyama,* and Ken Fye,* San Francisco, Calif. (introduced by Elliot Rapaport).

This histological and autoradiological study examines the early effects of UVL on cell division and macromolecule synthesis. The right flanks of 110 albino hairless mice were irradiated with 6.03×10^5 ergs/cm² of mid UVL energy. In group I the mice were sacrificed hourly for 6 hr, daily for 5 days, and at 7 days post irradiation. Each mouse received 40 μc of ^3H-thymidine i.p. 1 hr before autopsy. In groups II and III autopsies were performed at 1, 3, 7, 24, 48, and 72 hr. In group II 30 μg of Colcemid were injected i.p. 1 hr before autopsy, and in group III 10 μc of ^3H-cytidine, ^3H-histidine, and ^3H-methionine were injected i.d. into separate mice 30 min before autopsy. *Results:* DNA synthesis: depressed (1/25 to 1/2 of normal skin) by 2 hr, recovery started by 4 hr, almost complete by 6 hr, 3 to 5 times normal by 24 hr, peak at 72 hr, still almost double at 7 days. Mitosis: depressed (to 0) by 1 hr, persisting for 7 hr (1/6 of normal), recovery by 24 hr, 3½ times normal at 72 hr. RNA and protein synthesis: marked inhibition in upper one-third of epidermis by 3 hr, recovery by 48 hr, acceleration by 72 hr. These findings indicate that UVL interrupts (1) the mitotic cycle in the S and G2 phases, and (2) RNA and protein synthesis in the upper epidermis shortly after exposure. The inhibition is followed by acceleration of all these functions.

90. Kinin Generation Caused by Human IgG-Rheumatoid Factor Complex. Wallace V. Epstein, Kenneth L. Melmon,* Margaret Tan,* and Jeffrey Stoff,* San Francisco, Calif.

Circulating rheumatoid factors are a regular concomitant of rheumatoid arthritis. There are no established mechanisms by which such factors might initiate or sustain an inflammatory response. In the present study, the macroglobulin fraction of sera from 10 patients with rheumatoid arthritis and from 11 normals was isolated and individually combined with either aggregated or nonaggregated normal human IgG, and the mixture was added to fresh normal plasma. The kininogen content (trypsin-released kinin minus kinin in nontrypsinized plasma) was determined at 0 and 10 min. An average fall of $47.2 \pm 9.2\%$ in plasma kininogen resulted from the addition of aggregated IgG combined with rheumatoid macroglobulin. A significant difference ($P < 0.01$) was found when normal macroglobulin plus aggregated IgG caused a decrease of only $9.1 \pm 6.8\%$. Combination of nonaggregated IgG with rheumatoid or normal macroglobulin resulted in kininogen depletion of 9.8% and 5.5% respectively. Controls of normal or rheumatoid macroglobulin or of aggregated or nonaggregated IgG mdividually added to normal plasma caused a kininogen fall of less than 5%. Naturally occurring 22S complex isolated from two rheumatoid sera caused the depletion of 38% and 40% of plasma kininogen. The 7S and 19S

gamma globulins dissociated from the 22S complex were incapable of generating kinin, but their recombination restored this ability. Although the several rheumatoid macroglobulin fractions were high in rheumatoid factor hemagglutinating activity, the mixture of rheumatoid macroglobulin with aggregated IgG capable of kinin generation is itself devoid of such activity. These findings indicate that the antigen-antibody complex of aggregated IgG and rheumatoid factor is capable of initiating kinin production. It has been shown previously that kinin is to be found in rheumatoid synovial fluid in high concentrations. Since the kinin-generating complex is serologically inactive, this possible pathogenetic mechanism is applicable to seronegative as well as seropositive rheumatoid arthritis.

91. Significance of Glucose Intolerance in Noncoronary Heart Disease. PHILIP O. ETTINGER,* HENRY A. OLDEWURTEL,* AND TIMOTHY J. REGAN, Jersey City, N. J.

The specific etiologic relation of glucose intolerance, its mechanism, and its significance to the clinical course of patients with cardiac disease have not been defined. To evaluate this phenomenon, i.v. glucose tolerance tests (GTT) were performed in 30 sequential patients under 55 yr of age with valvular heart disease or cardiomyopathy, without myocardial ischemia or failure. They had no personal or family history of diabetes mellitus, were normotensive and nonobese, received a high carbohydrate diet, received no thiazides, and had normal plasma electrolytes. The glucose disappearance slope, G_K, of 1.08 $\pm$ 0.06 was lower than in normals of the same age, 1.95 $\pm$ 0.19 ($P < 0.0001$). Since fasting plasma FFA levels were not significantly correlated with G_K, an inhibitory effect of FFA on glucose utilization was not evident. Immunoassay of plasma insulin demonstrated a reduced and delayed insulin response to acute hyperglycemia in the cardiac patients. Further, plasma glucose and insulin response to i.v. tolbutamide were significantly reduced. To examine the possible role of enhanced sympathetic activity in cardiacs, norepinephrine was infused into normals during a GTT. G_K and plasma insulin declined to levels approximating those seen in the patient group. However, beta blockade with propranolol produced no increase of G_K in cardiacs. Cardiac index (CI) was reduced in most patients, without left ventricular filling pressure elevation, suggesting a hemodynamic determinant of G_K. After valvular surgery, five patients increased CI (1.59 ± 0.11 to 2.23 ± 0.20 liters/min per m^2, $P < 0.02$) but failed to achieve normal levels, and did not significantly alter the reduced G_K up to 3 months postoperatively. Thus, glucose intolerance may frequently be an acquired metabolic disorder unrelated to etiology of the cardiovascular disease. This appears to be due to decreased insulin secretion, through a mechanism related to diminished systematic blood flow, and is not readily reversible after surgery.

92. Inhibition of the Platelet-Surface Reaction in Endotoxin Shock and the Generalized Schwartzman Reaction. G. EVANS* AND J. F. MUSTARD, Hamilton, Ontario, Canada.

Transient platelet aggregates can cause organ dysfunction and tissue injury. Since endotoxin causes platelet aggregation, we examined the relationship between endotoxin-induced platelet aggregation, organ dysfunction, and tissue injury. Platelet aggregation induced by collagen, antigen-antibody complexes, and endotoxin is inhibited by nitrogen mustard, acetylsalicylic acid, phenylbutazone, and sulfinpyrazone. Particle-free endotoxin given intravenously to 20 rabbits caused a fall in the platelet count (60%), a drop in arterial blood pressure (−71 mm Hg), and an increase in venous pressure (+8 mm Hg). Histological examination of the lungs by light and electron microscopy showed the pulmonary vessels to be plugged with platelet aggregates, and pulmonary edema to be present. Rabbits (20) given any one of the above drugs in doses which inhibit endotoxin-induced platelet aggregation showed only a slight fall in platelet count (−9%) and arterial pressure (−6 mm Hg) and no rise in venous pressure with intravenous endotoxin. The lungs from these animals showed only occasional platelet aggregates in the pulmonary vessels and minimal pulmonary edema. The acute shock and lung change were also prevented in animals made thrombocytopenic with ^{32}P before administration of endotoxin. Phenylbutazone given to rabbits ½ hr before the preparatory and second injection of endotoxin caused a significant reduction in deaths (untreated 19 of 36, treated 7 of 28) and in number of animals developing the Schwartzman kidney (untreated 7 of 17, treated 1 of 21). These observations show that prevention of endotoxin-induced platelet aggregation diminished considerably the organ dysfunction and tissue injury associated with endotoxin infusion.

93. Cholesterol Lowering by Colchicine. WILLIAM W. FALOON,* DALE I. WEBB,* AND THOMAS F. RACE,* Syracuse, N. Y. (introduced by Eugene L. Lozner**).

In previous studies of the malabsorptive syndrome induced by colchicine, significant decreases in serum cholesterol were observed. The present study was therefore undertaken to determine the relationship of these changes to cholesterol intake, weight loss, triglyceride alteration, catharsis, and other parameters of absorption. Nine subjects receiving constant low calorie diets containing 200–500 mg daily of cholesterol and 20% or less polyunsaturated fat were given 1.9–3.9 mg of colchicine daily after 4- to 7-day control periods. After 6–8 days of colchicine the average decrease in cholesterol was 56 mg/100 ml (28%). In all subjects colchicine withdrawal was followed by rising cholesterol. Two subjects received colchicine for 21 days, resulting in decreases of 100 mg/100 ml. In a double blind study three patients receiving weight-maintaining diets (600–900 mg cholesterol) were given 6 day periods of colchicine and placebo. Cholesterol fell by 30 mg/100 ml or more during colchicine but rose during placebo periods in all

three. In five subjects both serum triglycerides and cholesterol were determined and there were no consistent correlations. Seven subjects were given magnesium sulfate or cascara for 4–6 days to induce cartharsis; cholesterol was unchanged in all, but subsequent colchicine induced decreases of 30–100 mg/100 ml in five. Fecal fat excretion rose slightly or not at all with colchicine and was unrelated to cholesterol, but other absorptive parameters (serum carotene, *d*-xylose, and ^{57}Co-B_{12} absorption) were regularly altered. These studies indicate that oral colchicine lowers serum cholesterol regardless of dietary cholesterol, triglyceride change, weight loss, or catharsis, but is associated with intestinal dysfunction producing cholesterol and bile salt malabsorption.

94. Cell Replication and Stabilization of Hemoglobin Synthesis in Differentiating Erythroid Cells. ANTONIO FANTONI,* ALBERT DE LA CHAPELLE,* AND PAUL A. MARKS, New York, N. Y.

One characteristic of differentiation of cells producing specialized protein is that protein synthesis becomes independent of continued RNA formation (stable protein synthesis). Studies to date have demonstrated stable protein synthesis in nonreplicating cells, e.g., reticulocytes, and lens, exocrine pancreas, muscle, mammary, and retinal cells, suggesting that stabilization is related to cessation of mitotic activity. We have examined the stability of hemoglobin (Hb) synthesis as related to cell multiplication during yolk sac erythroid cell (YSEC) differentiation in fetal mice (21 day gestation). YSEC are formed in blood islands by day 8 of gestation and enter the embryo circulation by day 9. On the basis of light and electron microscope observations, YSEC differentiate as relatively homogeneous populations, from large nucleated early forms on day 8, to mature cells with pycnotic nuclei by day 15. Between days 10 and 12, the number of YSEC per embryo increases at least 4-fold, with intense replicating activity (determined by mitotic index and incorporation of ^{3}H-thymidine into DNA). YSEC multiplication is accompanied by a 5-fold increase in Hb content per cell. YSEC of 10 and 11 day embryos were incubated with actinomycin. The antibiotic had no effect on the rate of Hb synthesis, while inhibiting RNA formation up to 95% and nonheme protein synthesis up to 80%. These studies suggest that hemoglobin synthesis, but not nonheme protein synthesis, is not dependent on RNA formation. Thus, selective stabilization of the capacity for Hb synthesis occurs at an early time during YSEC differentiation and is not related to a loss of capacity for cell multiplication.

95. Response of Renal Transit Time to Variations of Renal Blood Flow and Solute Excretion. MELVIN H. FARMELANT,* CHARLES BAKOS,* AND BELTON A. BURROWS,** Boston, Mass.

Differences between the kidneys in ^{131}I-Hippuran excretion in renovascular hypertension are reduced by osmotic loads. This response was studied in dogs by determining transit time of ^{131}I-Hippuran injected into one renal artery, and monitoring renal radioactivities. A plot of radioactivity of the injected kidney minus radioactivity of the contralateral kidney yielded a declining half-sigmoid curve skewed toward the tail. The interval between injection and 50% of peak radioactivity ($t_{\frac{1}{2}}$) and the splay of the curve around the midpoint (α) were measured. Partial clamping of the aorta reduced renal blood flow; infusions of normal saline or mannitol in saline produced a wide range of solute excretion (mOsm/min). Both in intact dogs and in those with reduced renal blood flow, $t_{\frac{1}{2}}$ and α were inversely correlated with creatinine clearance ($t_{\frac{1}{2}}$ vs. C_{cr}, $r = -0.81$; α vs. C_{cr}, $r = -0.84$). In intact dogs there was little relationship between mOsm/min and $t_{\frac{1}{2}}$ ($r = -0.50$) or α ($r = -0.24$), over an 8-fold range of solute excretion. However, these parameters were inversely correlated in dogs with reduced renal blood flow ($t_{\frac{1}{2}}$ vs. mOsm/min, $r = -0.66$; α vs. mOsm/min, $r = -0.75$). These findings suggest that the diameter of the renal tubules of intact dogs increases as the bulk flow of solute increases, to maintain the passage times relatively constant. As blood flow is reduced, the diameter of the tubules cannot constrict sufficiently to maintain linear velocity. In this "underfilled" condition of the tubules, increasing osmotic loads increase linear velocity (decreased $t_{\frac{1}{2}}$ and α). Measurements of each half-curve indicate that reduction in blood flow or increased solute excretion does not preferentially affect either a "slow" or a "fast" population of nephrons. This suggests that altered distribution of renal blood flow is not the cause of the renographic abnormalities in renovascular hypertension. These data explain the effect of mannitol loads in reducing the renographic differences between the kidneys in renal arterial stenosis.

96. Role of Plasma Amino Acids in the Regulation of Gluconeogenesis. PHILIP FELIG,* OLIVER E. OWEN,* AND GEORGE F. CAHILL, JR., Boston, Mass.

Prolonged starvation is characterized by a progressive reduction in gluconeogenesis, serving to minimize depletion of body protein. To determine whether hormonal or substrate alterations or both are responsible for the diminished gluconeogenesis, five healthy but obese volunteers were fasted for 21–44 days. In all subjects a marked increase in plasma valine, leucine, isoleucine, methionine, and α-NH_2 butyrate concentrations occurred, with a peak at 5 days and return to base line in 10–14 days. This elevation coincided with the sharp fall in serum immunoreactive insulin. Previous studies have demonstrated a significant depression of these amino acids after stimulation of endogenous insulin secretion and an elevation of the branched-chain amino acids in experimental and spontaneous diabetes. In contrast, plasma alanine declined progressively during starvation, particularly between days 1 and 5. The progressive diminution of excreted nitrogen after day 5 paralleled the decrease in plasma alanine. Despite a fall in plasma alanine to one-fifth of its initial level, net splanchnic extraction after 40–44 days exceeded that of any other amino acid and amounted to 50% of the arterial level. Surprisingly, glycine, threonine, and serine were elevated

by 10 days and reached higher levels at 21–44 days. The data suggest that the initial changes in amino acids are attributable to the fall in serum insulin. The later progressive decline in gluconeogenesis which occurs with no further decrease in insulin levels appears to be directly related to the decrease in alanine. Of significance is the failure to observe any comparable change in total plasma alpha amino nitrogen content, suggesting that the amino acid pattern, particularly the level of alanine, is critical in rate control of gluconeogenesis, and not the total concentration of all amino acids.

97. Cigarette Smoking and Manifestations of Lung Cancer. ALVAN R. FEINSTEIN AND NELSON A. GELFMAN,* West Haven, Conn.

In this survey, we have considered the role of cigarette smoking, not as a possible cause of lung cancer, but as a possible factor in its clinical course and histologic appearance. The case material, which was restricted to men for whom adequate histories were available, consisted of 410 patients with unequivocal lung cancer, observed in the combined services of the Yale–New Haven and West Haven Veterans Administration Hospitals. The amount of cigarette smoking was categorized as none, light (less than 1 pack per day), moderate (1 or 1–2 packs), and heavy (2 packs or more). For percentages of patients in each smoking category, the following trends were noted with increasing amount of smoking: an earlier age at diagnosis ($P < 0.05$); a higher incidence of chronic cough ($P < 0.05$) but a lower incidence of either new cough or recent changes in cough pattern; more patients with a mass noted at bronchoscopy; and fewer patients with abnormal respiratory reserve. No distinct trends were found for divers other aspects of clinical presentation. Histologic findings were difficult to evaluate because of marked discrepancies between the original interpretations and those made by another pathologist reexamining the slides for this study. Although epidermoid and small cell carcinomas were uncommon in nonsmokers, no consistent relationship was found—in either set of readings—between the cellular type of the cancer and the amount smoked. A striking finding was the absence of a distinct correlation between cigarette smoking and prognosis in regard to operability, 1 yr survival, or 5 yr survival. Regardless of the role cigarette smoking may play in causing lung cancer, these data suggest that antecedent smoking does not affect most of the manifestations or the outcome after the disease has occurred.

98. Neuromuscular Effects of the Porphyrin Precursor Delta Aminolevulinic Acid. DANIEL S. FELDMAN,* RICHARD D. LEVERE,* AND JAMES S. LIEBERMAN,* Brooklyn, N. Y. (introduced by Attallah Kappas).

Overproduction of delta aminolevulinic acid (ALA) is the basic abnormality in porphyrin synthesis in acute intermittent porphyria. However, the origin of the neurological symptoms is unknown. Alteration in properties of excitable membranes and synapses might underlie paralysis, cramps, and other symptoms. Since ALA is in a class of compounds with potent synaptic effects, its action in a "model synapse" was studied in vitro in rat and human muscle, employing intracellular microelectrodes. Acetylcholine (ACh) release was measured by spontaneous subthreshold activity at the neuromuscular junction (miniature end plate potential, m.e.p.p.) Frequency was unchanged when ALA was added to normal bathing solutions in concentrations up to 10 mg/ml (8 mM). Muscle membrane polarization and m.e.p.p. amplitude increased variably. When m.e.p.p. frequency was augmented by depolarization with 20 mM K^+ to 173 ± 10.3 (mean $\pm$ SE, $n = 11$), ALA, 1 mg/ml, decreased frequency to 124 ± 9.1 ($n = 10$, $0.005 > P > 0.001$). Increasing ALA resulted in greater inhibition of ACh release. Addition of ALA to depolarized preparations lacking calcium did not induce ACh release, indicating that ALA did not free "bound" calcium. Inhibition of ACh release could result from membrane hyperpolarization secondary to restricted Na^+ movement across the membrane. Muscle membrane resistance, substantially a measure of passive sodium permeability, declined from 1.1×10^5 ohms to 0.25×10^5 ohms when ALA, 2 mg/ml, was added, and returned toward control values upon its removal. These results indicate that ALA can "stabilize" the excitable membranes of nerve and muscle, and inhibit excitatory transmitter release by competition with Na^+ for passage through the cell membrane. Although a direct mechanism for neurological symptoms in acute porphyria remains speculative, ALA has a significant capacity for altering neuromuscular function and may play a role in their production.

99. Evaluation of Factors Regulating Hepatic Control of Insulin Homeostasis. JAMES B. FIELD, MARSHALL WEBSTER,* AND THEODORE DRAPANAS,* Pittsburgh, Pa.

