The Function of Conjugation in the Excretion of Bilirubin. ROGER LESTER AND PETER D. KLEIN, Boston, Mass., and Argonne, Ill. (introduced by Franz J. Ingelfinger †).

To determine if correlations exist between structure and glucuronide formation, we studied the excretion of bilirubin and several other bile pigments in rats. Biliverdin $\xrightarrow{+2H}$ bilirubin $\xrightarrow{+4H}$ mesobilirubin $\xrightarrow{+4H}$ mesobilirubinogen $\xrightarrow{-2H}$ i-urobilin, a series of tetrapyrrols differing only in the number of their double bonds, form a convenient spectrum with which to assess the effect of structure on conjugation. ¶ Radiochemically pure, specifically labeled, tritiated forms of the latter four tetrapyrrols were prepared and administered intravenously to rats with external biliary drainage. In normal Sprague-Dawley rats, 74 to 87% of each administered tetrapyrrol appeared in bile within 90 minutes of injection. Bilirubin and mesobilirubin were excreted as glucuronide conjugates, but conjugate formation did not appear to be requisite for the excretion of mesobilirubinogen and i-urobilin. Confirmation of this distinction was obtained in rats lacking the conjugating enzyme glucuronyl transferase (Gunn rats). In these animals, less than 10% of the injected bilirubin-^{3}H and mesobilirubin-^{3}H appeared in bile during the 90 minutes after injection. In a comparable period, however, 83 to 92% of injected mesobilirubinogen-^{3}H and i-urobilin-^{3}H was excreted by the liver. Thus, unlike biliverdin (previously shown to be excreted as the glucuronide in rabbits), bilirubin, and mesobilirubin, the excretion of mesobilirubinogen and i-urobilin occurs without glucuronide formation. ¶ When Dreiding molecular models of the tetrapyrrols were constructed, it was apparent that biliverdin, bilirubin, and mesobilirubin possessed a marked degree of steric rigidity resulting from the methyne (−CH=) bridges between the outer and inner pyrrol rings. These bridges are saturated (−CH_2−) in mesobilirubinogen and i-urobilin, and, as a result, models of these molecules exhibit a high degree of flexibility. ¶ These studies suggest that glucuronide conjugation is not an essential step in the excretion of all bile pigments, but is specifically associated with the excretion of those tetrapyrrols having unsaturated bridges linking their outer ring structures.

Clinical Detection of the Potential Allergic Reactor to Penicillin by Immunological Tests. B. B. LEVINE,* A. P. REDMOND, AND H. E. VOSS, New York, N. Y.

Eighty patients with past histories of penicillin allergy in whom penicillin therapy was necessary were evaluated for potential penicillin allergy by prospective immunological tests. These tests consisted of the following: *1*) Direct skin testing for wheal-and-flare reactions with penicilloyl-polylysine (BPO-PLL) and with a mixture of benzylpenicillin, benzylpenicilloic acid, and benzylpenilloic acid (minor determinant mixture). These reagents detect skin sensitizing antibodies to the BPO (major) and the minor determinants, respectively. *2*) Hemagglutination assays of sera for IgM and IgG circulating antibodies. Patients treated with penicillin were followed for a period of 1 to 2 weeks after penicillin was stopped. The following observations were made: *a*) Of 55 patients with negative skin tests and 19 S antibodies who were given courses of penicillin, 54 tolerated the drug without reaction, and 1 developed a late allergic reaction (maculopapular eruption) 3 days after the start of penicillin. *b*) Of 13 patients with negative skin tests and 7 S antibodies, all tolerated courses of penicillin without allergic reaction. *c*) Of 4 patients with positive BPO-PLL skin tests and 7 S antibodies, 1 tolerated penicillin therapy without allergic reaction, and 3 had mild accelerated urticarial reactions. These reactions occurred 12 to 48 hours after initiation of penicillin therapy and correlated temporally with low titers of BPO-specific IgG serum antibodies. *d*) Three other patients with positive skin tests to BPO-PLL, 2 patients with a positive test to the minor determinant mixture, and 3 patients with positive skin tests to both reagents were not treated with penicillin because of insufficient medical indication. These results are consistent with the view that negative skin tests to both major and minor determinant reagents rule out the occurrence of immediate and accelerated allergic reactions to penicillin, but do not completely rule out the possibility of a late allergic reaction. A positive skin test to BPO-PLL appears to indicate a higher probability of an accelerated urticarial reaction. The significance of positive tests to the minor determinants is not yet clear, but our other data indicate that these patients have the highest risk of immediate (anaphylactic) allergic reactions. Although large scale studies are necessary to fully evaluate their efficacy, these tests show promise as practical clinical methods to detect the potential immediate and accelerated allergic reactor to penicillin.