Repetitive blood samples drawn simultaneously from the portal vein, hepatic vein, and femoral artery were assayed for insulin (immunoassay) and glucose in anesthetized dogs to evaluate hepatic regulation of insulin homeostasis. Portal vein and hepatic artery blood flows were measured with electromagnetic flowmeters, permitting calculation of pancreatic insulin production and insulin and glucose flux across the liver. In the control period the liver retained during a single transhepatic circulation $37 \pm 8\%$ (SEM) of the $22{,}000 \pm 5200$ μU/min insulin presented. 5 min after intraduodenal glucose (50 g), pancreatic insulin production and hepatic insulin retention significantly increased. Pancreatic insulin production rose within 5 min after intraduodenal glucose when femoral artery glucose had only minimally increased. By 40 min, pancreatic insulin production increased 8- to 10-fold and hepatic insulin retention exceeded 90%. Intravenous glucose infusion, producing equivalent portal vein glucose but higher femoral artery glucose levels than those after intraduodenal glucose, increased pancreatic insulin production 3- to 4-fold. Hepatic insulin retention also increased, reaching a maximum of 65% at 50 min. These changes were less marked than after

intraduodenal glucose. There was good correlation between increased insulin presented to the liver, increased percentage of hepatic insulin retention, and initiation of glucose storage by liver. Tolbutamide (1 g i.v.) caused an immediate, transient (3–5 min) 8-fold increase in pancreatic insulin production, and hepatic insulin retention increased to 60–70%. After this, despite some persistence of increased insulin presented to the liver, the percentage retained returned to pretolbutamide levels. These results demonstrate the dynamic role of liver in insulin homeostasis and suggest that the process may be controlled by the amount of insulin reaching the liver. Portal vein–arterial glucose concentration difference and intestinal factors may also be important.

100. IgA Deficiency Associated with Partial Deletion of Chromosome 18. MURRAY FEINGOLD,* ROBERT S. SCHWARTZ, LEONARD ATKINS,* ROBERT ANDERSON,* CHRISTOS S. BARTSOCAS,* DAVID L. PAGE,* AND JOHN W. LITTLEFIELD, Boston, Mass.

In contrast to the studies on the X chromosome, there are few data concerning the mapping of autosomal loci. We have studied two patients, one with a ring chromosome replacing a chromosome 18 and the other with a partial deletion of the long arm of chromosome 18. In both patients IgA was not present, and in one the level of IgG was extremely low. The patient with the ring chromosome was a 3 yr old retarded boy with multiple congenital anomalies and a history of recurrent unexplained fevers. Repeated immunoglobulin analyses showed IgG 0.1 mg/ml, IgA absent, and normal IgM. The patient with a partial deletion of the long arm is a 9 yr old girl with mental retardation and cleft lip and palate. Repeated immunoglobulin analyses showed normal IgG and IgM values, but absent IgA. No other immunologic abnormalities were detected in this patient. Chromosome, immunoglobulin, and blood group determinations on the parents and siblings of both patients were normal. These findings suggest that a locus controlling the synthesis of IgA is on the long arm of chromosome 18. The marked deficiency of IgG in one patient might also relate to loss of material from this chromosome. There are various explanations by which a partial deletion of one of the pair of chromosomes 18 could lead to an apparent absence of IgA. Immunoglobulin levels have not been reported in the cases of ring 18 and partial deletion of chromosome 18 noted in the literature. Such studies should be done to determine the significance of our results; conversely, chromosome analyses should be done on patients with immunoglobulin deficiency states.

101. Arterial Oxygen Tension and Functional Severity in Acute Myocardial Infarction. SIDNEY J. FILLMORE,* MARIO SHAPIRO,* AND THOMAS KILLIP, New York, N. Y.

Oxygen (O_2) is commonly administered to patients with acute myocardial infarction (AMI). Although reduced arterial O_2 tension (Pa_{O_2}) may complicate AMI, the usefulness of O_2 therapy during the acute phase remains controversial. Moreover, low Pa_{O_2} in AMI has not been correlated with sequential change in cardiac function. 124 paired determinations of Pa_{O_2}, Pa_{CO_2}, arterial pH, and blood lactate have been analyzed serially in 29 patients with unequivocal AMI during inhalation of air and 28% O_2. The polarographic electrode was calibrated tonometrically before Pa_{O_2} determination. During each study the clinical status of the patient was graded as: I, no failure; II, mild failure; III, pulmonary edema; IV, shock. Chest X-rays obtained concurrently were graded by an observer unaware of the other data: A, normal; B, moderate congestion; C, pulmonary edema; D, pulmonary edema with effusion. Pa_{CO_2} and pH remained within normal limits in all four groups. Lactate was normal (8.1 ± 5 mg/100 ml blood) in groups I and II, but averaged 18.0 ± 11 mg/100 ml in group III and 19.0 ± 10 mg/100 ml in group IV ($P < 0.025$). Pa_{O_2} correlated with clinical grade when all data were pooled and during serial studies in individual patients. Pa_{O_2} of 76 ± 11 mm Hg during air inhalation and increment (Δ) of 28 ± 13 mm Hg during 28% O_2 were within normal limits in group I. Pa_{O_2} during air inhalation and Δ with 28% O_2 declined as functional severity of infarct increased (mm Hg): group II, 63 ± 9, Δ $+20 \pm 9$; group III, 53 ± 9, Δ $+10 \pm 4$; group IV, 51 ± 11, Δ $+10 \pm 7$. Arterial oxyhemoglobin saturation and oxygen content were significantly reduced in groups III and IV. Clinical and roentgen classification correlated well. Abnormalities in groups III and IV were surprisingly parallel and not relieved by high inspired O_2 concentration. Thus arterial hypoxemia is common in AMI and correlates with functional severity as judged by clinical criteria. Serial Pa_{O_2} analysis provides a sensitive and useful indicator for evaluating patient progress and response to therapy. Marked reduction of Pa_{O_2} is associated with poor prognosis and may adversely affect cardiac performance. Failure of increased concentration of inspired O_2 to relieve hypoxemia suggests that radical techniques may be necessary to improve tissue oxygenation in patients with functionally severe myocardial infarction.

102. In Vitro Interferon Induction with Various Synthetic and Naturally Occurring Inducers. MARTIN S. FINKELSTEIN,* GERALD H. BAUSEK,* AND THOMAS C. MERIGAN,* Palo Alto, Calif. (introduced by Frederic L. Eldridge).

Our previous studies in mice revealed differences in the in vivo antiviral protection induced by a synthetic carboxylate copolymer (pyran) and a double-stranded ribonucleotide homopolymer (poly rC/rI). In the present investigations, these interferon inducers also demonstrated differences in their pattern of in vitro interferon production with different cells. The unique ability of peritoneal macrophages, described below, to respond to pyran may explain the prolonged and potent protection afforded the mouse by pyran against intraperitoneal virus challenge. Synthetic and naturally occurring agents were studied as interferon inducers on monolayers of different cell types (permanent aneuploid cells, primary diploid cells, and macrophage explants). It was found that the

different cell species could be divided into several discrete categories depending upon their reactivity to the various inducers. Interferon was detected in permanent cells (Hela or L_{929} cells) only when they were stimulated by viruses or statolon; primary chicken or human fibroblast cells could also be stimulated by poly rC/rI; mouse peritoneal macrophages, the most generally susceptible cells studied, also could be induced by endotoxin or pyran (when complexed to an organic cation, arginine). In permanent and primary cells, actinomycin D inhibited virus-induced interferon synthesis; statolon-induced interferon production was moderately inhibited, whereas poly rC/rI–, endotoxin-, and pyran-induced interferons were least affected by actinomycin D. Additional studies indicated that poly rC/rI, pyran, and endotoxin interferons were released rapidly (2–8 hr), whereas Newcastle disease virus– and statolon-induced interferon appeared later (18–24 hr). Studies are now in progress of the uptake and intracellular localization of radiolabeled inducers. These results are compatible with the hypothesis, advanced by others from in vivo studies, that there are several mechanisms for interferon production, including both rapid release of preformed material and slower *de novo* synthesis; each of these mechanisms may not be present in every cell type.

103. Metabolic Clearance and Production Rates of Human Growth Hormone. JOSEPH L. FINSTER,* ANDREW L. TAYLOR,* AND DANIEL H. MINTZ,* Pittsburgh, Pa. (introduced by I. A. Mirsky**).

Basal metabolic clearance rates (MCR) and production rates (PR) of human growth hormone (HGH) were determined in seven hospital control subjects by the constant infusion technique of Tait, employing ^{125}I-HGH. Calculations of MCR and PR were based upon data from four plasma samples, obtained 15 min apart, in which immunoprecipitable ^{125}I-HGH and immunoassayable HGH concentrations were constant. The MCR were 178 ± 40 (mean $\pm$ SD) ml/min and the PR were 375 ± 157 mμg/min. The 24-hr basal HGH production of 0.5 mg compares favorably with the replacement requirements for linear growth in pituitary dwarfs. Less than 0.5% of the daily basal production appeared in the urine in immunoreactive form. Three hypothyroid patients had reduced MCR (125 ± 45 ml/min) and PR (221 ± 41 mμg/min), suggesting that decreased peripheral metabolism contributes to their maintenance of normal basal plasma HGH concentrations. In contrast, one hyperthyroid patient demonstrated a markedly increased MCR (350 ml/min) and PR (1050 mμg/min). These preliminary data suggest that the reported diminished HGH responses to hypoglycemia in altered thyroid function may be due to increased peripheral degradation in hyperthyroidism and decreased hypothalamic-pituitary responsiveness in hypothyroidism. Three acromegalic patients had MCR of 126, 176, and 325 ml/min with corresponding PR of 1512, 3520, and 47,775 mμg/min. Increasing MCR and PR correlated directly with the severity of the disease process. The MCR in five insulin-dependent and four insulin-independent diabetic patients were 138 ± 19 and 148 ± 30 ml/min, respectively. The corresponding PR were 366 ± 197 and 238 ± 86 mμg/min. Neither the MCR nor the PR of either diabetic group was significantly different from that of the control subjects. These data provide additional support for the absence of abnormalities in HGH metabolism in diabetic patients.

104. The Response of the Right Ventricle to Systolic Loads at Birth and Later. R. W. M. FRATER,* S. YUAN,* H. WEXLER,* D. ROTHMAN,* C. WEBER,* AND L. SILVER,* New York, N. Y. (introduced by Stanley Levenson**).

80 calves and 20 piglets were studied from birth to 14 months: control groups, with and without thoractomy; pulmonary stenosis groups, one operated when newborn, one operated at 1 month; some had stenosis to maximum, others to a standard 50% reduction in external diameter. Right- and left-sided pressures, cardiac outputs, ventricular chamber weights, and cardiac anatomy were studied. In the control groups the right ventricular (RV) pressures dropped from 55% to 25% of systemic in 1 month. The RV/LV weight ratio changed from 1.0 to 0.6. The newborns tolerated pulmonary stenosis by generating more stroke work, maintaining better cardiac output, and producing higher systolic pressures with lower end diastolic pressures than the 1 month group. In long-term studies of equal duration, in the newborn group the right ventricles generated more stroke work and developed systemic pressure levels, and the RV/LV weight ratios reached 1.5, whereas in the 1 month group the right ventricles generated less work and developed less than systemic pressures although the RV/LV weight ratios reached 1.3. In both groups the right ventricles took on a circular cross-section, and in no instance was infundibular hypertrophy seen. Histological and histochemical studies showed gross myocardial hypertrophy and great increases in adenosine triphosphatase. The RV coronary supply increased with the hypertrophy. This study has produced a preparation of pulmonary stenosis with resting RV pressures at systemic levels and has shown differences in the acute and chronic responses of newborn and older right ventricles to pressure loads. These differences were not related to the degree of stenosis produced, but may be due to relative RV dominance at birth and rapid growth in the 1st month of life. The study fails to support an acquired origin for infundibular hypertrophy seen clinically.

105. β-Aspartyl-ε-lysine in Human Urine. GEORGE W. FRIMPTER, New York, N. Y.

A new syndrome manifested by failure to grow and intractable vomiting was encountered. Both urine and plasma of the patient were found to contain the unique dipeptide β-aspartyl-ε-lysine. An infant with undiagnosed disease, even after exploratory laparotomy, was found to have a prominent peak between methionine and isoleucine on the ion exchange amino acid chromatogram of urine and plasma. This complicated area of the

chromatogram reveals several ninhydrin-positive unknowns. Purification of the compound from the patient's urine was effected by multiple passages through resin columns. Hydrolysis yielded equimolar amounts of aspartic acid and lysine. β-Aspartyl-ε-lysine was synthesized by both the acid chloride and the aspartic anhydride methods and shown to chromatograph with the unknown. The α-aspartyl-ε-lysine isomer was eluted from the 150 cm column later, as were β-arpartyl-α-lysine and the more basic α-aspartyl-α-lysine and α-lysyl-α-aspartate. β-Aspartyl-ε-lysine, the most acidic dipeptide, elutes from the ion exchange columns in the same general area as other compounds with two free terminal alpha amino groups, such as cystathionine, α,ε-diaminopimelic acid, and the mixed disulfide of cysteine and homocysteine. β-Aspartyl-ε-lysine has been identified in bacterial products. There was, however, no evidence of infection in this patient. Urine from normal subjects probably contains the dipeptide in trace amounts. Although β-arpartyl dipeptides have been found in urine, the epsilon lysine linkage is unique and may indicate a new class of compounds. There was no significant family history and the mother had a normal urinary chromatogram. Apparent but slow recovery has been accompanied by gradual decrease of β-aspartyl-ε-lysine peptiduria and peptidemia.

106. Factors Controlling the Early Steps of Epidermal Protein Synthesis. IRWIN M. FREEDBERG* AND KATSUIHIKO MATSUI,* Boston, Mass. (introduced by Irving H. Goldberg).

Previous work from our laboratory has indicated that control of protein synthesis in epidermis depends upon variables in both the ribosomal and the soluble fractions of the tissue. In order to define the role of the soluble fraction, an investigation of transfer RNA and the amino acid–activating enzymes in epidermal tissue has been undertaken. Transfer RNA prepared by phenol extraction of the high-speed supernatant fraction of adult guinea pig epidermis and hair root cells cannot be charged with amino acids and inhibits the transfer reaction in a liver amino acid–activating system. In contrast, direct phenol extraction of unfractionated epidermal homogenates yields an active transfer RNA preparation which has been further purified by DEAE cellulose and Sephadex chromatography. The resulting monodisperse material accepts amino acids as actively as does similarly prepared liver transfer RNA. Amino acid–activating enzymes have been prepared by fractional precipitation of an epidermal cytoplasmic extract at pH 5.0. Such enzymes from adult epidermis are significantly less active than those from liver despite efforts at further chromatographic purification. The reactions involved in the early steps of epidermal protein synthesis have been investigated in vitro with these preparations. Cofactor requirements and time course are similar to those in analogous systems prepared from other mammalian tissues. Incubation of the components with either epidermal or hair root ribosomes results in the incorporation of amino acids into peptide linkage. The characteristics of both the transfer RNA fraction and the activating enzyme preparation vary with the age of the animals. The yield and activity of transfer RNA, which is high in newborn animals, is decreased in adults and further diminished in senescent animals. Similarly, the specific activity of the amino acid–activating enzymes declines with age. These early steps of amino acid activation and transfer, therefore, represent potential sites for control of protein synthesis in normal and pathologic epidermis.

107. Leukocyte Collection by an In Vivo Continuous-Flow Centrifuge. EMIL J FREIREICH,* Houston, Texas (introduced by Emil Frei, III).

Leukocyte (WBC) collections from hematologically normal patients and patients with blood disorders have been studied with a centrifuge developed in collaboration with International Business Machines Corp. Venous blood is anticoagulated in vitro with citrate, passed through 50 *g* centrifugal field at 40–60 ml/min, with continuous removal of buffy coat and return of WBC-depleted blood to the donor. In 16 studies of hematologically normal patients, median collection efficiency was 30% of WBC which passed through the instrument. A higher proportion of lymphocytes (37%) are recovered than of polymorphonuclear WBC (18%), and 76% of monocytes are extracted. Only 1% of red cells and 7% of platelets passing through the instrument are removed. Thus, up to 11 liters of whole blood can be processed in a single procedure. In 33 studies of patients with chronic lymphocytic leukemia, an average of 54% of lymphocytes were collected in a single pass through the instrument. Using leukapheresis and processing up to 100 liters of blood in a 40-day period, circulating lymphocytes, lymph node and spleen enlargement could be diminished. The WBC from patients with chronic granulocytic leukemia behave like normal WBC; only 16% of polymorphonuclear cells are recovered, whereas 38% of lymphocytes are recovered. Immature cells resembling blasts are recovered with high efficiency (87%) from patients with acute leukemia or chronic granulocytic leukemia. Lymphocytes collected with the instrument show unimpaired in vitro transformation with antigen or phytohemagglutinin. This instrument permits relatively rapid processing of large volumes of blood from individuals and collection of WBC with very small loss of other formed elements of blood. Collection of large quantities of isogenic WBC should prove useful for study of the effect of leukapheresis on donors, for study of WBC in vitro, and for study of allogenic WBC transfusions.

108. Increased Isoproterenol Responsiveness: A Test for Hyperdynamic Beta Adrenergic Circulatory State. EDWARD D. FROHLICH,* ROBERT C. TARAZI,* AND HARRIET P. DUSTAN,** Cleveland, Ohio.

Hyperdynamic beta adrenergic circulatory state is manifested by exaggerated heart rate response and cardiac awareness to various physiological and pharmacological interventions; hypertension may often occur. Though

patients with symptoms referable to hyperkinetic circulation have long been recognized, opportunity for detailed neuropharmacological investigation and specific treatment has only recently been possible. Hemodynamic and pharmacological characteristics of 14 such patients (2 normotensive and 12 hypertensive) were compared with those of 25 normal volunteers and 14 asymptomatic hypertensive patients. Patients with symptoms had higher resting cardiac index (3.6 ± 0.06 liters/min per m^2; $P < 0.001$) and heart rate (85 ± 4 beats/min; $P < 0.001$) than normotensive (3.0 ± 0.09 liters/min per m^2; 68 ± 2 beats/min) or asymptomatic hypertensive individuals (2.9 ± 0.1 liter/min per m^2; 71 ± 2 heats/min); left ventricular ejection rate was higher, but not significantly so ($P < 0.10$). With graded infusion of isoproterenol (1–3 μg/min, intravenously), heart rate increased by 34 beats/min in patients with symptoms, but by 10 and 13 beats/min (3 μg/min) in the normal and asymptomatic hypertensive groups, respectively ($P < 0.001$). In 9 of the 14 patients with symptoms, isoproterenol produced a hysterical outburst, almost uncontrollable, which was reversed immediately with propranolol (i.v.) but not with placebo. Response of arterial pressure and peripheral resistance to upright tilt, and of arterial pressure and heart rate to carotid sinus stimulation, ocular pressure, Valsalva maneuver, cold, tyramine, trimethaphan, and atropine were normal; and since catecholamine and porphobilinogen excretion and thyroid function were likewise normal in patients with symptoms, it seems reasonable to ascribe the syndrome to increased beta adrenergic receptor site responsiveness. This conclusion is further strengthened by remission of symptoms and by arterial pressure reduction (in hypertensives) with propranolol treatment in all treated symptomatic patients. These results suggest that increased response to isoproterenol infusion may provide evidence for increased beta adrenergic reactivity as a mechanism in hyperdynamic beta adrenergic circulatory state, hyperkinetic heart syndrome, juvenile systolic hypertension, or neurocirculatory asthenia.

109. The Metabolism of Deoxythymidylic Acid (dTMP) in Human Leukocytes: Evidence for Membrane Localization and Leakage of dTMP Phosphatase. ROBERT C. GALLO,* CARLA DAVIS,* AND SEYMOUR PERRY,* Bethesda, Md. (introduced by C. Gordon Zubrod**).

In bacteria it has been established that dTMP and other nucleotides do not enter the cell as a unit. Instead, dTMP is dephosphorylated at the cell membrane, forming deoxythymidine (TdR). TdR then enters the cell, and dTMP is formed by rephosphorylation before incorporation into DNA. We have observed that myeloblasts from patients with acute leukemia (AL) metabolize dTMP in a similar manner. Radioactivity from ^{3}H-TMP (5 μM) was incorporated into DNA at a rate of 10–50 $\mu\mu$moles/10^8 WBC, identical with the rate of incorporation when ^{3}H-TdR was used. However, no ^{32}P-TMP was incorporated, ^{32}P rapidly appearing in the medium as inorganic phosphate, a fact which demonstrates that the cell although impermeable to dTMP still metabolized this nucleotide. Studies utilizing leukocytes from patients with AL and CML have demonstrated that dTMP phosphatase leaks into the media. This leakage occurs with leukocytes obtained either by sedimentation of whole blood at 22°C (dextrose-dextran) or by centrifugation (40 *g* for 30 min) regardless of the anticoagulant used, and is found within 1 hr after the blood is obtained. Deoxythymidine phosphorylase, the enzyme catalyzing the reversible synthesis and catabolism of TdR, was also found in the medium but in less quantity than the phosphatase. Other catabolic enzymes were not found in the media. The observations that dTMP phosphatase leaks from intact leukocytes, and that dTMP does not enter the leukocytes but yet is a substrate for dTMP phosphatase of the intact cell, suggest that this enzyme is localized at the cell membrane. Leukocyte alkaline phosphatase (LAP), which can dephosphorylate dTMP, may be responsible for these findings rather than a specific dTMP phosphatase, since we have found that *p*-nitrophenyl phosphate is also dephosphorylated by an enzyme in the medium. If this is the case, it is of interest to speculate that differences in LAP found in various disorders may be a result of alterations in membrane function.

110. Renal Papillary Hypertrophy and the Urinary Concentrating Defect in Kaliopenic Rats. KENNETH D. GARDNER, JR.,* Palo Alto, Calif. (introduced by David A. Rytand**).

Kaliopenic nephropathy is characterized by renal hypertrophy and a reduced efficiency of the urinary concentrating mechanism in man and the rat. We have examined the relationship between changes in renal papillary structure and maximum urinary concentrating ability (Umax) in kaliopenic rats. During 14 days of dietary potassium deprivation, rats displayed progressive decreases in Umax and progressive increases in the volumes of dry solids and contents of deoxyribonucleic acid (DNA) in their renal papillary regions. There was a positive ($r = +0.83$) and significant ($P < 0.001$) correlation between the volumes of dry solids and the contents of DNA in their papillae, and a negative ($r = -0.67$) and significant ($P < 0.05$) correlation between the volumes of papillary dry solids and Umax. Radioautographs of ^{3}H-thymidine–labeled papillae demonstrated a 3-fold increase in cell labeling at 24 hr (Umax had decreased 21%) and peaks of labeling on the 2nd and 10th days. By the 14th day of potassium deprivation, the volume of papillary dry solids had increased 52% ($P < 0.001$) and papillary DNA content had increased 263% ($P < 0.001$) above normal values. When intratubular distances (between basement membranes) were measured in photomicrographs of cross-sections taken at four different levels through papillae, a mean increase of 29% ($P < 0.01$) above normal distances was recorded in the kaliopenic kidney. These findings indicate that in the renal papilla of the rat, an increase in DNA content, an increase in the volume of solids, and a separation of tubules accompany the development of kaliopenic nephropathy. According to the mathematical model of Wirz and Hargitay, such

changes could account for the reduction in Umax which characterizes renal function in these animals.

111. Roles of ATP and of Peroxidative Metabolism during Phagocytosis in Alveolar Macrophages (AM). J. Bernard L. Gee,* Eugene D. Robin,** James B. Field, Jan D. Smith,* Anthony R. Tanser,* and James Kaskin,* Pittsburgh, Pa.

Alveolar macrophages (AM) are important pulmonary cells involved in lung defense mechanisms. It is generally accepted that AM are obligatory aerobes; that phagocytosis depends on O_2-requiring metabolic pathways and stimulates cellular O_2 consumption. The potential roles of oxidative phosphorylation and peroxidative metabolism and their relationship to the energy requirements for phagocytosis were studied in rabbit AM obtained by pulmonary lavage. Measurements of ATP, Q_{O_2}, and $^{14}CO_2$ output from ^{14}C substrates were performed under "resting" conditions and during phagocytosis of heat-killed *S. albus* in the presence of glucose and serum. Particle entry was assessed by light microscopy. Data indicate the following: (1) Exposure of resting AM to antimycin A (0.05 μg/ml), DNP (5×10^{-4} M), and oligomycin (5 μg/ml) reduced ATP to 30%, 70%, and 30% of control values. (2) Particle entry was normal in the presence of these inhibitor concentrations. Phagocytosis was associated with a mean reduction in ATP of 0.8 mμmoles/10^6 cells. Whereas the ATP reduction associated with phagocytosis was variable in uninhibited cells ($P < 0.05$), it was consistent in the presence of either antimycin or DNP ($P < 0.01$). (3) Menadione reduced particle entry, stimulated Q_{O_2}, and reduced ATP to 75% of control values in resting AM. (4) The well known augmented ^1C- and ^6C-labeled glucose conversion to $^{14}CO_2$ during phagocytosis was confirmed. (5) The importance of peroxidative mechanisms involving both catalase and H_2O_2 was suggested by the stimulation of ^{14}C-formate conversion to $^{14}CO_2$ during phagocytosis, and by reduction of Q_{O_2} and formate oxidation with minimal reduction in ATP by aminotriazole (catalase inhibitor). Aminotriazole did not reduce particle entry. Energy for some component of phagocytosis is derived from ATP. Peroxidative pathways, involving catalase, are not associated with particle entry, but appear related to the increased Q_{O_2} of phagocytosis and possibly serve a bactericidal function.

112. Proteolytic Enzymes in Human Pancreatic Juice in Acute Pancreatitis. M. C. Geokas,* H. Rinderknecht,* P. Wilding,* Y. Lillard,* A. L. Baker,* C. J. Berne,* and B. J. Haverback, Los Angeles, Calif.

Pancreatic juice aspirated from the pancreatic duct or obtained via a pure pancreatic fistula has no spontaneous tryptic activity and contains substantial amounts of trypsin inhibitor. However, in ethionine-induced pancreatitis in dogs and in patients with acute pancreatitis, free proteolytic activity has been identified in pancreatic secretion obtained from the pancreatic duct. Furthermore, we recently detected free elastase activity in extracts of pancreatic tissue, obtained at autopsy, from patients with acute pancreatitis. No attempt has been made to date, however, to determine and characterize the main pancreatic proteases, in pancreatic juice, in their free or zymogen form, in patients with acute pancreatitis. Also, the identification and quantitation of elastase in pancreatic secretion has never been adequately established, because of a significant potentiating effect of trypsin and chymotrypsin on the elastase/elastin system. In this study, pancreatic secretion was obtained (via a pure pancreatic fistula) from two patients with acute pancreatitis; quantitative determinations were carried out which included total protein, amylase, and five pancreatic peptidases: trypsin, chymotrypsin, elastase, and carboxypeptidase A and B. Specific substrates were used for each enzyme, and in the case of elastase, an assay system was designed to account for the potentiating effect of trypsin and chymotrypsin. The bulk of pancreatic proteases was found to exist in the active form throughout the period of pancreatic juice collection in the first case (M.G.). In the second case (P.M.), the amount of amylase and the ratio of proteolytic proenzyme to active enzyme fluctuated in accordance with the degree of pancreatic inflammation. It is concluded that (1) pancreatic juice from two patients with acute pancreatistic contained substantial amounts of trypsin, chymotrypsin, elastase, and carboxypeptidase A and B in their free form, a fact which strongly suggests their role in the autodigestive process in this disease; (2) elastase activity has been definitely identified in human pancreatic secretion.

113. Hormonal Regulation of Gene Expression in Tissue Culture. Thomas D. Gelehrter* and Gordon M. Tomkins,** Bethesda, Md.

A line of rat hepatoma cells (HTC) has been established in which dexamethasone (Dex) and other adrenal steroid hormones induce a 10-fold increase in the activity of tyrosine aminotransferase (TAT). Measurement of labeled amino acid incorporation into antibody-precipitable TAT has demonstrated that the hormone increases the rate of synthesis of new enzyme molecules. Studies on the mechanism of induction have suggested that one action of Dex is to regulate enzyme synthesis at the translational level. Other experiments, with inhibitors, however, which have demonstrated a requirement for RNA synthesis, suggest that another action of the hormone is to stimulate the accumulation of specific messenger RNA for TAT. The subject of this report is a description of the hormonal effects on RNA synthesis and on polysome distribution in HTC cells. Sensitive double-labeling and sucrose density gradient studies have demonstrated that Dex does not stimulate the synthesis of ribosomal or transfer RNA under conditions in which TAT is induced. Experiments with low doses of actinomycin D have confirmed that no new ribosomal RNA synthesis is necessary for enzyme induction. The double-labeling studies have suggested, however, that the hormone increases the labeling of a small amount of non-ribosomal cytoplasmic RNA. The nature of this RNA has been analyzed further by sucrose density gradient and polyacrylamide gel electrophoresis techniques. The

results of these studies are consistent with the requirements for new RNA synthesis demonstrated in inhibitor experiments. In addition, recent studies have shown that the hormone causes a shift in polysome profiles to heavier aggregates. Whether this shift results solely from an enhanced synthesis of heavy messenger RNA, or from an independent effect on ribosome distribution as well, has been investigated further and will be discussed.

114. Complement (C′) Consumption by Endotoxic Lipopolysaccharide (LPS) in Immunoglobulin-Deficient Sera. H. GEWURZ,* R. SNYDERMAN,* H. S. SHIN,* L. LICHTENSTEIN,* AND S. E. MERGENHAGEN,* Bethesda and Baltimore, Md. (introduced by C. S. Stetson**).

Endotoxic LPS has potent ability to induce C′ fixation in normal mammalian serum. "Lesions" indicative of terminal C′ component activity appear on LPS after incubation in normal serum. The six terminal C′ components are consumed, and C′-dependent biologically active by-products (neutrophil chemotactic factor; anaphylotoxin) are generated. Hence, certain toxicities induced by endotoxins may be mediated via C′. The present investigation was concerned with the role of classical humoral antibody in this interaction. Attempts to deplete antibodies to LPS by selective absorptions proved noncritical, in part because of solubilization of LPS. Therefore, LPS-C′ interactions were investigated in certain agammaglobulinemia porcine, bovine, and human sera. LPS isolated from *Veillonella alcalescens, Serratia marcescens,* and *Salmonella minnesota* were reacted with precolostral piglet serum containing $< 2.5 \times 10^{-6}$ mg/100 ml γ-globulin and with sow serum (500 mg/100 ml γ-globulins) derived from pathogen-free Minnesota miniature pigs. Comparable C′ fixation was observed in both groups of specimens. Over 80% of the piglet C′ was fixed, neutrophil chemotactic factors and anaphylotoxin were produced, and characteristic C′-mediated lesions appeared on LPS. Similar results were obtained when LPS was incubated with immunoglobulin-deficient sera from (*a*) developing bovine embryos as early as the 40 cm stage, (*b*) developing porcine embryos as early as the 90th gestational day, and (*c*) humans with Bruton and late-occurring types of agammaglobulinemia. The only sera regularly deficient in reactivity with LPS were from individuals with Swiss type lymphopenic agammaglobulinemia. However, unlike other human specimens, these sera were markedly deficient in the C′1q component of C′ and in "properdin." Hence, the basis of their impaired reactivity with LPS is not yet clear. We conclude that LPS-C′ interactions can occur in sera markedly deficient in classical immunoglobulins and perhaps without their participation. Alternative pathways for C′ activation are being investigated.

115. The Demonstration of Previously Undetected γG Autoantibody on Human Red Cells. BRUCE C. GILLILAND,* JOHN P. LEDDY,* AND JOHN H. VAUGHAN,** Rochester, N. Y.

RBC showing positive direct Coombs reactions with anticomplement (C′) sera but not with anti-γ-globulin sera have been described in patients with connective tissue diseases or acquired hemolytic disease (AHD). Attachment of C′ to RBC surfaces by nonimmune mechanisms has been postulated. Alternatively, very small amounts of C′-fixing antibodies undetectable by ordinary methods may be involved. A more sensitive method for detection and quantification of γG globulin on RBC was developed by combining quantitative C′ fixation with the antiglobulin consumption technique. As few as 25 molecules of cell-bound γG globulin can be detected, which is at least a 25-fold increase in sensitivity over the Coombs test. Normal RBC have invariably shown less than 30 molecules of γG per RBC. A positive direct Coombs test in our hands requires more than 700 molecules of γG per RBC. C′-coated RBC in 5 of 6 systemic lupus erythematosus (SLE) patients and 4 of 6 AHD patients showing negative Coombs reactions with potent anti-γG serum exhibited 48–625 molecules of γG per RBC. RBC that were Coombs negative with both anti-C′ and anti-γG reagents were shown to have 47–119 molecules of γG per RBC in 9 of 10 SLE patients, 3 of 9 patients on Aldomet therapy, 3 of 5 AHD patients in clinical remission, and 0 of 10 patients with rheumatoid arthritis. In 10 patients, eluates were prepared from 100–250 ml of blood and concentrated 50- to 100-fold. All 10 eluates gave positive classical indirect anti-γG Coombs reactions with human RBC. Three of these eluates were from patients whose RBC had shown only 50–54 molecules of γG per RBC. Eluates from 3 SLE patients so tested gave negative indirect Coombs tests with rabbit and sheep RBC. It is likely that (1) few cases of RBC sensitization by C′ have a nonimmunologic basis, and (2) RBC autosensitization occurs in SLE even more frequently than has previously been recognized.

116. Nitrogen Loss in Hemodialysis. H. EARL GINN,* ANN B. FROST,* B. J. MATTER,* AND WILLIAM W. LACY,* Nashville, Tenn. (introduced by David E. Rogers).

The rate of nitrogen loss in hemodialysis patients is significantly greater than in most chronic uremics. The diet of dialysis patients must, therefore, be altered to allow replacement of essential substances, particularly amino acids, that are removed during dialysis. Studies on clearances of individual amino acids showed that the dialyzing capacity of the amino acids is similar to that of creatinine and that as much as 20% of the nitrogen of the dialysate solution is α-amino nitrogen. In patients undergoing twice-weekly hemodialysis on limited amounts of high-quality protein (HQP), 0.5 g protein/kg per day, α-amino nitrogen loss was sufficiently great to reduce the plasma total α-amino nitrogen by 50% after 1 month of dialysis and to decrease the level of all essential amino acids in the plasma. On this diet the patients further showed a fall in serum albumin and were in negative nitrogen balance. When the protein content of the diet was increased by adding low-quality protein (LQP), a striking increase in blood urea levels resulted, accompanied by a fall in serum albumin and a negative nitrogen

balance. When the protein content of the diet was 0.75 g HQP/kg per day or greater, nitrogen balance was positive and the plasma total α-amino nitrogen and serum albumin levels were normal. It was found that in anephric patients an increase in HQP above 0.75 g/kg per day produced an increase in plasma urea without significantly altering nitrogen balance, a result which suggests an optimum level of protein intake at 0.75 g/kg per day for anephric patients on twice-weekly dialysis.

117. Clinical Experience with Lipid Dialysis. H. Earl Ginn,* Billy J. Matter,* James H. Shinaberger,* and Joel B. Mann,* Nashville, Tenn., Los Angeles, Calif., and Miami, Fla. (introduced by A. Gorman Hills**).

Dialysis is a well established procedure in the treatment of many drug or chemical intoxications. However, many such intoxicants with high oil solubility, such as glutethimide, are relatively inefficiently removed by standard dialysis utilizing aqueous solutions. In an effort to increase removal of these compounds, we developed a lipid dialysate system utilizing soybean oil, found to be effective, nontoxic, inexpensive, and readily available. Owing to the high lipid:water partition coefficients of these compounds, e.g., 100:1 for glutethimide, 56:1 for seconal, etc., only 5–10 liters need be used. Kiil, Klung, and twin-coil dialyzers have been successfully utilized. This technique has now been successfully employed in the dialytic treatment of severe intoxications from glutethimide, secobarbital, pentobarbital, trifluoperazine (TFP), and camphor with no adverse effects. Eight comatose, areflexic, hypotensive patients who had ingested 8–30 g of glutethimide from 8 to 36 hours before dialysis were treated, with six survivals. One patient with severe secobarbital intoxication was treated successfully as the blood level dropped from 8 to 1 mg/100 ml during a 6 hr dialysis. Another patient with pentobarbital intoxication had a similar response. Aqueous and lipid dialysate systems were connected in series to manage successfully a young woman who had ingested both meprobamate and TFP. An elderly man who had mistakenly ingested 12 g of camphor regained consciousness after the removal of 6.5 g during a 4 hr dialysis. The use of lipid dialysate requires minimal modification of standard hemodialysis equipment and should be useful for removal of many toxic agents.

118. Effects of Intraduodenal Perfusions of Dextrose, Fatty Acid, and Amino Acid on Total Pancreatic Enzyme Output in Man. Vay L. W. Go,* Alan F. Hofmann,* and W. H. J. Summerskill,* Rochester, Minn. (introduced by Eric E. Wollaeger**).

To compare pancreatic enzyme responses to defined stimuli, we developed a method which measures total pancreatic enzyme output. Through a four-lumen tube, positioned fluoroscopically, markers were perfused into the gastric fundus ($^{51}CrCl_3$) and proximal duodenum (polyethylene glycol). Determinations of marker concentration in aspirates from the antrum and distal duodenum permitted calculation of the fractions of gastric content entering the duodenum (11.3 ± 2.2%, mean ± SE; 228 20 min collections), of duodenal reflux into the stomach (8.3 ± 1.3%), and of recovered duodenal content (28.9 ± 2.5%). These figures, together with those of enzyme determinations on duodenal samples, were used to compute total output of lipase, trypsin, and amylase in response to isotonic solutions of saline, dextrose (277 mM), fatty acid (10 mM micellar monoolein and oleic acid), and amino acid (simulated beef hydrolysate of 18 mM, 182 mM, or 272 mM) perfused in random order in 14 normal fasting subjects. The response to dextrose during the 1st hour (units × 10^3/hr) (trypsin 15.9 ± 3.4, amylase 86.9 ± 10.1, lipase 57.3 ± 12.2) was significantly greater than that to saline (trypsin 5.7 ± 1.6, amylase 50.0 ± 14.5, lipase 26.8 ± 4.3) ($P < 0.05$), but enzymes fell to control levels during the 2nd hour (trypsin 5.2 ± 1.2, amylase 59.8 ± 4.2, lipase 27.1 ± 4.8) ($P > 0.10$). The 1st-hour responses to fatty acid (trypsin 19.1 ± 2.8, amylase 117.0 ± 15.8, lipase 67.3 ± 8.4) and amino acid (trypsin 17.9 ± 1.9, amylase 99.0 ± 19.4, lipase 68.3 ± 8.5) were similar to those to dextrose ($P > 0.10$), but remained elevated during the 2nd hour of perfusion (fatty acid: trypsin 10.7 ± 0.6, amylase 68.2 ± 2.5, lipase 48.7 ± 2.7; amino acid: trypsin 17.0 ± 1.1, amylase 104.3 ± 6.2, lipase 72.8 ± 8.4) ($P < 0.05$). All three hydrolytic products caused an initial increase in enzyme output and possibly a discharge of preformed enzymes, but only fatty acid and amino acid caused continued enzyme secretion equal to that reported for maximal exogenous pancreozymin stimulation. This technique supplements previous methods which measure changes in concentration alone, and should permit quantitative evaluation of pancreatic function in disease.