The Influence of Hypoxemia on the Defense of pH in Chronic Hypercapnia. DAVID Z. LEVINE, DANIEL G. SAPIR, AND WILLIAM B. SCHWARTZ,* Boston, Mass.

Recent observations in the unanesthetized dog have defined the response of extracellular hydrogen and bicarbonate concentrations to graded degrees of chronic hypercapnia. On the basis of the "titration curve" provided by such studies it has been proposed that a rational approach can be made to the quantitative evaluation of complex acid-base disturbances, i.e., disturbances in which both respiratory and metabolic components may be present. However, the possible applicability of such data to chronic respiratory acidosis in man has been open to question in view of the fact that all studies have been carried out with a normal O_2 tension in the inspired air. The present study was undertaken for the purpose of evaluating the influence of chronic hypoxemia on the response to chronic elevations of carbon dioxide tension. ¶ After an appropriate control period, dogs were placed in a large environmental chamber in which ambient O_2 tension was reduced sufficiently to lower arterial O_2

saturation to 70 to 80% and P_{O_2} to 40 to 50 mm Hg. During the initial 9 days, arterial P_{CO_2} was maintained at normal levels by raising ambient carbon dioxide tension to approximately 25 mm Hg. Subsequently, carbon dioxide tension in the chamber was increased in a stepwise fashion at 1-week intervals in order to produce levels of arterial carbon dioxide tension ranging up to 95 mm Hg. The data demonstrate that hypoxemia of a degree as great as that usually encountered in severe pulmonary insufficiency does not significantly alter the pattern of change in acid-base equilibrium that has been found in uncomplicated (nonhypoxemic) hypercapnia; with progressive increase in P_{CO_2}, bicarbonate concentration rose in a curvilinear fashion and H^+ concentration increased linearly by 0.33 nanomole of hydrogen per mm Hg increment in P_{CO_2}. The results suggest that the degree of hypoxemia ordinarily encountered in patients with chronic pulmonary insufficiency is not an important variable in determining the adjustments of acid-base equilibrium to the hypercapneic state.

The Antidiuretic Action of Adenosine 3′,5′-Monophosphate (3′,5′-AMP) in Man. ROBERT A. LEVINE, Brooklyn, N. Y. (introduced by John F. Mueller *).

Vasopressin increases the intracellular concentration of 3′,5′-AMP in dog kidney homogenates and in the isolated toad bladder, and 3′,5′-AMP mimics the effect of vasopressin on the permeability of the toad bladder to water. This study was undertaken to determine if 3′,5′-AMP induces antidiuresis in man. Acute water load studies were performed in eight recumbent subjects who consumed 25 ml per kg of water, after 10 hours of hydropenia. With indwelling Foley catheters in place, intravenous infusions of 5% dextrose and water were started at least 1 hour before study. Urinary volume and osmolar concentration were determined on 15-minute collections for at least 1 hour before and 3 hours after intravenous administration of 3′,5′-AMP (6.25 to 10.0 mg per kg for 1 minute). Prompt antidiuresis followed 3′,5′-AMP injection in all subjects. Mean urinary volume (ml per minute) decreased and osmolar concentration (mOsm per L) increased, respectively, from control values of 15.3 ± 10.7 and 62.8 ± 8 to 8.8 ± 5.6 ($p < 0.025$) and 69 ± 25 at 15 minutes; 5.1 ± 4.8 ($p < 0.001$) and 133 ± 66 ($p < 0.025$) at 30 minutes; and 7.4 ± 6.3 ($p < 0.010$) and 142 ± 97 ($p < 0.050$) at 45 minutes. As noted in our previous human studies with 3′,5′-AMP, transient side effects, consisting of cardioacceleration, abdominal pain, and headache, frequently occurred. To determine if the antidiuretic response to 3′,5′-AMP was due to a direct renal action of vasopressin, studies were performed in a posthypophysectomized, catheterized patient with diabetes insipidus and normal tests of renal function. 3′,5′-AMP (8 mg per kg for 1 minute) repeatedly simulated the time course and effects of 10 mU of Pitressin on urinary excretion and osmolarity and did not alter endogenous creatinine clearance. This study shows that 3′,5′-AMP promotes antidiuresis in man and supports *in vitro* data that 3′,5′-AMP may be the intracellular mediator of the action of vasopressin.

The Effect of Angiocardiographic Dye on Left Ventricular Volumes. GILBERT E. LEVINSON, MANOUCHEHR NADIMI, WILLIAM A. CONRAD, AND KHALDOON I. HILMI, Jersey City, N. J. (introduced by Philip H. Henneman *).