119. Measurement of Plasma Angiotensin II and Correlation with Renin and Aldosterone in Normal and Hypertensive Man. David J. Gocke,* Inge Oppenhoff,* Joan N. Gerten,* Louis M. Sherwood,* and John H. Laragh, New York, N. Y.

To measure plasma angiotensin a very sensitive and specific radioimmunoassay has been developed, making it possible for the first time to demonstrate a close positive correlation between renin activity and angiotensin in a variety of physiologic circumstances. Furthermore, when highly purified human renin is incubated with plasma in vitro, the amounts of angiotensin measured by bioassay and by this immunoassay are identical, providing additional validation of the procedure. Antibodies were produced by immunization of rabbits with aspartyl-angiotensin II–albumin complex. Dextran-coated charcoal was utilized to separate antibody-bound from free ^{125}I-angiotensin. The technique has an absolute sensitivity of 1 picogram ($\mu\mu$g). Angiotensin is routinely detectable in 0.1 ml or less of unextracted normal plasma. In addition to having greater sensitivity, this method is an improvement in that losses from extraction and concentration are avoided. Subjects were studied under conditions of controlled sodium balance, and parallel measurements of plasma renin and aldosterone secretion were made. In normals, on unrestricted diets, angiotensin

ranged from 2.5 to 100 $\mu\mu$g/ml. After sodium depletion, values were 100–300 $\mu\mu$g. Concurrently, renin activity increased from 0.4–1.8 ng/ml per hr to 8–18 ng/ml per hr. Commensurate changes in aldosterone occurred. Angiotensin and renin levels were rapidly depressed to subnormal values by saline infusions. In 12 patients with essential hypertension, basal angiotensin values were within the normal range. In 3 hypertensives, diazoxide induced serial and parallel increases in renin and angiotensin as blood pressure fell. Plasma angiotensin was markedly increased in renal artery stenosis, malignant hypertension, and especially in juxtaglomerular hyperplasia. High values, unexplained by concurrent renin levels, were observed in 3 females and 2 males given oral contraceptives. These data document the role of plasma angiotensin in the renin-aldosterone interaction. The ability to measure minute quantities of circulating angiotensin in human plasma and correlate these values with pressor activity will also facilitate differentiation of hypertensive disorders.

120. Separation of Humoral from Hemodynamic Factors Affecting Proximal Sodium Reabsorption during Blood Volume Expansion. MARTIN GOLDBERG, BARRY B. STAUM,* AND JAMES L. CRISTOL,* Philadelphia, Pa.

Acute expansion of the vascular volume results in a rise in Na excretion ($U_{Na}V$) secondary to reduced tubular Na reabsorption (T_{Na}). Although a natriuretic hormone has been postulated, such a factor has not been shown to be physiologically important in the absence of changes in renal hemodynamics or plasma proteins. To isolate the factors affecting proximal T_{Na}, studies were performed in dogs in which one denervated kidney was perfused in situ with blood from the dog's own femoral artery through a blood pump. Renal blood flow (RBF) and perfusion pressure (PP) were rigorously controlled during spontaneous changes in renal vascular resistance (RVR). Ethacrynic acid and chlorothiazide were administered to inhibit distal T_{Na}. Eight dogs were acutely expanded with 500 ml saline intravenously, and eight received 500 ml isooncotic human or dog plasma. Urinary water and electrolyte losses were replaced, so that observations were made during steady-state sustained expansion at two levels of controlled PP to the experimental kidney: one level at the preexpansion value (equal to aortic P) and the second at reduced PP, RBF, GFR, and filtered load (F_{Na}). Saline loading produced a natriuresis (mean $\Delta T_{Na}/F_{Na} = -12\%$) at stable PP and also an absolute increase in $U_{Na}V$ at reduced PP, RBF, and F_{Na}. No consistent change occurred in RVR. Plasma loading also increased $U_{Na}V$ at unchanged PP. Although RVR fell transiently, it rose again above control values during steady-state expansion when mean $U_{Na}V$ was 78% above control and mean T_{Na}/F_{Na} was reduced 7% ($P < 0.01$). At lowered PP (−17%), reduced GFR (−7%), and reduced RBF (−19%), RVR was increased +6% while $U_{Na}V$ was still 47% above control. Infusion of red blood cells to raise hematocrit to control values did not alter T_{Na}/F_{Na}. The data clearly reveal the presence of a humoral factor which decreases proximal T_{Na} in the expanded animal while all known hemodynamic factors have been controlled.

121. Comparison of Lysostaphin and Oxacillin as Initial Therapy in Canine Staphylococcal Endocarditis. LEONARD M. GOLDBERG,* CHATRCHAI WATANAKUNAKORN,* AND MORTON HAMBURGER,** Cincinnati, Ohio.

Lysostaphin, a lytic enzyme that hydrolyzes staphylococcal cell walls, has a salutory effect in the early treatment period of *Staphylococcus aureus* endocarditis in dogs. We have examined retrospectively the early treatment period of dogs with endocarditis given oxacillin or intravenous lysostaphin. Numbers of staphylococci in blood and tissue, the clinical course, and the mortality were studied in comparable animals from each group. 43 untreated dogs served as controls; 34 were killed at the time therapy would ordinarily start, and 9 were permitted to die in the natural course of the infection. Lysostaphin treatment improved dogs clinically faster than oxacillin. The initial reduction in circulating staphylococci was comparable for the two drugs. Lung, liver, spleen, kidney, and myocardium were sterile in 57%, 9%, and 0% of specimens from dogs given oxacillin, lysostaphin, and nothing, respectively. Cultures of aortic and mitral valves were sterile in less than 2% of untreated dogs and in 50% of dogs treated 5 or 6 days with either drug. Nonsterile valve specimens grew more than 1×10^5 staphylococci/g in 1 of 4 specimens from lysostaphin-treated and 4 of 4 specimens from oxacillin-treated dogs. Staphylococci were observed in stained sections of heart valves of 82% of 34 untreated dogs, 78% of 9 dogs treated with oxacillin for 5–7 days, and 12.5% of 8 dogs treated with lysostaphin for 4–6 days. Death occurred within 4 days of the time dogs were considered ill enough to treat in 75% of 8 untreated dogs, 21% of 37 oxacillin-treated dogs, and 0% of 8 lysostaphin-treated dogs. Although oxacillin excelled in eradicating staphylococci from lung and solid tissues, lysostaphin was superior in producing more rapid clinical improvement, eradicating staphylococci from heart valves, and reducing mortality in the early treatment period of canine endocarditis.

122. Rapidity of Renal ^{42}K Exchange: Tissue Studies of Isotope Deposition and Washout. ARTHUR G. GOLDMAN,* A. AARON YALOW,* HAROLD GARELICK,* AND C. EVANS PATRICK,* New York, N. Y. (introduced by Louis Leiter**).

^{42}K was injected into the right renal artery of 10 dogs and circulation to the right kidney was occluded 0.25–10 min later. A semilogarithmic plot of each kidney's specific activity (SA) as a function of time of occlusion gave a 10-point disappearance curve for renal ^{42}K, whose fractional decrease rate, λ, was evaluated by least squares analysis. Each kidney's fractional potassium turnover rate, k, was calculated as (arterial plasma potassium concentration) (P-aminohippurate (PAH) clearance per mEq renal potassium). The mean k ($\bar{k}$) was found to equal λ: $\bar{k} = 0.159 \pm 0.037$ min^{-1} (SD),

$\lambda = 0.157 \pm 0.020$ min^{-1} (sp of estimate). In two animals, initial and final SA were determined using ^{42}K and ^{86}Rb as tracers for potassium. The radioactivity loss rate, λ, again agreed closely with k: dog 83, $\lambda = 0.154$ min^{-1}, k = 0.157 min^{-1}; dog 84, $\lambda = 0.121$ min^{-1}, k = 0.122 min^{-1}. On the other hand, the renal ^{42}K content 0.25–0.7 min after arterial injection was found to be 15–20% lower than the arteriovenous extraction ratio of PAH or renal content of simultaneously injected ^{131}I-iodo-Hippuran. We conclude that after bolus injection of ^{42}K into the renal artery, a significant fraction of the isotope rapidly diffuses back into the plasma, possibly from extracellular fluid, as the bolus passes down the renal capillary. During washout of the deposited isotope, plasma SA changes only slowly, and ^{42}K disappearance is determined solely by the rate at which the potassium in arterial plasma flows through the organ. ^{42}K exchange with renal potassium is thus limited by the plasma flow rate during isotope washout but not during isotope deposition after bolus injection.

123. Analysis of Late Distal Tubular Function during Saline and Urea Infusions in Dog. MARVIN H. GOLDSTEIN,* JOHN K. MAESAKA,* HERBERT I. JERNOW,* AND MARVIN F. LEVITT,** New York, N. Y.

Stop flow studies were performed in hydropenic dog to evaluate the proposal that sustained saline infusions limit distal sodium transport. In one group 2.5% saline was infused for 2 hr; in the second group 3.6% urea was infused. U/P inulin, UOsm (mOsm/kg), U_{Na} (mEq/liter), and Uurea (mmoles/liter) were measured during free flow, and in the initial specimens after 3 or 8 min of ureteral obstruction. In 12 saline studies, U/P inulin, UOsm, and U_{Na} averaged 7.5, 473, and 245 during free flow, and 8.0, 495, and 243 after 3 min obstruction, respectively. When saluresis was produced by distal tubular blockade (thiazide and ethacrynic acid), postobstruction U/P inulin and UOsm also remained unchanged as compared with free flow. During six urea infusions, U/P inulin, UOsm, and Uurea averaged 7.0, 465, and 338 in free flow and increased after 3 min obstruction to 12, 552, and 474, respectively. U_{Na} fell, however, from 52 to 39. Thus, with 3 min ureteral obstruction during urea infusions some 50% of the late distal tubular sodium and water was reabsorbed, but less than 10% was reabsorbed during saline infusions. When stop flow was prolonged for 8 min during saline infusions, U/P inulin increased from 9.3 to 24.5, UOsm from 474 to 558, with little change in U_{Na}. During urea infusion, with 8 min obstruction, U/P inulin increased from 11.5 to 32, UOsm from 465 to 825, and Uurea from 445 to 770. After prolonged ureteral obstruction, therefore, 62% of the distal tubular sodium and water was reabsorbed during saline infusions and 64% during urea infusions. These data suggest that saline infusions limit distal tubular sodium reabsorption during free flow but that this limit disappears with prolonged ureteral obstruction. In addition, when distal reabsorption continues during stop flow, postobstruction Uurea and UOsm increase comparably, implying that stop flow does not impair the capacity of the medulla to trap solute.

124. Regulation of Glycolysis by K^+ Transport across Cell Membrane and Mitochondrial Membrane. EDWIN E. GORDON, New York, N. Y.

Stimulation of glycolysis by active monovalent cation transport has been convincingly documented for erythrocytes. In respiring cells, there is an energy-dependent mitochondrial K^+ transport system in addition to the cell membrane K^+ transport system. The cell membrane system is coupled to Na^+ transport, is ATP dependent, and is inhibited by ouabain; the mitochondrial system is activated by valinomycin (a dodecadepsipeptide antibiotic), is energy dependent (but ATP independent), and is insensitive to ouabain. In the present study, the influence of each of these two K^+ transport mechanisms on the rate of glycolysis was examined. Ehrlich ascites tumor cells were harvested from the peritoneal cavity of mice 7–10 days after inoculation and depleted of K^+ by repeated washing in a K^+-free medium at 5°C. The depleted cells were incubated aerobically at 37°C in K^+-free medium containing 5 mM glucose. Stimulation of the cell membrane system by addition of K^+ (5.1 mM) resulted in an increase in glucose utilization from 3.4 to 10.4 mμmoles/mg protein per min and in lactate production from 5.6 to 15.9 mμmoles/mg protein per min. Inhibition of cell membrane K^+ transport with ouabain (10^{-3} M) reduced the rate of glucose utilization and lactate production to values slightly above those found in the K^+-depleted state. Stimulation of mitochondrial K^+ transport with valinomycin (10^{-7} M) increased glucose utilization to 24.8 mμmoles/mg protein per min and lactate production to 39.8 mμmoles/mg protein per min in K^+-repleted cells, but not in K^+-depleted cells. When cell membrane K^+ transport was blocked with ouabain, stimulation of mitochondrial K^+ transport enhanced glucose utilization to 13.3 mμmoles/mg protein per min and lactate production to 18.7 mμmoles/mg protein per min. These experiments lead to the conclusion that cell membrane and mitochondrial transport of K^+ are about equally effective in regulating the rate of glycolysis in respiring Ehrlich ascites tumor cells.

125. Protection of Alveolar Macrophages from the Cytotoxic Activity of Cigarette Smoke by Glutathione and Cysteine. GARETH M. GREEN,* Boston, Mass. (introduced by Edward H. Kass**).

The depressant action of cigarette smoke on the phagocytic activity of alveolar macrophages may be a significant mechanism in the pathogenesis of certain pulmonary diseases. The mechanism of this toxic depression is unknown. Because cigarette smoke contains many substances that might have an oxidant activity in biologic systems, various reducing agents were examined for their protective activity against cigarette smoke in an in vitro system. Rabbit alveolar macrophages, staphylo-

cocci, and cigarette smoke were admixed in tissue culture flasks according to a method previously described. Macrophage activity was assessed by measurement of rates of phagocytic destruction of the staphylococci. Glutathione and cysteine at concentrations of 2.5 μmoles/ml provided complete protection against phagocytoxic quantities (6 ml) of cigarette smoke. Further quantitation showed that 0.4–0.8 μmoles of cysteine and somewhat less of glutathione were required per milliliter of cigarette smoke for this protection. The toxic effect of cigarette smoke on the macrophages and the protective action of glutathione and cysteine required the presence of protein in the flask medium; comparable effects were found when bovine serum albumin was substituted for autologous rabbit serum. The rapid drop in redox potential caused by cigarette smoke in aqueous protein solutions was moderated by glutathione. However, phagocytosis was unaltered by changes in or levels of redox potential per se. A more specific action of cigarette smoke on sulfhydryl groups was suggested by the observation that neither the disulfide forms of glutathione and cysteine nor reducing agents such as ascorbic acid and ferrocyanide showed any protective effect. This proposed action of cigarette smoke might serve as a prototype for the damaging action to lung tissues caused by certain other oxidant gases and air pollutants.

126. Evidence for Hydrogen Ion Transport by the Turtle Bladder in the Presence of Ambient Bicarbonate. HOWARD H. GREEN,* PHILIP R. STEINMETZ,* AND HOWARD S. FRAZIER, Boston, Mass.

Acidification by the isolated urinary bladder of the water turtle *Pseudemys scripta elegans* could be accomplished either by secretion of H^+ into, or transport of HCO_3^- out of, the medium bathing the mucosal surface. The observation of acidification of bathing fluid which is free of added CO_2 or HCO_3^- supports the former hypothesis, whereas the report of a reduction of P_{CO_2} in HCO_3^--containing mucosal media which had been acidified bl bladder sacs is strong evidence for the latter mechanism. Direct measurements of the P_{CO_2} in the mucosal medium were performed by mounting over the end of a P_{CO_2} electrode bladder sacs containing 6–8 ml of 25 mM HCO_3^- Ringer's solution. The preparation was suspended in a constant-temperature bath of Ringer's solution identical with the mucosal medium except for the addition of 2 mM glucose. The serosal medium was stirred continuously by bubbles of 2.5% CO_2 in air, and the transbladder potential difference and P_{CO_2} of the mucosal medium were monitored during the ensuing 5–6 hr of incubation. The bladder sac was then removed from the electrode and the latter was allowed to equilibrate with the serosal medium. In seven experiments, the mean P_{CO_2} of the mucosal medium was 1.8 ± 0.36 mm Hg ($P < 0.005$) greater than that of the serosal medium. This result supports the hypothesis that acidification by the isolated turtle bladder results from the transport of H^+ into, rather than removal of HCO_3^- from, the mucosal medium.

127. Meningococcal Carrier State in "Normal" Families. SHELDON GREENFIELD* AND HARRY A. FELDMAN,** Syracuse, N. Y.

This study of the epidemiology of the *N. meningitidis* carrier state in a population of "normal" families was initiated in December 1966 in Syracuse, New York. Posterior nasopharyngeal swabs are obtained bimonthly (seven surveys have been completed) from approximately 200 members of 37 families. Prevalence rates, despite loss of carriage and new acquisitions, have remained relatively constant, ranging from 3 to 8%. The cumulative incidence of carrier acquisitions is 13%. More than half of the carriers have remained so for 6 months or longer. More males than females and more adults than children (<20 yr) are carriers. When the two factors are combined, 15–26% of males aged 20 and over are found to be carriers, whereas no more than 3% of female children were positive in any one survey. Of the 15 families which had carriers in at least one survey, 8 had one and only 3 had three or more (median family size five to six persons). This experience suggests that in ordinary families, in a nonepidemic period, meningococcal carrier rates are not high. No cases of meningococcemia or meningitis have been detected in this population during this study. All meningococci isolated were of group B except for a few nongroupables. Of the meningococci isolated, 28% were resistant to 0.1 mg/100 ml or more of sulfadiazine. Since cases of meningococcal disease often occur in children, the data suggest that carriage of meningococci without further invasion may be associated with immunity. Similar data have been collected from persons attending a rheumatic fever prophylaxis clinic. The meningococcal carrier state in them has been independent of the kind of streptococcal prophylaxis provided.

128. Viral Induction of Hyperlipidemia. SIDNEY E. GROSSBERG,* Milwaukee, Wis. (introduced by W. W. Stead**).

A striking hyperlipidemia could be induced in chicken embryos as a result of infection with certain RNA viruses containing structural lipoproteins. A number of viruses and other microbial agents were tested, and the greatest increase in plasma lipids, manifest as progressive lactescence, was produced by the immunologically related subgroup B arboviruses, viz., Japanese encephalitis (JE), St. Louis encephalitis (SLE), or West Nile (WN) viruses. After inoculation into the allantois, an extraembryonic excretory organ, they produced disseminated infection. In normal embryos, ether/ethanol-extractable plasma lipid tended to increase from 8–9 mg/ml to 10–11.5 mg/ml; plasma triglycerides, phospholipids, cholesterol, and free fatty acids normally decreased with time, whereas cholesterol esters increased, as measured by thin-layer chromatography. During JE viral infection, total plasma lipid weighed as much as 30 mg/ml, and both triglycerides and phospholipids increased as cholesterol esters fell. An increase in β-lipoproteins and chylomicrons was revealed by electrophoresis. Intravenous administration of heparin failed to clear lactescent plasma,

suggesting a deficit in lipoprotein lipase. Plasma glucose values rose abnormally as shown by the *d*-glucose oxidase test. Insulin administration did not clear the hyperlipidemia. Hepatomegaly was associated with histologic evidence of a hepatitis detectable by day 3–4; no histologic lesions in brain were noted. After SLE and WN viral infections, total extractable lipids increased to attain maximum levels of 30 mg/ml and 65 mg/ml, respectively; further, in these two infections only triglycerides disproportionately increased. It is proposed that dynamic imbalances of lipid anabolism and catabolism result from enzymic alterations induced during viral replication primarily in liver cells infected with certain RNA, lipid-containing viruses.

129. Metabolism of Cyclic AMP by Epithelial Cells of the Toad Bladder. PAUL F. GULYASSY,* San Francisco, Calif. (introduced by J. H. Comroe, Jr.).

The antidiuretic action of vasopressin is mediated within the target cell by adenosine-3′,5′-monophosphate (cyclic AMP). The chemical steps by which cyclic AMP changes resistance of membranes to flow of water along osmotic gradients is unknown. To gain insight into this final phase in the antidiuretic pathway, we studied the chemical fate of cyclic AMP in one target tissue, the toad bladder. In whole bladders incubated with ^{3}H–cyclic AMP, more than 95% of radioactivity was extracted by 5% TCA and consisted of residual ^{3}H–cyclic AMP, ^{3}H–5′-AMP, ^{3}H-ADP, and ^{3}H-inosine. In contrast, on exposure of ^{3}H–cyclic AMP to a crude homogenate of the epithelial cells scraped from the mucosal face of the bladder, the principal metabolite was ^{3}H-inosine, whereas ^{3}H–5′-AMP was either absent or present in undetectable amounts. Metabolism of ^{3}H–cyclic AMP by homogenates of the epithelial cell scrapings from the bladder was strongly stimulated by alkalinization over the pH range 6–9. Theophylline inhibited metabolism of cyclic AMP only to a limit of 50%, the inhibition being limited to the OH^--stimulated component. These results suggest the possibility that a second pathway for metabolism of cyclic AMP may exist. If such is the case, its relationship, if any, to the ultimate biological effects of cyclic AMP within cells remains to be defined.

130. The Effect of Bilateral Nephrectomy upon Hemodynamics and Body Composition in Chronic Renal Failure. C. L. HAMPERS,* R. M. ZOLLINGER, JR.,* J. J. SKILLMAN,* J. R. W. GUMPERT,* G. L. BAILEY,* AND J. P. MERRILL,** Boston, Mass. (introduced by James P. O'Hare**).

Six patients with severe chronic renal failure (CRF) and hypertension were evaluated before and again 1 and 4 months after bilateral nephrectomy while being maintained with regular hemodialysis. Body composition was also measured with each study. A mean femoral artery BP of 142 mm Hg fell to 136 mm Hg (P NS) at 1 month and to 120 mm Hg at 4 months ($P < 0.005$). This was mainly reflected in a drop in mean diastolic pressure of 13 mm Hg at 4 months ($P < 0.025$). There was a slight fall in total peripheral resistance with time, but resting cardiac index (CI) did not appear to be affected. After nephrectomy (4 months) there was a greater rise in BP over resting levels with standard exercise ($P < 0.005$). In comparing the CI calculated from the femoral artery with that from the brachial artery (A-V shunt), a close correlation ($r = 0.97$) was found in the 18 pairs. Changes in exchangeable Na^+ ($^{24}Na^+$), extracellular fluid (ECF) ($^{82}Br^-$), total body water (THO), and Ke ($^{42}K^+$) did not correlate with the change in BP. Plasma volume, on the other hand, showed a tendency to fall with the decrease in mean BP after nephrectomy despite constant body weight ($P < 0.1$). It is concluded that bilateral nephrectomy has a salutary effect on the hypertension of CRF, but that this effect may not be immediately apparent. The fall in BP would appear to be independent of changes in ECF and exchangeable Na^+ and to correlate only roughly with a fall in plasma volume.

131. Mechanism of Effort Syncope in Aortic Stenosis. E. W. HANCOCK,* M. D. FLAMM,* B. E. BRANIFF,* AND R. W. KIMBALL,* Palo Alto, Calif. (introduced by Herbert N. Hultgren**).

There are few observations during exercise in patients with aortic stenosis to support the usual explanations for effort syncope—sudden arrhythmia or inability to increase cardiac output. On the basis of exercise studies during supine right heart catheterization in 36 patients, supine left heart catheterization in 19, and upright treadmill exercise in 23, a third mechanism is suggested as the most frequent explanation. Syncope or hypotension did not occur during supine exercise, but during treadmill exercise 5 of 23 patients showed a decrease in arterial systolic pressure of 20 mm Hg or more, and 2 developed near syncope associated with marked hypotension of brief duration. The cardiac output in one of these patients increased from 4.6 liters/min at rest to 14.5 during treadmill exercise, with an abrupt fall to 4.4 at the time or near syncope. In another patient with a history of effort syncope, the cardiac output rose from 5.3 liters/min at rest to 14.9 during treadmill exercise. Cardiac output during supine exercise increased normally in 12 of 55 patients and failed to rise at all in only 3 patients, all of whom had advanced congestive heart failure. Left atrial or pulmonary artery wedge mean pressure increased abnormally in 52 of 55 cases, and left ventricular end diastolic pressure increased abnormally in 18 of 19 cases, exceeding 35 mm Hg in 6 patients. Significant arrhythmias did not occur in any of these studies. We conclude that effort syncope in aortic stenosis occurs in association with acute left ventricular failure which develops at critical levels of exercise in the upright position. Cardiac output falls abruptly, as if a descending limb of Starling's curve were transiently entered; in the presence of the vasodilatation of exercise this results in a sharp fall in arterial pressure and sharp reduction of cerebral blood flow.

132. The Effect of Aldosterone on the Permeability Response of the Toad's Urinary Bladder to Vasopressin. J. S. HANDLER, S. URAKABE,* A. PRESTON,* AND J. ORLOFF,** Bethesda, Md.

Aldosterone is known to increase the rate of sodium transport (short-circuit current (scc)) by the toad bladder and the scc response to vasopressin. In the present studies it has been observed that the osmotic water flow response (P_{os}) to vasopressin and cyclic 3',5'-AMP of steroid-depleted bladders (incubation for 16 hr) is considerably less than that of aldosterone-treated paired tissues. Base-line P_{os} and diffusional permeability to H_2O (P_{H_2O}) are unaffected by steroid depletion or by aldosterone. However, a fall in diffusional permeability to urea (P_{urea}) is observed in steroid-depleted bladders, which is prevented by aldosterone. As with P_{os}, the effect of vasopressin on P_{urea} is greater in aldosterone-treated tissue. Adrenal glucocorticoid hormones have a greater effect on the P_{os} response to vasopressin than on base-line scc, whereas the reverse is true for aldosterone. For example, when concentrations of aldosterone and corticosterone which have equal effects on the P_{os} response to vasopressin are tested on scc, the effect of aldosterone is greater. Conversely, when concentrations of aldosterone and dexamethasone with equivalent effects on scc are examined for their effects on the P_{os} response to vasopressin, dexamethasone is more active. Thus, in addition to their effects on sodium transport, the adrenal steroids also influence the vasopressin-induced changes in osmotic permeability to H_2O and diffusional permeability to urea. The latter changes may be primarily related to the glucocorticoid activity of the steroids.

133. Mechanisms of Human Trombokinetics. L. A. HARKER* AND C. A. FINCH,** Seattle, Wash.

Total thrombopoiesis has been calculated from the total number of megakaryocytes multiplied by their volume (total megakaryocyte mass), and effective thrombopoiesis from platelet turnover (platelet count/platelet survival) using methods previously described. Megakaryocytopoiesis was also evaluated in terms of altered nuclear lobe number and the directly related cytoplasmic volume changes. The results of such measurements in 75 selected patients characterize the quantitative and qualitative aspects of thrombopoiesis. In patients with thrombocytopenia due to increased platelet destruction, measurements of total and effective production were identical at rates of 2–4 times normal acutely, and 4–10 times normal in chronic states. Ineffective thrombopoiesis, amounting to as much as 90% of the total production, was found in B_{12} and folate deficiency, in di Guglielmo's syndrome, and as an isolated defect producing thrombocytopenia. Increased thrombopoiesis was accomplished by an increase in cytoplasmic volume of the individual megakaryocytes and/or an increase in the number of megakaryocytes. For example, in 14 patients with ITP megakaryocyte number increased 3-fold and megakaryocyte volume doubled. On the other hand, in 5 patients with the thrombocytosis of infection or malignancy there was a 3-fold increase in megakaryocyte number, but cytoplasmic volume was decreased by a third. Characterization of these two independent mechanisms permits differentiation of clinical disorders of platelet production.

134. Pressure-Flow Relationships in Atrial Fibrillation. ALEXANDER HARLEY* AND JOSEPH C. GREENFIELD, JR., Durham, N. C.

Atrial fibrillation is characterized by irregular variation in cycle length and the absence of an atrial contribution to ventricular filling. The inability to measure accurately either phasic aortic blood flow or instantaneous left ventricular volume has limited the scope of earlier work on the hemodynamics of atrial fibrillation. In 13 patients having atrial fibrillation, phasic pressure and flow were measured in the ascending aorta by the pressure gradient technique. Relationships between cycle length (previous RR interval), stroke volume, peak velocity, peak flow, pulse pressure, stroke work, peak power, and the phases of systole were evaluated statistically. Through a wide range of stroke volume there was little change in the duration of total systole in an individual patient. Duration of ejection (DE) varied directly with stroke volume (SV) whereas preejection period varied inversely, and both showed good correlation with stroke volume, stroke work, and peak power. The pooled data from all 13 patients gave the relationship $\log_e SV = 2.912 \log_e DE + 8.386$ ($r = 0.91$). There was marked variation from patient to patient in the duration of both the preejection period and total systole. Correlation of stroke volume with previous cycle length varied from $r = 0.53$ to $r = 0.93$ for individuals and was $r = 0.57$ for the group. In six patients with continuous data, a better correlation was found between stroke volume and the two previous cycle lengths (range $r = 0.72$–0.96) than with the preceding cycle length alone. The relationship between pulse pressure and stroke volume was good for individuals but poor for the group ($r = 0.75$). No relationship was noted between the size of stroke volume and the percentage ejected during the first half of ejection. The beat-to-beat measurement of aortic blood flow and pressure has allowed a closer analysis of the determinants of the phases of systole during atrial fibrillation than was previously possible.

135. Stimulation by Colchicine of Collagenase Production by Rheumatoid Synovium in Culture. EDWARD D. HARRIS, JR.,* JOHN M. EVANSON,* AND STEPHEN M. KRANE, Boston, Mass.

It has been shown in this laboratory that rheumatoid synovial tissue survives in serum-free culture medium and releases an enzyme which degrades native collagen at neutral pH. Below 27°C collagen molecules in solution are cleaved into three-quarter and one-quarter length fragments, and at 37°C both collagen fibrils and native fibers are further broken down to low molecular weight polypeptides. To study mechanisms of production of collagenase in vitro, rheumatoid synovium obtained at

operation was incubated at 37°C in modified Eagle's medium. Medium harvested daily was analyzed for hydroxyproline and protein content; after dialysis and concentration, collagenase was assayed by the release of radioactivity from ^{14}C-labeled reconstituted collagen fibrils. Collagenase was usually undetectable until day 3 of culture and was maximal on days 4–6. Release of hydroxyproline from tissue collagen paralleled collagenolytic activity; total protein concentration in the medium decreased after day 2 of culture. Media from days 1 and 2 contained nondialyzable inhibitor of collagenase released on subsequent days. Addition of colchicine (0.1 μg/ml) to the medium each day resulted in a marked increase (approximately 3- to 10-fold) in collagenolytic activity. Colchicine addition *only* on day 1 was sufficient to increase collagenase release on days 3 through 5. Mitoses were not observed in sections of tissue incubated in the presence or absence of colchicine. Colchicine did not alter the pattern of $^{14}CO_2$ and ^{14}C-lactate production from 1-^{14}C-glucose added to the incubation medium on day 4 of culture. However, iodoacetate (10^{-3} M) added on day 4, when enzyme activity was already detectable, completely inhibited both glucose utilization and enzyme production. Neither iodoacetate (10^{-3} M) nor colchicine (0.1 μg/ml) affected activity of the isolated collagenase. These observations suggest that collagenase is synthesized by living rheumatoid synovial tissue in culture, and that colchicine, by some mechanism other than inhibition of cell division, stimulates production of this enzyme.

136. Inhibitory Effect of Kidney Homogenates from Uremic Human Subjects on Erythropoietin Activity. FRED E. HATCH,* JAMES W. FISHER,* BUYNG L. ROH,* AND BOBBY J. KELLEY,* Memphis, Tenn. (introduced by James W. Culbertson**).

Previous studies have suggested that erythropoietin (ESF) inhibition, possibly by some toxic product of uremia, may play a causative role in the pathogenesis of the anemia of chronic renal insufficiency (CRI). In the present study, human kidney homogenates from two patients with chronic glomerulonephritis and one patient with irreversible tubular necrosis of unknown etiology were injected in varying concentrations into mice made polycythemic according to a modification of the method of De Gowin and coworkers. Kidney homogenates from two normal human subjects were similarly prepared and injected as controls. 50 mg of the CRI kidney homogenate produced 31.5% and 76% inhibition of the effects of 0.2 units ESF on ^{59}Fe incorporation in RBC of polycythemic mice; 100 mg produced 100% and 80% ESF inhibition. In contrast, 200 mg of the normal kidney homogenate produced only 33% and 27% inhibition of ESF. Uremic plasma completely blocked the effects of ESF, whereas normal plasma accentuated ESF. Additional studies revealed that both renal cortical and medullary homogenates from patients with CRI produced significant inhibitory effects on ESF. The renal inhibitor was only partially inactivated by boiling, and appeared specific for kidney tissue, since human liver homogenate, prepared similarly to the kidney homogenate, produced no ESF inhibition. Using the technique of Schneider to separate the kidney subcellular fractions, the inhibitor was localized in the soluble cytoplasmic portion and was still present after in vitro dialysis. These data confirm the presence of an ESF inhibitor(s) in renal tissue and plasma of patients with CRI, which suggests a possible role of uremia in the mechanism of the anemia.

137. Effect of Epinephrine on Distribution of Radiothyroxine in Man. MARGUERITE T. HAYS* AND DAVID H. SOLOMON, Los Angeles and Torrance, Calif.

Ten normal young men were given repository epinephrine repeatedly for 4 days during the course of a radiothyroxine (^{131}I-T_4) disappearance curve. During epinephrine administration, serum ^{131}I-T_4 disappearance rate (k) decreased, fecal ^{131}I decreased slightly, and urinary iodide-131 "clearance" (excretion divided by serum ^{131}I) was initially unchanged but later decreased slightly. Hepatic ^{131}I, estimated by external counting, was constant. Indices of serum T_4-binding activity were studied. Serum TBG capacity increased 14% during epinephrine administration ($P < 0.01$). This increase appeared on day 2 of epinephrine and persisted at least 1 day after epinephrine was discontinued. TBPA capacity dropped 9%. Serum-free T_4 and resin T_3 uptake decreased from the 2nd day of epinephrine through the 1st postepinephrine day. Three indices, namely, free T_4, the reciprocal of the TBG capacity, and urinary ^{131}I-T_4 "clearance" changed almost exactly in parallel, suggesting that the increase in TBG is responsible for the later phase of the reduction in T_4 disappearance and catabolism. However, these changes are temporally dissociated from the decrease in k, which begins and ends abruptly with initiation or discontinuance of epinephrine administration. Comparison of the quantity of ^{131}I leaving the serum with that accounted for by various routes of clearance indicates that the decrease in net disappearance from serum during the first 2 days of epinephrine is not due to decreased efflux but to increased influx, a shift of ^{131}I-T_4 from tissues to serum. This shift is reversed during the first 2 postepinephrine days. Such shifts of ^{131}I-T_4 without concordant changes in urinary iodide-131 suggest a change in T_4 binding by cellular binding sites not involved in deiodination of T_4. This has led to a new physiologic model of T_4 distribution which postulates the presence of two types of cellular binding sites, one leading to deiodination and one not.

138. Mechanism of Sodium Excretion with Reduced Nephron Population. JOHN P. HAYSLETT,* MICHAEL KASHGARIAN,* AND FRANKLIN H. EPSTEIN, New Haven, Conn.

As the population of individual nephrons is reduced by renal disease, sodium excretion per nephron must increase to maintain normal sodium balance. This adjustment has been ascribed to a humoral, natriuretic "third factor" that decreases sodium reabsorption in the proximal tubule.

or, alternatively, to hyperfiltration at the glomerulus. Micropuncture studies were performed in well hydrated rats on high Na diets, (*a*) with intact kidneys, (*b*) after 50% nephrectomy, (*c*) after 80–90% nephrectomy, when BUN averaged 52 mg/100 ml. Sodium excretion per nephron increased 2-fold after uninephrectomy, while GFR per nephron rose 75%. When more kidney tissue was removed, sodium excretion per nephron increased to 5–6 times normal, even though there was no further rise in GFR per nephron. Hyperfiltration is therefore not responsible for the augmentation in sodium excretion by individual nephrons when renal mass is progressively reduced. The half-time of reabsorption in proximal tubules blocked with oil was entirely unchanged by renal ablation (9.3 sec in groups *a* and *b*, 8.9 sec in group *c*). Since prolongation of proximal reabsorptive half-time is the hallmark of "third factor" in the rat, these experiments exclude the participation of this factor in adjustments made by these rats to experimental renal insufficiency. The stepwise increase in sodium excretion per nephron seen as renal insufficiency progresses is not caused by a parallel increase in GFR per nephron or by changes in proximal reabsorption, but reflects a change in absorption in the distal tubule or collecting duct.

139. The Interaction of Erythropoietin and Iron Supply in the Control of Marrow Production. ROBERT S. HILLMAN,* Seattle, Wash. (introduced by E. Donnall Thomas).

The normal erythroid marrow responds to the increased erythropoietin stimulation of a severe hemorrhagic anemia by a production increase of 3–4 times normal. In contrast, the sudden onset of a hemolytic anemia can result in a production response of 5–8 times normal in a similar period of time. This difference may now be explained as an expression of iron supply. Normal individuals, iron deficients, and hemachromatotics were studied at a standard erythropoietin stimulation level by maintaining a constant anemia, hematocrit 25–30, with daily, graded phlebotomy. At various times, the iron required to support increased red cell production was supplied either from endogenous stores (reticuloendothelial stores alone or both reticuloendothelial and parenchymal iron), oral iron, iron dextran infusions, or senescent red cells. The maximum marrow production level for each iron supply situation was then determined by serial plasma iron turnovers, reticulocyte indices, and phlebotomy balance. Production data were also collected in iron-deficient individuals for comparison. Maximum production varied for each iron source. Whereas iron deficients were unable appreciably to increase production (0.5–1.5 times normal), individuals relying on either reticuloendothelial stores or oral iron attained a maximum production level of 2.8–4.0 times normal. If iron was available from two sources, i.e. reticuloendothelial plus oral iron or reticuloendothelial plus parenchymal iron, production increased to 4.1–5.2. When the same subjects received either intravenous iron dextran or senescent red cells to mimic hemolysis, there was an immediate increase in iron supply, serum irons rose to 200–300 μg/100 ml, and production increased to 4.9–7.8 times normal. It is therefore concluded that each potential source of iron demonstrates a characteristic maximum iron delivery rate to transferrin, and the magnitude of erythropoietin effect on marrow production is governed by the level of iron supply.