Quantitative angiocardiography yields slightly but systematically smaller estimates of left ventricular volume than indicator dilution. To study this discrepancy, measurements were made in nine dogs to determine the effects of volume-measuring technics on heart rate, left ventricular pressures, and dP/dt, stroke volume measured by electromagnetic flowmeter, and left ventricular dimensions measured by myography. Blood sampling from left ventricle or aorta (at rates to 2 ml per second), continuous infusions of indocyanine green dye into left ventricle (at the same rates), and sudden injections of 1 or 2 ml of dye or iced saline produced no significant effects. Injections of 3 to 5 ml of green dye or iced saline produced negligible effects confined to the injection interval. However, injections of contrast material (0.25 to 1 ml per kg) produced appreciable dose-related rises ($p < 0.001$) in left ventricular systolic and diastolic pressures, dP/dt, and stroke volume, evident 1 beat after onset of injection. This enhancement of ventricular performance was accompanied by significant ($p < 0.001$) decrease in left ventricular end-diastolic and end-systolic dimensions that appeared during injection, persisted after termination of injection, and in all instances was present for an interval of at least 12 beats. The fall in end-diastolic fiber length ranged from 5% during injection and 6% thereafter with injections of 0.25 ml per kg to 9% and 15% with injections of 1 ml per kg. The fall in volume with rise in pressure indicates a decrease in compliance of the ventricle. We conclude that indicator-dilution methods produce no change in left ventricular performance or dimensions, but that contrast materials in doses conventionally employed produce a decrease in left ventricular volumes consistent with the reported discrepancy between indicator dilution and quantitative angiocardiography.

Acid Base Changes Associated with Potassium Deficiency: Evidence for Impaired Renal Conservation of Chloride. HOWARD LEVITIN * AND ROBERT G. LUKE, New Haven, Conn.

The pathogenesis of hypochloremia in potassium deficiency was studied in rats treated with deoxycorticosterone acetate (DOCA) while on a low potassium, low chloride diet. When severe potassium depletion (20% reduction in muscle potassium) develops in such animals, balance studies reveal marked impairment of the ability of the kidneys to conserve chloride. Urinary leakage of chloride is not present with lesser degrees of potassium deficiency, but when it appears, it leads to, and persists despite the presence of, mounting chloride depletion. It

is evident from comparison with control animals that the chloride leak is related to potassium deficiency and not to the administration of DOCA or the presence of hypochloremic alkalosis. It is also independent of the concomitant sodium balance. The ability of the kidneys of potassium-depleted rats to conserve chloride was rapidly restored to normal when potassium was administered. ¶ The metabolic alkalosis observed in animals with severe potassium and chloride depletion cannot be corrected by the administration of either ion alone. Thus potassium administration without chloride resulted in a 50% correction in the alkalosis without a significant change in the renal excretion of acid. Chloride repletion without potassium also induced a 50% correction in the alkalosis that could be accounted for by a significant reduction of acid excretion in the urine. Repletion with both potassium and chloride resulted in complete correction of the extracellular alkalosis. ¶ It seems evident that severe potassium depletion, in addition to causing a movement of hydrogen ion into cells, is associated with a defect in renal chloride conservation.

Pendelluft: **Fact or Fiction?** BENJAMIN M. LEWIS,* EDWARD GAFFNEY, AND BURLEIGH DETAR, Detroit, Mich.

If different regions of the lung have different compliances and resistances, these regions have, in terms of electrical analogy, different time constants. During repetitive breathing a region with a short time constant will begin to inspire earlier than a region with a long time constant, and such a region will also expire earlier and receive more of the inspired breath. Since regions with different time constants are out of phase, some ventilation will pass from one region to the other and not appear externally (*Pendelluft*). ¶ We have investigated the occurrence of this phenomenon by introducing a bolus of hydrogen at the beginning of an inspiration, which consisted otherwise of 1% helium while the subject was breathing repetitively at functional residual capacity. After this inspiration the subject expired slowly to residual volume while five samples were taken directly from the expiratory gas stream by a "six shooter" gas sampler. These samples were analyzed for helium and hydrogen by a gas chromatograph. ¶ In theory, the region (or regions) with a short time constant, since it begins inspiration first, should receive more hydrogen than the region with the long time constant, and since the short time constant region also expires first, the concentration of hydrogen plotted against volume should fall more rapidly than that of helium, once the subject's dead space is cleared. Actually, in five normal subjects, six patients with airflow obstruction, and five patients with other pulmonary disease the reverse occurred: helium concentration fell more rapidly than hydrogen. In only three patients, two with airflow obstruction and one without, did the hydrogen concentration fall more sharply. Thus, in most instances, gas is not distributed on inspiration in a way compatible with the occurrence of *Pendelluft.*

Sodium Balance in the Human Renal Transplant. EDMUND LEWIS, M. DONALD BLAUFOX, PAUL JAGGER, DAVID LAULER, EHSAN QURESHI, AND JOHN P. MERRILL,† Boston, Mass.