140. Ribosome Stability in Tumor Cells. CARL A. HIRSCH,* Boston, Mass. (introduced by Howard H. Hiatt).

In contrast to most normal tissues, many tumors remain in nitrogen balance in a fasted host. Under nutritional deprivation such tumors also undergo little diminution in their content of ribosomes, essential components of the cell's protein-synthesizing complex. We have previously shown that ribosome levels in normal mammalian tissues reflect a balance between synthesis and degradation, and that the ribosome complement in rat liver is markedly decreased in starvation because of accelerated degradation and slowed synthesis. We now have investigated two possibilities that could account for the differential effect of malnutrition on host and tumor ribosome content, an effect that could differentially alter the nitrogen balance of the tissues: (1) The tumor cells lack a ribosome-degrading system and hence cannot lose ribosomes. (2) The tumor cells are capable of degrading their ribosomes, but catabolism is balanced by synthesis. We have measured ribosomal RNA and protein turnover relative to DNA, which is conserved, in a mouse ascites tumor line. During cell growth in a fed host, the ribosomes were stable, but during growth in culture, they were renewed with a half-time of about 7 days, a rate similar to that obtaining in several other cultured neoplasmas and in normal rat liver. The tumor ribosomes were not stable under all conditions in vivo, however. Host starvation led to degradation at approximately the in vitro rate. Nevertheless, in contrast to liver, the tumor underwent little net change in ribosome content, degradation being offset by synthesis. Thus, the tumor cells do not lack a ribosome-degrading system. The mechanisms modulating ribosome synthesis and degradation in tumor cells differ quantitatively from those in normal cells in their response to the same environmental signals and are being investigated.

141. The Mode of Action of Sodium on the Contractile Proteins of the Arteries. WILLIAM HOLLANDER AND NOBUHIKO SHIBATA,* Boston, Mass.

A number of studies suggest that sodium ions may alter the blood pressure by having a direct effect on the contraction of arterial smooth muscle. In the present study, the effect of Na^+ on the superprecipitation (contraction) of arterial actomyosin was investigated. Actomyosin was isolated from glycerinated canine arteries with 0.05 M KCl containing 1.0 mM ATP, by the method of Rüegg. The purity of the actomyosin preparation was established by electron microscopy, sedimentation coefficient, viscosity measurements, and ATPase activity. Superprecipitation was measured by the turbidometric method

in a Hitachi spectrophotometer. Calcium ions added to an incubation medium containing 0.5 mM ATP, 3.3 mM Mg^{++}, 0.06 M K^+, and 20 mM Tris maleate (pH 6.8) caused superprecipitation of the actomyosin. The rate and extent of superprecipitation varied directly with the Ca^{++} concentration and were maximal at 10^{-5} M Ca^{++}. When the K^+ in the incubation medium was partly replaced by Na^+, the superprecipitation caused by fixed amounts of Ca^{++} increased strikingly. The responsiveness of arterial actomyosin to Ca^{++} appeared to vary directly with the ratio of Na^+ to K^+ in the incubation medium. Sodium also had an effect on the calcium uptake and release by the arterial microsomal fraction which was isolated by differential ultracentrifugation between 12,000 and 40,000 *g*. The microsomal fraction, incubated with $^{45}Ca^{++}$, ATP, Mg^{++}, and K^+, accumulated calcium and reduced the Ca^{++} concentration in the incubation medium. The partial replacement of K^+ by Na^+ decreased calcium uptake and increased calcium release by the microsomes. When the microsomal fraction was incubated with actomyosin without sodium, it inhibited the superprecipitation of actomyosin by Ca^{++}. The microsomes lacked this inhibitory effect when Na^+ was added to the incubation medium. In conclusion, sodium ions appear to augment the contraction of arterial actomyosin by sensitizing the contractile protein to Ca^{++} ions and by inhibiting calcium uptake and increasing calcium release by the microsomes.

142. Nutritional and Hormonal Control of DNA Synthesis in Adipose Tissue. C. H. HOLLENBERG AND A. VOST,* Montreal, Canada.

Since little is known about regulation of new fat cell formation, a study was made of factors influencing incorporation of ^{3}H-thymidine into rat adipose tissue DNA. After in vitro incubation of adipose slices, the specific activity of DNA was measured in fat cells and stromal cells. Only stromal elements contained radioactive DNA, and prior fasting reduced incorporation. After in vivo injection of ^{3}H-thymidine, adipose tissue was sampled at various times. Radioactivity appeared in stromal DNA within 1 hr but was not apparent in fat cells for 2 days. Thereafter, fat cell radioactivity increased, reaching a plateau by 5 days. Hence fat cell maturation was rapid. To study the effect of fasting, animals were starved for 2 days, injected with thymidine, and fed for 2 weeks before sacrifice. Prior fasting diminished thymidine incorporation into stromal DNA and nearly abolished incorporation into fat cells. 48 hr of fasting beginning either 12 hr or 2 wk after injection did not reduce fat cell DNA radioactivity. Hence, fasting reduced DNA synthesis in primordial fat cells without destroying newly formed or mature fat cells. When fasted rats were refed for 36 hr and then injected, fat cell DNA radioactivity was less than in fed animals. Thus reexpansion of adipose mass with refeeding was not accompanied by accelerated new cell formation but was due to filling of existing cells. With fed hypophysectomized animals, fat cell DNA radioactivity was negligible 2 wk after injection; stromal labeling was variable. Prior growth hormone treatment increased stromal DNA radioactivity 5 times but had no effect on fat cell DNA label. Similarily, in intact animals, preliminary data suggest that growth hormone has its major effect on stromal DNA formation. Hence this hormone may differentially affect fat and stromal cell proliferation.

143. The Relationship between Intrarenal Distribution of Blood Flow and Sodium Balance in Normal Man. N. K. HOLLENBERG,* M. EPSTEIN,* R. GUTTMANN,* R. I. BASCH,* AND J. P. MERRILL,** Boston, Mass.

The relationship between sodium intake and the intrarenal distribution of blood flow has been determined during sodium balance studies in 17 normal subjects. Intrarenal blood flow distribution was measured by the ^{133}Xe washout method at the time of arteriography in potential kidney donors. The rapid or cortical component (C_I) represented $66.8 \pm 3.0\%$ (SEM) of the total renal blood flow in eight subjects in balance on a 10 mEq Na intake, significantly less than the $83.8 \pm 1.3\%$ in six subjects on a 200 mEq Na diet ($P < 0.005$). The percentage of total renal blood flow in the second component (C_{II})—juxtamedullary blood flow identified by radioautography in the dog—was much greater in the Na-deprived ($23.4 \pm 2.7\%$) than in the Na-loaded ($8.2 \pm 1.8\%$) group ($P < 0.005$). The rate of blood flow was also much higher in C_{II} on a low salt diet (113 ± 5.4 ml/100 g per min) than on a high salt diet (50 ± 8.6 ml/100 g per min) ($P < 0.001$). A combined increase of flow rate and percentage of flow to C_{II} indicates an increase in absolute flow to a compartment in the kidney. Plasma volume deficit in the low salt group did not account for the difference, since the infusion of 1000 ml of 6% dextran just before the flow study in three subjects on a low salt diet did not reverse either the blood flow redistribution (C_I, $68.7 \pm 9.2\%$; C_{II}, $24.3 \pm 7.8\%$) or the high flow in C_{II} (134.7 ± 16.4 ml/100 g per min). We conclude that changes in intrarenal distribution of blood flow must be considered among the factors involved in renal sodium regulation in man.

144. Studies of the Regulation of Plasma Aldosterone in Normal Man and in Primary Aldosteronism. R. HORTON,* Los Angeles, Calif., and Birmingham, Ala. (introduced by Harold T. Dodge).

Our knowledge of the physiology of aldosterone in man is based upon analysis of urinary metabolites of the hormone. This approach does not allow study of acute changes. The excretion of aldosterone metabolites is relatively slow, and the two major conjugates are synthesized in and influenced by disorders of the liver and kidney. Plasma aldosterone was estimated by the method of Brodie and Tait and coworkers, 1967. This new double-isotope derivative assay uses ^{14}C-aldosterone indicator and ^{3}H-acetic anhydride (800 mc/mmole) which is vacuum distilled before reaction. Four thin-layer and paper chromatography steps are necessary for purification. In our hands, the nonspecific blank is 0.1 ± 0.07 (SD)

mμg per sample. No measurable aldosterone is present in human adrenalectomized plasma. In the sitting position for 1 hr, plasma aldosterone is 7.3 ± 2.1 (SD) mμg/100 ml; supine values are 3.8 mμg. Angiotensin II in minimal pressor dose (7–10 mμg/kg per min) increases plasma aldosterone 3-fold within 1 hr, without change in plasma cortisol. ACTH 1 unit/hr causes a 5-fold rise in both plasma aldosterone and cortisol. Fludrocortisone 0.9 mg/day orally for 3 days suppresses plasma aldosterone (but not cortisol) to < 3 mμg/100 ml. In four cases of primary aldosteronism, plasma aldosterone was 20–67 mμg/100 ml, although in two, urinary aldosterone was borderline. There was no major change in plasma aldosterone after angiotensin infusion despite pressor hypersensitivity, or after fludrocortisone administration, suggesting fixed secretion with these stimuli. This work indicates that the measurement of aldosterone in plasma allows study of the acute physiology and control mechanisms in normal and hypertensive man.

145. The Presence of DNA-Synthesizing Blood Mononuclear Leukocytes in Inflammatory Disease. DAVID A. HORWITZ,* PETER STASTNY,* AND MORRIS ZIFF,** Dallas, Texas.

Although mononuclear leukocytes do not normally proliferate in the blood, increased numbers of DNA-synthesizing cells have been seen in certain viral, bacterial, and connective tissue disorders. The presence of DNA-synthesizing leukocytes in the blood of patients with various inflammatory diseases was investigated in these experiments. White blood cells were cultured for 5 hr with tritiated thymidine (^{3}H-TdR) and incorporation was determined by liquid scintillation counting. Results were expressed in cpm per million mononuclear cells. The cells of 18 normal individuals incorporated negligible amounts of ^{3}H-TdR (mean, 5.7 ± 2.8), as did those of 9 patients with noninflammatory diseases (mean, 6.7). Small increases were seen in 3 of 11 patients with rheumatoid arthritis (mean, 8.9) and 4 of 10 patients with granulomatous disease (mean, 16). Marked increases were observed in 11 patients with miscellaneous inflammatory diseases (mean, 87); 5 patients with viral hepatitis (mean, 122); and 27 patients with systemic lupus erythematosus (mean, 124). Elevation of the mononuclear ^{3}H-TdR uptake was most consistently observed in active inflammatory disease, and, when sequential studies were performed in the same patient, the values measured appeared to reflect the clinical course. Increases in mononuclear ^{3}H-TdR uptake were associated with parallel increases in the percentage of tritium-labeled blood mononuclear cells in radioautograph preparations. Though labeled cells resembled immature lymphocytes, a significant number assumed morphologic and functional characteristics of macrophages during a 3-day period in tissue culture. Others have shown that the mononuclear cells of inflammatory exudates after acute tissue injury are hematogenous in origin and are derived from rapidly dividing precursors in the bone marrow. The observed appearance of DNA-synthesizing mononuclear leukocytes in the blood of patients with inflammatory diseases is consistent with these observations. It is suggested that the mononuclear ^{3}H-TdR uptake may reflect acute tissue injury and may, therefore, serve as a measure of inflammatory activity in human disease.

146. The Effect of Complement on the Phagocytosis of Sensitized Red Cells by Human Monocytes. HEINZ HUBER,* MARGARET J. POLLEY,* AND WILLIAM D. LINSCOTT,* San Francisco and La Jolla, Calif. (introduced by Frank J. Dixon**).

Human monocytes isolated from the peripheral blood in high purity were used to study the in vitro interaction between these macrophage-like cells and red cells sensitized with γG or γM antibodies with and without the addition of purified human complement components. After reaction with C'1,4,2,3, red cells sensitized with either γG or γM antibodies became attached to monocytes and phagocytosis was demonstrable. In the absence of complement, red cells sensitized with γM antibodies showed no affinity for the monocytes and no phagocytosis occurred. Cells sensitized with γG antibodies were phagocytized without the addition of complement. However, the amount of antibody required for this reaction was at least 100 times greater than that needed when complement was present. γG in the incubation medium at concentrations far below its serum level specifically inhibited the non–complement-dependent phagocytosis of red cells sensitized with γG antibody, but had no effect on the attachment and phagocytosis reaction of complement-coated red cells. Only the complement-dependent reaction was markedly reduced by prior treatment of the monocyte layer with trypsin. Adherence and phagocytosis were due to bound C'3 on the red cell; cells having reacted only with C'1,4,2 were negative with respect to both reactions. We conclude that adherence of red cells to monocytes is an essential step in the complement-dependent phagocytosis reaction.

147. "Fingerprints" in the Liver: Loss of Methionine-Activating Enzyme. GEORGE HUG,* LEO J. CUSSEN,* WILLIAM K. SCHUBERT,* AND GAIL CHUCK,* Cincinnati, Ohio (introduced by Edward L. Pratt**).

A white girl, age 7 months, had hepatosplenomegaly, ascites, prominent abdominal veins, rickets, unusually fine hair, and hypochromic-microcytic anemia. The only episode of jaundice was between age 1 and 3 wk. Hepatomegaly was noted at age 6 months. Ceruloplasmin and erythrocyte activity of galactose-1-phosphate uridyl transferase were normal. Urinary methionine was as high as 100 μg/ml. She died in coma at age 12 months. The autopsy 2 hr later revealed a nodular liver with portal cirrhosis, fatty change, and many coarse granules resistant to diastase, glucosidase, phospholipase, ribonuclease, trypsin, pepsin, pectinase, hyaluronidase, glucuronidase, and lipoxidase, and demonstrable only with PAS, eosin, and burnt Sudan black stain. On electron microscopy, numerous mitochondria were observed to have a coarsely flocculent matrix. Some mitochondria appeared

fused and their internal structures were replaced with an amorphous substance. There were many large lipid droplets. Membranes of the endoplasmic reticulum frequently were arranged on either side of a single row of jagged black granules about 100 mμ in diameter. Uranyl acetate alone stained comparable granules, making it doubtful whether they are glycogen and suggesting that they might be RNA. The picture resembles "fingerprints" as observed by Steiner and coworkers in liver of ethionine-fed rats. The activity of methionine-activating enzyme (MAE) was measured according to Mudd and coworkers and expressed as millimicromoles of methionine converted per gram per minute. MAE of the patient was: liver 24.4, kidney 12.7, pancreas 6.2. MAE of a comparable "normal" autopsy was: liver 189, kidney 14.7, pancreas 10.2. The apparent k_m in liver was 8.6×10^{-7} M for the patient and the "normal" control. Because of the lability of MAE, its loss in the patient's liver is difficult to interpret despite the normal presence of other enzymes (glucose-6-phosphatase, amylo-1.4→1.6-transglucosidase) and despite normal MAE in kidney and pancreas. However, in vivo loss of MAE might impair transmethylation affecting RNA and phospholipids, and thus result in the observed ultrastructure.

148. The Mechanism of Action of 6-Mercaptopurine in Inflammation. ERIC R. HURD* AND MORRIS ZIFF,** Dallas, Texas (introduced by J. Donald Smiley).

Current evidence suggests that the mononuclear cells of inflammatory skin lesions are short-lived cells derived from bone marrow. It has been demonstrated by others that treatment with 6-mercaptopurine (6-MP) results in the disappearance of mononuclear cells from such lesions. To elucidate further the mechanism of this effect, the circulating mononuclear cells of animals demonstrating a 6-MP-induced local anti-inflammatory response have been examined from a number of points of view. Rabbits were treated with 6-MP (18 mg/kg per day) for 8–10 days, and the following studies were carried out in eight treated and eight control animals: (1) total and differential counts of blood leukocytes; (2) differential counts of cytocentrifuge preparations of blood leukocyte suspensions after incubation with India ink; (3) measurement of DNA synthesis in leukocyte suspensions utilizing in vitro tritiated thymidine (^{3}H-TdR) incorporation (1 hr pulse labeling) as measured by liquid scintillation counting and radioautography; and (4) biopsy of subcutaneous inflammatory lesions induced by injection of egg white and India ink. Relatively to control animals, a striking decrease in large mononuclear cells (monocytes and large lymphocytes) in the blood was observed in the 6-MP–treated animals, even though the numbers of polymorphonuclear leukocytes and small and medium lymphocytes were unchanged. In cytocentrifuge preparations, total monocytes (majority carbon labeled) were reduced by half. In vitro incorporation of ^{3}H-TdR by mononuclear cells was reduced 40%. Radioautography demonstrated that the large lymphocyte was the predominant cell type labeled, and confirmed a significant decrease in numbers of labeled mononuclear cells. Close correlation between numbers of circulating large mononuclear cells and percentage of mononuclear cells in the inflammatory lesion (most of which phagocytosed carbon particles) was observed. The data obtained suggest that the anti-inflammatory effect of 6-MP, reflected by a decrease in large mononuclear cells in tissue lesions, results from suppression of a bone marrow response to local inflammation, with a consequent fall in proliferating large mononuclear cells in the blood.

149. The Mechanism of Fluid Production in Experimental Cholera Toxin–Induced Diarrhea. Frank L. IBER,* THOMAS J. MCGONAGLE,* HAROLD A. SEREBRO,* EVELYN H. LUEBBERS,* THEODORE M. BAYLESS,* AND THOMAS R. HENDRIX,** Baltimore, Md.

It has not been established whether the diarrhea of cholera is due to increased intestinal secretion of fluid, decreased intestinal absorption, or a combination of these two factors. To evaluate these possibilities, chronic Thiry-Vella loops of jejunum and ileum in the dog were studied to determine (1) glucose absorption and (2) unidirectional fluxes of Na, before and during cholera toxin–induced diarrhea. 39 studies were completed in five dogs (two jejunal and three ileal). A jejunal solution containing Na 140, K 10, Cl 140, CO_2 10 μEq/liter, 65 mM glucose, ^{131}I-labeled Rose Bengal as a nonabsorbable marker, and $^{22}Na^+$ was instilled into the loop and immediately withdrawn to obtain a sample at zero time. The solution was returned to the loop and subsequent samples were taken at 10 and 20 min intervals. Each loop was studied before (control) and during cholera toxin–induced diarrhea. The diarrhea was produced by placing the cholera exotoxin (Inaba 569b) in each loop for 3 hr. In the jejunum, fluid was absorbed during the control period (0.241 ml/min). During cholera, 0.260 ml/min of fluid was produced. Unidirectional fluxes were calculated after the method of Berger and Steele. Sodium movement from lumen to blood (α) during the control period was 117.4 μEq/min, and 120.7 μEq/min during cholera. Sodium movement from blood to lumen (β) was 84.4 μEq/min during the control period, but was increased to 176.6 μEq/min during cholera. Glucose absorption during the control period was 0.0027 mmole/min. This was unchanged during cholera (0.0028 mmole/min). Comparable results were obtained in the ileum. In the jejunum and ileum, (β) increased during the cholera periods. We conclude that during cholera diarrhea (a) glucose and Na absorption are unchanged in the jejunum and ileum, and (b) movement of Na and fluid into the lumen is significantly increased and is the result of increased flux into the intestine and not due to any alteration of absorption.

150. Nucleic Acid Metabolism in the Gastrointestinal Tract of Mice during Restraint Stress. ANTHONY R. IMONDI,* M. EARL BALIS,* AND MARTIN LIPKIN, New York, N. Y.

Recent studies have suggested that inhibition of epithelial cell proliferation in the presence of continued cell

loss contributes to the development of stress erosions in the gastric mucosa. In the present investigation nucleic acid metabolism was studied in all regions of the gastrointestinal tract of mice subjected to restraint stress and to fasting. The incorporation of ^{3}H–thymidine-methyl and 5-^{3}H-uridine into DNA and RNA was measured 16 and 40 hr after treatment. The findings were correlated with relevant histologic and microradioautographic observations. A series of changes not seen in fasted mice were present in the stomachs of stressed mice. The data revealed an initial decrease in the rate of DNA synthesis accompanied by a loss of total stomach RNA, without a change in the rate of synthesis of new RNA. Later, the rate of RNA synthesis decreased, PAS-positive mucoprotein content of gastric surface cells was markedly diminished on histological examination, and gastric erosions and extravasation of blood into the gastric lumen were observed. The early decrease in rate of DNA synthesis noted in stomach was also observed in small and large intestine. However, RNA metabolism in these tissues was not significantly affected by restraint stress. The findings indicate that alterations in nucleic acid metabolism in gastric cells during restraint stress lead to a loss of ribosomal RNA and a consequent inhibition of mucoprotein synthesis. These events, which contribute to a continued inhibition of DNA synthesis, result in decreased cell production, an inability to compensate for the observed cell loss, and the development of mucosal erosions in the stomach.

151. Glutathione Reductase Activity and Hemolysis in Hemoglobin C Disease. ERNST R. JAFFÉ, EGMOND E. RIEBER,* HELEN M. ANDERSON,* NECHAMA S. KOSOWER,* AND JOHN L. PENNY,* Bronx, N. Y.

Structurally altered hemoglobins and decreased activities of enzymes are associated with hereditary hemolytic disorders, but the mechanisms by which these abnormalities cause various degrees of hemolysis are incompletely understood. Homozygous hemoglobin C usually is not associated with marked anemia. Significant hemolysis was observed in a Negro woman homozygous for hemoglobin C (hemoglobin 6.2–11.3 g/100 ml, reticulocytes 5.5–10%). Glutathione reductase activity in dilute hemolysates of her erythrocytes was low (1.9–3.0 μmoles NADPH oxidized per min per g hemoglobin (IU), 8 determinations), as compared with 4.5 ± 0.8 (SD) IU in normal hemolysates and 5.2–8.9 in hemolysates with comparable levels of reticulocytes. No abnormality, however, could be demonstrated in the metabolic activity of her intact erythrocytes in vitro. The concentration of GSH, the stability of GSH and the formation of Heinz bodies upon incubation with acetylphenylhydrazine, the rate of reduction of endogenous GSSG after complete, rapid oxidation of GSH by methyl phenylazoformate, and the reduction of nitrite-induced methemoglobin were normal. Utilization of glucose and production of lactate from glucose or inosine were consistent with the reticulocytosis. Hemoglobin C trait was demonstrated in both parents and two siblings. Glutathione reductase activity was decreased in hemolysates of the brother's erythrocytes and was low normal in the mother's, but neither had evidence of hemolysis. Two independent hereditary abnormalities were assumed to be present in this family: hemoglobin C and an alteration in the glutathione reductase system. The latter abnormality could be demonstrated only in hemolysates. No direct extrapolation, therefore, could be made from altered enzymatic activity observed in hemolysates to the behavior of intact erythrocytes. Although not yet demonstrated in intact cells in vitro, changes may occur in vivo to produce increased hemolysis of homozygous hemoglobin C erythrocytes which have an altered system for the reduction of oxidized glutathione.

152. The Role of Glucose in the Steroidogenic Action of Adrenocorticotropic Hormone (ACTH). DOUGLAS J. JONES,* WENDELL E. NICHOLSON,* AND GRANT W. LIDDLE,** Nashville, Tenn.

The steroidogenic action of ACTH is mediated by cyclic AMP, which stimulates conversion of cholesterol to pregnenolone; pregnenolone is then readily converted to progesterone and then to hormonal steroids without further need for ACTH or cyclic AMP. The hypothesis that cyclic AMP might act through promoting the formation of NADPH is controversial. If NADPH plays such a mediating role, then glucose or glycogen might be an important source of the glucose-6-phosphate used as substrate in the generation of NADPH. Studies were performed to determine whether glucose facilitates the steroidogenic action of ACTH. Rat adrenals were incubated in vitro. Steroidogenesis was assessed in terms of corticosterone production. The role of glucose was evaluated, first by observing consequences of using high or low concentrations of glucose in the incubation medium, and, second, by measuring rates of conversion of ^{14}C-glucose to $^{14}CO_2$ while steroidogenesis was stimulated by various means. In studies with quartered, halved, or whole adrenals, reducing the glucose from 10 to 1 mM greatly diminished the amount of corticosterone formed in response to ACTH or cyclic AMP but did not diminish the amount of corticosterone formed in response to exogenous NADPH or progesterone. In the presence of glucose, stimulation of corticosterone synthesis with ACTH or cyclic AMP was accompanied by parallel increases in glucose utilization, but stimulation of corticosterone synthesis by addition of NADPH or progesterone was not accompanied by increased glucose utilization. Adrenal homogenates and frozen-thawed adrenals failed to respond to ACTH or cyclic AMP with increases in corticosterone production or glucose utilization. Such preparations responded to NADPH with increases in corticosterone production without increased glucose utilization. Since ACTH-dependent steroidogenesis is facilitated by glucose and NADPH-dependent steroidogenesis is not, it is concluded that a role of glucose might be to serve as a substrate for ACTH-regulated NADPH formation.

153. The Role of the Kidney in Blood Glucose Homeostasis during Prolonged Starvation. ANDERS JONSSON* AND LEONARD L. MADISON,** Dallas, Texas.

This study was designed to define the role of the kidney in blood glucose homeostasis in dogs during prolonged starvation by measuring the balance of glucose across the kidney in 13 dogs after overnight fasting, in 7 dogs after a 3–6 day fast, and in 6 dogs after 14 days starvation. In five additional studies hepatic glucose output was determined after prolonged fasting (>2 wk) by the hepatic venous catheter technique. Renal venous blood was obtained via a catheter passed into the right renal vein. At least five arterial and renal venous integrated blood specimens were drawn simultaneously over 7 min periods in each study. Glucose was measured enzymatically in quadruplicate on duplicate Somogyi filtrates (each renal A-V glucose difference was calculated from 16 glucose determinations). Freedom from contamination of renal venous with inferior vena cava blood was assured by high (83%) PAH extractions used for renal blood flow estimation. Results indicate that in the postabsorptive state and after a 3–6 day fast the kidneys utilize glucose (3.18 and 3.22 mg/min) rather than supplying it for use by other tissues. In contrast, after 14 days starvation renal glucose output occurred but averaged only 0.55 mg/min (0.03 mg/kg per min). Although during prolonged starvation hepatic glucose output fell about 60%, it nevertheless averaged 15.5 mg/min (1.23 mg/kg per min), an amount 40 times greater than that supplied by the kidneys. These data indicate that prolonged starvation results in the conversion of the kidney from an organ of glucose utilization to one of glucose output. However, despite its ability to produce glucose, these data indicate that the kidney is not a quantitatively important source of glucose even after starvation of 14 days duration.

154. Hereditary Hypochromic Microcytic Anemia in Belgrade Rats. MARK E. KAHN,* HELEN M. RANNEY,** D. SLADIĆ-SIMIĆ,* AND P. N. MARTINOVITCH,* Bronx, N. Y., and Belgrade, Yugoslavia.

In 1966, Sladić-Simić and coworkers described a profound hereditary hypochromic microcytic anemia in offspring of an irradiated laboratory rat. The autosomal recessive inheritance, growth retardation, abnormal peripheral blood smear, and progressive splenomegaly were reminiscent of thalassemia. In contrast to its effects in thalassemia in man, parenteral iron therapy produced significant improvement in anemia, although little change in red cell morphology was observed. No expression of the genetic factor has been detected in heterozygotes. The present studies have confirmed and extended the original observations, in Belgrade as well as in a small colony in New York derived from crossing Belgrade anemic animals with Carworth Farms Wistar rats. Non–iron-treated anemic rats from the Belgrade colony have been found to have high values for serum iron, iron-binding capacity, and saturation of transferrin with absence of stainable marrow iron. Persistent reticulocytosis and shortened red cell survival are not corrected by splenectomy. After parenteral iron therapy, moderate improvement in anemia is accompanied by erythrocytosis and normoblastic erythroid hyperplasia, and bone marrow iron becomes apparent, particularly in ringed sideroblasts. Once the initial growth period is past, anemic rats in New York have a spontaneous partial improvement in anemia comparable to that which follows parenteral iron in Belgrade. The complex Hb pattern of some normal rats (four components) and that of anemic rats were indistinguishable by starch gel electrophoresis. Patterns of ^{14}C–amino acid incorporation into the polypeptide chains of rat globin after in vitro incubation of erythrocytes of anemic and of APH-treated normal and heterozygote rats were obtained by the methods of Clegg and Weatherall. No differences were apparent among anemic homozygotes, heterozygotes, or normal rats with comparable reticulocytosis. Thus, this severely hypochromic anemia of the Belgrade rats appears to represent neither simple iron deficiency nor imbalance in polypeptide chain synthesis.

155. Shwartzman Reaction with Microbial Protoplasts. GEORGE M. KALMANSON,* MIKE KUBOTA,* AND LUCIEN B. GUZE, Los Angeles, Calif.

Although microbial protoplasts (L forms) have been isolated from a variety of clinical materials and deliberately produced in experimental animals, their role in pathogenesis of disease is uncertain. It would be important to demonstrate the potential for producing toxic effect in vivo by any mechanism. Experiments were designed to elicit an endotoxic effect by the Shwartzman reaction. Rabbits were prepared by intradermal inoculation of either a stable protoplast of *P. mirabilis,* the parent bacterial form, or purified *E. coli* endotoxin. Intravenous challenge was with protoplast or endotoxin. When animals were challenged with endotoxin, 1 of 8 rabbits which received 3.5×10^6 protoplasts in the skin, 8 of 18 with $2.3–6.1 \times 10^7$ protoplasts, and 6 of 8 with 4.3×10^8 protoplasts had positive reactions. 3 of 10 animals which received 9.2×10^7 bacteria and 14 of 18 animals prepared with endotoxin had positive reactions after challenge with endotoxin. When similarly prepared animals were challenged with $1.5–2.3 \times 10^8$ protoplasts, 4 of 18 protoplast-prepared animals, 1 of 10 bacteria-prepared animals, and 2 of 18 endotoxin-prepared animals had positive but less intense reactions. 6 of 8 animals prepared with lysed protoplasts had positive reactions after challenge with endotoxin. These studies suggest that pathogenic potentiality of protoplasts may reside in the presence of Shwartzman reactive material.

156. Biochemical Heterogeneity of a Normal Human Platelet Population. S. KARPATKIN,* New York, N. Y. (introduced by Angelo Taranta).

Considerable controversy exists as to whether human platelets die by senescence or by random removal. Kinetic data from platelet survival studies suggest that both processes may apply. Evidence for metabolic hetero-

geneity of platelet populations would be consistent with the theory of metabolic depletion leading to platelet senescence and ultimate death. Washed human platelets obtained from healthy blood donors were separated by centrifugation into two extreme density populations by employing various inert specific gravity suspending media. An upper 25%, lighter layer, sp gr 1.042, and a lower 25%, heavier layer, sp gr 1.052, were separated. When the number of platelets was expressed per ml packed platelets, the heavy platelets were noted to be 1.7 times larger than the lighter platelets. Measurements of high-energy phosphates (ATP, ADP, AMP; glycogen; and P_i) were 1.5-, 1.2-, 1.6-; 2.3-; and 2.0-fold greater respectively in heavy-large platelets when these data were expressed per platelet (10 experiments). The rate of lactate production in the presence and absence of glucose, as well as glucose uptake over a 1 hr period, was also investigated. Again, heavy-large platelets had a 2-fold greater rate for glycogenolysis, glycolysis, and glucose uptake (five experiments). The incorporation of uniformly labeled ^{14}C-L-leucine into hot TCA-precipitable material was 4-fold greater for heavy-large platelets (two experiments). These data reveal that large-heavy platelets are metabolically different from smaller-lighter platelets. (In clinical situations associated with rapid platelet turnover, an increased number of larger platelets, presumably younger platelets, are frequently noted on peripheral blood smear.) The data are consistent with the thesis that these large-heavy platelets, with higher metabolic energy stores and "increased protein synthesis," are younger platelets. Accordingly, these "young" platelets are better equipped glycolytically to maintain the ionic gradients of the platelet and the external environment.

157. Evidence for Different Adrenergic Mechanisms in Heart and Brain. RICHARD I. KATZ* AND IRWIN J. KOPIN, Bethesda, Md.

Neurons in the peripheral sympathetic nervous system, when stimulated, release norepinephrine as a neurotransmitter. Because of a number of similarities in synthesis, storage site, and response to drugs of catecholamines in the sympathetic and central nervous systems, adrenergic neurons in brain are thought to function in a manner analogous to those in the periphery. It has been suggested that affective disorders (endogenous depression and mania) are associated with altered cerebral catecholamine metabolism, and drugs which influence these amines have been used in the treatment of such psychiatric illnesses. Lithium salts have been reported to be effective in the treatment of mania and also appear to influence catecholamine metabolism. However, no peripheral sympatholytic actions have been reported in patients treated with lithium. Slices of brain or heart actively accumulate ^{3}H-norepinephrine from incubation media. Under these conditions, uptake occurs primarily in the adrenergic neurons, which appear to remain intact. In vitro release of labeled norepinephrine during electrical field stimulation from tissue slices previously incubated with the tritiated amine has been used to study the pharmacology of adrenergic neurons. During electrical field stimulation with rectangular DC pulses (9 ma, 100 Hz, 4.0 msec duration) ^{3}H-norepinephrine is released into superfused Krebs-Ringer bicarbonate solution. The presence of lithium chloride in the perfusate, in a concentration found in brain and plasma during treatment for mania (1–3 mEq/liter), markedly reduced the stimulation-induced release of these labeled amines from brain slices, but had no effect on release of norepinephrine from intact atria. These observations provide the first evidence of a distinct difference in response of adrenergic neurons in the peripheral sympathetic and central nervous systems.

158. Reversal by Copper of the Lathyrogenic Action of D-Penicillamine. HARRY R. KEISER,* ROBERT I. HENKIN,* AND MORLEY KARE,* Bethesda, Md., and Raleigh, N. C. (introduced by Albert Sjoerdsma).

A lathyrogenic effect of D-penicillamine (D-Pen) has been demonstrated recently in both laboratory animals and man. Since D-Pen is a chelator of copper, the role of copper in the lathyrogenic actions of D-Pen on collagen metabolism was evaluated. 40 male weanling Holtzman rats were divided into groups of eight animals each and fed a diet of ground Purina chow containing 15% dextrose. One group served as controls, another group received D-Pen (10 g/kg diet) for 28 days, and the other three groups received D-Pen for 52 days. The last two groups were given copper in either a low dose (50 mg/kg diet) or a high dose (10 g/kg diet) for the last 24 days. On the 52nd day, the following indices of collagen metabolism were ascertained in all groups: urinary hydroxyproline (HOPr), salt-soluble collagen, total collagen, proline hydroxylase activity, and monoamine oxidase (MAO) activity in the skin. Animals treated with D-Pen alone, in comparison with controls, had a lower body weight (mean $\pm$ SEM, 131 ± 8 vs. 298 ± 7 g), higher urinary HOPr (100 ± 6 vs. 66 ± 4 μg/mg creatinine), higher soluble collagen (7.2 ± 1.3 vs. 0.5 ± 0.1%), lower total collagen (22 ± 2 vs. 37 ± 2 μg HOPr/mm^2), lower proline hydroxylase activity (85 ± 22 vs. 274 ± 20 units), and lower MAO activity (0.47 ± 0.04 vs. 0.83 ± 0.05 units.) The low dosage of copper partially corrected and the high dosage almost completely corrected all the abnormalities produced by D-Pen alone. These data demonstrate that the lathyrogenic action of D-Pen results from copper chelation and that copper plays an important role in mechanisms of collagen synthesis.

159. Adenine Phosphoribosyl Transferase Deficiency: A Previously Undescribed Genetic Defect in Man. WILLIAM N. KELLEY,* ROBERT I. LEVY,* FREDERICK M. ROSENBLOOM,* J. FRANK HENDERSON,* AND J. EDWIN SEEGMILLER,** Bethesda, Md.

A previously undescribed deficiency of an enzyme of purine metabolism, adenine phosphoribosyl transferase (A-PRTase), was demonstrated in four members representing three generations of one family. The A-PRTase activity in their dialyzed erythrocyte lysates ranged from 21 to 31% of normal. The mutant enzymes could not

be distinguished from normal by electrophoretic migration, thermal stability, gel filtration, pH optima, or kinetic properties. Analysis of the genetic data allows the conclusion that the four affected individuals are heterozygous for this enzyme defect despite enzyme activity which is substantially less than the expected 50% of normal. Heterozygotes for orotic aciduria exhibit a quantitatively similar reduction in activity (20–35% of normal) of another phosphoribosyl transferase, orotidylic pyrophosphorylase, which has been attributed to the presence of an abnormal regulator gene product. We have no evidence to support the postulation of such a mechanism in our patients. A closely related purine phosphoribosyl transferase, hypoxanthine-guanine phosphoribosyl transferase (HG-PRTase), is essential for the normal regulation of purine metabolism in man, since its deficiency is associated with an accelerated rate of purine biosynthesis and hyperuricemia. In addition, clinical and biochemical studies have established that the HG-PRTase locus is on the X chromosome. Despite its close functional similarity to HG-PRTase, a partial deficiency of A-PRTase in these subjects was not consistently associated with any clinical abnormalities or any detectable aberration in purine metabolism, and its inheritance in two of three female offspring of an affected male suggests that the A-PRTase locus is on an autosome rather than on the X chromosome.

160. The Mode of Action of Vitamin K in Stimulating Prothrombin Synthesis. ROGER K. KIPFER,* LAN-FUN LI,* AND ROBERT E. OLSON,** St. Louis, Mo.

Vitamin K stimulates the appearance of the coagulation proenzymes factors II, VII, IX, and X in the plasma of birds and mammals deficient in this vitamin. This report deals with recent experiments in this laboratory demonstrating the in vitro control of coagulation proenzyme synthesis by vitamin K_1. The synthesis of prothrombin and factor X has been demonstrated in the isolated rat liver perfused with oxygenated Krebs-Ringer buffer containing 3% bovine albumin, amino acids, glucose, terramycin, and 20% washed rat red cells. In the presence of 10 μg/ml of warfarin sodium, no synthesis occurred. The addition of vitamin K_1 (10 μg/ml) overcame this inhibition and resulted in the biosynthesis of amounts of prothrombin and factor X equivalent to 100 and 30% respectively of the rat's normal content of circulating factors. Puromycin at 200 μg/ml abolished the response. The kinetics of coumarin drug–vitamin K_1 relationship in the perfused rat liver suggests an allosteric interaction with a single regulatory protein. The immunochemical assay of prothrombin was accomplished with rabbit antibody to purified rat prothrombin. When ^{14}C-leucine was added to the perfusate before vitamin K_1, prothrombin precipitated with specific antibody from the perfusate after 4 hr was radioactive. These results suggest that vitamin K stimulates de novo prothrombin synthesis and that the control exercised by both vitamin K and the coumarin drugs is applied at the translational level of protein synthesis.

161. Epinephrine Production in Human Adrenal Medulla and Pheochromocytoma Tissue. ABBAS E. KITABCHI,* JAMES E. VANCE,* NANCY ROCK,* AND ROBERT H. WILLIAMS,** Seattle, Wash.

To investigate the mechanism of epinephrine synthesis in man, phenylethanolamine-*N*-methyl transferase (PNMT), which converts norepinephrine (NEP) to epinephrine (EP) in the adrenal medulla, was studied in crude and partially purified preparations of adrenals of five normal subjects and of pheochromocytoma tissues of five patients. PNMT activity from normal adrenals was stimulated by EDTA and sulfhydryl compounds and was inhibited by PO_4, *p*-hydroxymercuribenzoate, heavy metals, and an inhibitor which was elaborated from four pheochromocytoma tissues. The inhibitor from the neoplastic tissues was dialyzable and heat stable. The inhibition was immediate and noncompetitive and was irreversible by metal chelators or sulfhydryl compounds. The PNMT activity in pheochromocytoma tissue from four of five patients was inhibited; it was reactivated by dialysis but not by EDTA. The dialysate of four neoplastic tissues inhibited the purified PNMT of the normal adrenal. Determination of catecholamines in the neoplastic tissues (data supplied by Dr. Wurtman and Drs. Sjoerdsma and Engelman) revealed increased NEP:EP in four tissues, which correlated with urinary catecholamine patterns. These four tissues contained inhibitors, whereas one pheochromocytoma tissue, which had a decreased NEP:EP, had no PNMT inhibitor. Polyacrylamide gel electrophoresis (by Dr. J. Kemp) of the neoplastic and normal PNMT preparations showed alteration of protein band components of the neoplastic tissue. This finding, along with abnormal changes in pH optima and thermolability curves obtained with the PNMT studies of neoplastic tissue, suggests possible alteration of the pheochromocytoma enzyme by the inhibitor. It is concluded that pheochromocytomas elaborate an inhibitor of PNMT which may exert a regulatory influence on EP production by altering the enzyme molecule, thus in part explaining the increased NEP:EP ratio in pheochromocytoma tissues and urines of these patients.

162. Investigation of the Coupling of Metabolism to Sodium Transport: The Question of a High-Energy Compound Other Than ATP as the Immediate Source of Energy for Transport. SAULO KLAHR,* ALLEN SHAW,* KUO HWANG,* JACQUES BOURGOIGNIE,* AND NEAL S. BRICKER, St. Louis, Mo.

In studying the coupling of metabolism to cation transport we have employed model systems capable of anaerobic energy conservation, in order to limit the number of potential metabolic pathways. Using the turtle bladder (TB), we found that dinitrophenol (DNP) profoundly inhibited anaerobic sodium transport despite persistence of appreciable ATP stores. Simultaneously glycolysist was stimulated and ADP/ATP ratios increased. Both the inability of residual ATP to sustain transport and the metabolic changes require explanation. One interpreta-

tion is that a high-energy intermediate, rather than ATP, provided energy for sodium transport. If DNP hydrolyzed this compound, it could block transport and initiate dephosphorylation of ATP, ADP accumulation, and stimulation of glycolysis. Another system with oxidative metabolism capable of anaerobic sodium transport was sought. Dogfish RBC were used. DNP effects were inconstant, but oligomycin, an antibiotic affecting high-energy intermediate synthesis in oxidative systems, reproduced the entire constellation of effects noted in TB. Thus the same phenomena occurred in two different cell types from widely separated species. To examine organ vs. species differences, turtle RBC were studied. Oligomycin "uncoupled" glycolysis from phosphorylation. In all three systems, therefore, sodium transport was inhibited in the presence of ATP whereas metabolism was stimulated. Further studies were performed on TB. Sodium azide, a mitochondrial ATPase inhibitor, did not block the DNP effects; thus DNP does not stimulate glycolysis by activating mitochondrial ATPase. DNP also did not activate (or inhibit) plasma membrane ATPase. However, inhibitors of plasma membrane ATPase blocked the DNP effects. Both ouabain and ethacrynic acid inhibited turtle bladder ATPase, and both inhibited the DNP-induced effects on glycolysis and ADP/ATP ratios. These data suggest that a high-energy intermediate may be evolved from ATP via plasma membrane ATPase. If so, the intermediate rather than ATP may represent the definitive source of energy for sodium transport.

163. Starvation and Gluconeogenesis in Pregnancy. ROBERT KNOPP,* EMILIO HERRERA,* AND NORBERT FREINKEL, Chicago, Ill.

The developing conceptus siphons maternal fuels throughout gestation. Failure to modify fetal weights by 2 days of maternal starvation in late pregnancy attests to the primacy of fetal needs. Accelerated mobilization of maternal fat during fasting has been recognized, but gluconeogenic contributions have not been evaluated. 19 day pregnant (P) and age-matched virgin (V) rats were compared after feeding or 48 hr fast. Plasma glucose was 75.3 ± 2.2 vs. 99.3 ± 2.7 in fed and 43.2 ± 1.6 vs. 63.7 ± 1.4 in fasted P and V respectively. "Steady-state" levels of key metabolites in liver (per μg DNA-P) did not differ in fed groups. After fasting, liver FFA and acetyl CoA were higher ($P < 0.02$) in P, suggesting greater fat mobilization for gluconeogenic activation. Gluconeogenesis was documented in vivo with intravenous 3-^{14}C-pyruvate (1 mmole). In fed groups, pyruvate conversion to ^{14}C-glucose did not differ. Fasting increased the conversion. Moreover, extracellular ^{14}C-glucose was 78.4% and 52.1% greater in P 5 and 10 min after pyruvate ($P < 0.05$), and 6.1% of injected counts were in hepatic glycogen in P vs. 0.5% in V after 30 min ($P < 0.001$). Urinary nitrogen and cations were compared in starvation during greatest fetal growth (days 19–21) and postpartum (days 6–8). Nitrogen, urea, uric acid, ammonia, and K were greater ($P < 0.02$) during day 1 of fasting ante- than postpartum ($P < 0.05$). Exaggerated nitrogen ($P < 0.02$), uric acid ($P < 0.05$), ammonia ($P < 0.001$), and K ($P < 0.05$) losses persisted during day 2. Creatinine and Na excretions did not differ. We conclude that besides mobilizing more fat during starvation in late gestation, the mother compensates for the conceptus' drain of glucose and amino acids by greater gluconeogenic efficiency and by depositing more gluconeogenic products as glycogen when exogenous precursors (i.e. pyruvate) are delivered. The disparately rising urinary ammonia suggests some additional activation of renal gluconeogenesis to provide base for increased ketonuria.

164. Elution Studies on Tissues from Patients with Goodpasture's Syndrome and Other Forms of Subacute Glomerulonephritis. DAVID KOFFLER,* JOHN SANDSON,* AND HENRY G. KUNKEL,** New York, N. Y.

Elution studies performed by Lerner and coworkers on kidneys from two patients with Goodpasture's syndrome have demonstrated the presence of anti-basement membrane antibodies which appear to be involved in the fulminating glomerulonephritis observed. The pathogenesis of the pulmonary lesions, however, has not been elucidated. Lung tissues were obtained at autopsy from a patient with Goodpasture's syndrome. Immunofluorescence studies of this tissue revealed that gamma globulin and β_1C-globulin were localized in a focal membranous distribution outlining segments of alveolar septa. In order to determine the specificity of the tissue-bound gamma globulin, aliquots of lung tissue were treated with acid buffer. Antibodies eluted from the lung were shown to stain alveolar septal membranes, glomerular basement membranes, and renal tubular basement membranes. Antibodies eluted from the nephritic kidney of the same patient also exhibited reactivity with pulmonary and renal basement membranes. These results suggest that anti-basement membrane antibodies eluted from the lung and the kidney react with similar antigenic determinants and mediate both the glomerulonephritis and the hemorrhagic pulmonary lesions observed in Goodpasture's syndrome. In contrast, eluates containing high concentrations of gamma globulin obtained from kidneys of other patients with severe subacute glomerulonephritis did not manifest anti-basement membrane antibodies. No antibodies reactive with nuclei or epithelial cells were noted, although in one eluate antibodies reactive with thyroid colloid were demonstrated. Eluates obtained from kidneys of patients with systemic lupus erythematosus (SLE) manifested high titers of antinucleoprotein antibodies, but no antibodies directed against membrane were present. In this limited experience, antibodies to basement membrane were found to be unique for the kidney and lung lesions of Goodpasture's syndrome, and antinucleoprotein antibodies for SLE. The specificity of the gamma globulin deposits in most other forms of glomerulonephritis remains to be determined.

165. Human Monoclonal γG Cryoglobulins with Anti-γ-Globulin Antibody Activity. PETER F. KOHLER,* WILLIAM D. TERRY,* AND HOWARD M. GREY,* La Jolla, Calif. (introduced by Richard S. Farr**).

Seven human myeloma proteins which were also cryoglobulins were examined with regard to possible antibody activity to human γ-globulin. Three test systems were used for this purpose: (1) agglutination of γ-globulin–coated latex particles; (2) coprecipitation of radioiodinated γ-globulin in the cryoprecipitate; and (3) ultracentrifuge analysis of the interaction between the Fab fragments of the cryoglobulins and 7S γG proteins. Five of the seven cryoglobulins studied showed anti–γ-globulin activity in at least two of the three tests employed. As would be expected if the observed anti–γ-globulin activity were indeed due to antibody, ultracentrifuge analysis indicated that the combining site on the cryoglobulins resided in the Fab fragment. Further, it was shown that the Fab combining site on the cryoglobulins recognized antigenic determinants present on the Fc fragment of human and other primate γG globulin, but they did not interact with other human immunoglobulins or nonprimate γG proteins. In two instances the cryoglobulins showed preferential reactivity toward one γG heavy chain subclass over the others. It was also found that the cryoprecipitation of those proteins with anti–γ-globulin activity could be specifically inhibited by the addition of excess amounts of normal γG globulin in a fashion similar to that observed in the quantitative precipitin reaction in the zone of antigen excess. In summary, it has been found that a high percentage of γG myeloma cryoglobulins behave as antibodies to γG globulin. It is postulated that in these cases the cryoprecipitate represents antigen-antibody complexes of such a nature that they precipitate only in the cold.

166. Early Reinnervation of the Heart after Cardiac Autotransplantation. HERMES A. KONTOS* AND RICHARD R. LOWER,* Richmond, Va. (introduced by David W. Richardson).

The responses to electrical stimulation of the vagus and stellate ganglion and to intravenous norepinephrine, nitroglycerin, tyramine, phenylephrine, atropine, and propranolol were studied in 32 dogs with cardiac autotransplantation 3 wk to 5 yr after the procedure. Parasympathetic reinnervation of the heart occurred as early as 37 days after transplantation; it was rare in the first 2 months after the procedure, occurred most frequently during the 3rd and 4th months, and was almost invariably present by the 6th month after transplantation. Sympathetic reinnervation occurred as early as 33 days after transplantation and was almost invariably present by the 4th month after the procedure. The responses of heart rate to nitroglycerin and phenylephrine indicated that the tachycardia in response to hypotension was dependent only on sympathetic reinnervation and that the bradycardia in response to hypertension was dependent only on parasympathetic reinnervation. The tachycardia in response to norepinephrine in dogs with both sympathetic and parasympathetic denervation was significantly greater than that in dogs with only parasympathetic denervation or in normal dogs after atropine, suggesting hypersensitivity to norepinephrine during sympathetic denervation. Despite confirmed parasympathetic reinnervation, most dogs showed no tachycardia in response to intravenous atropine, indicating that resting vagal tone was low. Yet the animals were able to effect an increase in vagal tone in response to reflex stimuli. Dogs with sympathetic reinnervation showed greater reduction in heart rate in response to propranolol than dogs with sympathetic denervation, suggesting the presence of resting sympathetic tone. The results show that reinnervation of the heart after cardiac autotransplantation occurs much earlier than was previously thought to be the case. Even in the early months after autotransplantation, reinnervation is sufficiently developed to enable the heart to respond appropriately to reflex stimuli.

167. The Initial Interaction between Estradiol and the Uterus: Binding to Cytosol Receptors. STANLEY G. KORENMAN * AND B. RAMANATH RAO,* Torrance, Calif. (introduced by William D. Odell).

Assignment of importance to the estrogen-specific receptors in uterine cytosol in initiation of estradiol (E_2) action requires proof that binding occurs before the earliest biochemical response to the hormone. That response, an increase of 3′,5′-AMP, occurs within 15 sec (Szego and Davis). Using the cytosol from preimplantation pregnant rabbit uteri, the kinetics of association and dissociation of ^{3}H-E_2 and receptor were studied employing activated charcoal to separate unreacted free steroid from bound steroid. The cytosol receptor had a molecular weight of 220,000 on sucrose density gradient ultracentrifugation (SDG) and was specific for steroidal and nonsteroidal estrogens. At 37°C measurable binding occurred routinely within 5 sec and peaked at 6 min, yielding an average association constant of 6.1×10^9 liters/mole per min. At 37°C dissociation of preformed complex was rapid and biphasic, suggesting binding sites of differing thermal lability. Association of ^{3}H-E_2 to cytosol receptors was even more rapid during incubation of uterine minces than with cytosol alone. The binding reaction was stopped by the addition of activated charcoal and an excess of nonradioactive E_2, followed by quick-freezing in dry-ice-acetone. The cytosol, prepared in the presence of charcoal, was centrifuged in a SDG to characterize the bound counts. Binding to cytosol receptors occurred within 5 sec at 37°C, reaching the maximum by 10 sec. At a temperature (23°C) at which E_2 binding in cytosol alone had been maximal at 1 hr, the peak was reached after 5 min of uterine mince incubation. We conclude that hormone-specific E_2 binding to uterine receptors in the cytosol preceds every biochemical response measured to date.

168. The Intracellular Catabolism of Hemoglobin by the Liver. STUART KORNFELD,* BARBARA CHIPMAN,* AND ELMER B. BROWN, St. Louis, Mo.

Hemoglobin (Hgb) injected intravenously into rats is selectively removed by the liver and subsequently digested. To determine the intracellular site of Hgb degradation, we injected rats i.v. with Hgb (3 mg) labeled in both the globin (^{75}Se-selenomethionine) and heme (^{59}Fe) moieties, killed them at timed intervals, and perfused their livers. The livers were fractionated by differential centrifugation into nuclear (I), mitochondrial (II), "lysosomal" (III), microsomal (IV), and supernatant (V) fractions. In a typical experiment, 46% of the ^{75}Se-methionine activity of the liver homogenate was recovered in fractions III and IV and 26.5% in fraction V at 10 min. At 3 hr 27% of the ^{75}Se activity was in fractions III and IV and 44% in fraction V. Total ^{75}Se activity in the liver increased progressively for 4 hr and then gradually declined. The distribution of ^{59}Fe activity followed a similar pattern. By 3 hr, 80% of the ^{59}Fe in fraction V was in a nonheme, heat-stable form, whereas only 30% of the ^{59}Fe in fractions III and IV was nonheme iron, suggesting that ^{59}Fe is removed from heme as it moves from fractions III and IV to fraction V. The particles involved in Hgb digestion had different sedimentation characteristics from classic lysosomes identified by acid hydrolase activity. This was clearly shown by pretreatment of rats with Triton WR-1339, which caused lysosomes to float in sucrose gradients but did not change the sedimentation of Hgb-containing particles. Infection, simulated in animals by sterile turpentine abscesses, blocks reutilization of Hgb iron. Catabolism of Hgb by rats with sterile abscesses was the same as in normal rats except for progressive accumulation of nonheme iron in fraction V. These observations suggest that Hgb catabolism in the liver occurs in small particles distinct from the larger lysosomes. Heme and globin are rapidly digested, with the release of iron and amino acids to the soluble fraction of the cell, where selenomethionine is used for new protein synthesis while iron is stored as ferritin or released from the cell; this release of iron is blocked in infection.

169. Determination of Muscle Mass by Isotopic Dilution of ^{14}C-Creatine. R. A. KREISBERG,* B. BOWDOIN,* AND C. K. MEADOR,* Birmingham, Ala. (introduced by T. J. Reeves).

Because of the localization of creatine in muscle, we investigated the feasibility of using isotopic dilution of ^{14}C-creatine to estimate muscle mass in vivo. Rats were sacrified at daily intervals after receiving 1–2 μc of 1-^{14}C-creatine IV. Routes of ^{14}C excretion, distribution in body tissues, and equilibration time were examined. Urine and stool were the major routes of ^{14}C excretion (negligible $^{14}CO_2$ found in expired air). ^{14}C calculated to be remaining in the body from "^{14}C injected $-$ ^{14}C excreted" was $100 + 2\%$ of that found by carcass analysis. By 144 hr, 94% of body ^{14}C was in carcass muscle, 4% in skin, and 2% in gut, kidney, and liver. Although not all ^{14}C was in muscle, we accepted the discrepancy since only a 6–7% overestimate of muscle mass could be introduced Uniformity of distribution throughout the muscle compartment was examined by determining the ^{14}C concentration in four small muscle pieces removed from separate sites of the skinned eviscerated carcass. The coefficient of variation of ^{14}C concentration between muscle sites in a single rat ranged from 3 to 17% (24 rats) with a mean of 10%. We accepted the mean ^{14}C concentration of the four muscle pieces as representative of the concentration in the muscle compartment. Paired comparison revealed that there was no significant difference between the muscle mass values obtained by isotopic dilution (^{14}C remaining in body $\div$ ^{14}C/g fat-free wet muscle) and the anatomic muscle mass values (obtained by dissection) in 24 rats weighing 200–400 g. These results indicate that dilution of ^{14}C-creatine can be used to measure muscle mass in vivo in rats, and suggest that with appropriate modification this method may be applicable to man and other large animals.

170. Inactivation of LATS by Antikappa and Antilambda Antisera. JOSEPH P. KRISS,* Palo Alto, Calif. (introduced by Norman Kretchmer).

Two current different hypotheses of the nature of the long-acting thyroid stimulator (LATS) state (*a*) that LATS is an antibody of the IgG type, and (*b*) that LATS is an IgG globulin but not an antibody; rather, it is the product of a cell line derived by clonal selection. This experiment was designed to test the latter hypothesis. The B chains of each IgG antibody molecule may be characterized as either kappa (κ) or lambda (λ) in type, but specific antibodies formed by a given individual possess both types of molecules. On the other hand, the clonal selection hypothesis would predict the formation of an IgG product which is of either κ or λ type, but not both. Specific anti-κ and anti-λ antisera were obtained, neither of which cross-reacted with components of the A chain. Equal aliquots of each of seven LATS-positive sera obtained from different subjects were incubated separately with anti-κ and anti-λ antisera. After centrifugation of the mixture, the supernatant solutions were tested for LATS activity by a bioassay procedure and this activity was compared with that of a control sample of the starting amount of the same LATS serum. In each case, LATS activity was reduced by both anti-κ and anti-λ sera. The degree of inactivation of LATS by a given antiserum varied from patient to patient within the range 25–75%, but the calculated combined inactivation of a given LATS sample by both antisera approximated 100%. The data add further evidence of the immunoglobulin nature of LATS and prove that LATS is not the product resulting from a monoclonal selection process.