Sodium balance was studied in six donor-recipient pairs at intervals ranging from 2 months to 2 years after renal transplantation. One of the grafts was an isotransplant; the remainder were allografts. Average inulin clearance was 72 ± 6.7 (SE) ml per minute for donors and 59 ± 4.9 ml per minute for recipients. Average PAH clearance was 292 ± 19.7 (SE) ml per minute and 281 ± 21.5 ml per minute for donors and recipients, respectively. The filtration fractions were identical in the twins (0.26) while being consistently lower in the allografts [0.20 ± 0.011 (SE)] compared to the donors (0.245 ± 0.017). Exchangeable sodium and potassium values were within normal range in all cases; no significant difference between donor and recipient was noted. ¶ Balance studies were performed on high (200 mEq per day) and low (10 mEq per day) sodium diets for 5 days each. Potassium intake was maintained at 100 mEq per day. Sodium balance was achieved within 4 days in all subjects. Donor peripheral plasma renin activity averaged 372 ± 118 (SE) nanograms (ng) angiotensin (A) % on the high sodium diet and 817 ± 129 ngA% on the low sodium diet. Transplant plasma renin values were 368 ± 133 ngA% and 856 ± 116 ngA% on the high and low salt regimens, respectively. Aldosterone secretory rates were performed on each of the subjects. The aldosterone secretory rate was 230 μg per day on the high salt and 1,110 μg per day on the low salt diet in the isograft. The allografts also showed similar physiologic response to salt loading and deprivation. ¶ We conclude that the renin-angiotensin-aldosterone mechanism is intact after renal transplantation and that grafted kidney responds normally under conditions of sodium loading and sodium deprivation.

Glycine Decarboxylation in the Erythrocyte: A Sensitive Assay for Aminolevulinic Acid Synthesis. M. LEWIS, G. R. LEE, AND M. M. WINTROBE,† Salt Lake City, Utah.

The initial and rate-limiting step in heme synthesis is the formation of delta-aminolevulinic acid (ALA) from glycine and Kreb's cycle intermediates. It has been proposed that this step is defective in certain experimental and clinical disorders; for example, pyridoxine deficiency and pyridoxine-responsive anemia. However, investigation of this proposal has been hampered by the lack of a sensitive assay method. Such a method, based on the measurement of the decarboxylation of glycine, is the subject of this report. The method to be presented is capable of quantitating ALA-synthetase activity in 10 ml of normal blood containing 1% reticulocytes. ¶ The specimen is preincubated with transaconitate in order to deplete the endogenous substrates. During the preincubation, the enzyme source is also incubated with glycine (0.2 M) and either alpha-ketoglutarate (α-KGA) or succinate. After 1 hour, glycine-1-^{14}C is added, and

the $^{14}CO_2$ evolved over the next 90 minutes is trapped in Hyamine and counted in a liquid scintillation system. A blank consists of an identical flask without added α-KGA. ¶ The amount of $^{14}CO_2$ liberation is proportional to enzyme concentration and is linear with time; sharp maxima exist for pH (7.0) and the concentration of α-KGA (0.024 M). A Lineweaver-Burke plot of glycine concentration versus velocity is linear. Specificity of the assay was demonstrated by *1*) absolute dependence on the presence of α-KGA or succinate, *2*) inhibition by hemin (1×10^{-4} M), and *3*) direct comparison with the incorporation of glycine-2-^{14}C into ALA in porcine reticulocyte-rich blood. ¶ In eleven normal volunteers, the mean activity was 33 mμmoles of glycine decarboxylated per 90 minutes per 10^8 reticulocytes, with a range from 17 to 71.

Leukocyte Reactivity, Serum Antibodies, and Clinical Symptoms during Specific Desensitization Therapy of Ragweed Pollinosis. LAWRENCE M. LICHTENSTEIN, PHILIP S. NORMAN,* WALTER L. WINKENWERDER, AND ABRAHAM G. OSLER, Baltimore, Md.

The capacity of isolated leukocytes to release histamine on interaction with ragweed pollen antigen has been studied in relationship to clinical symptomatology and response to therapy of 66 ragweed-sensitive patients during two seasons of pollen exposure. In placebo-treated patients, the response of the leukocytes to antigen E (purified from ragweed pollen) was closely correlated with the severity of symptoms during the ragweed season. The cell sensitivity therefore provided a quantitative prognostic index of the severity of the allergic condition. The sensitivity of the leukocytes did not change significantly in patients treated with placebo or with ragweed antigen. In one untreated donor, however, spontaneous loss of clinical symptoms coincided with a decreased ability of cells to release histamine. Serum anti-E levels after therapy were also measured, in terms of their ability to bind antigen and thereby decrease the *in vitro* allergic response. The levels of this antianaphylactic antibody did not change in the placebo-treated group, but rose in individuals treated with E. The increase in serum antibody activity ranged between 3- and 3,000-fold and was correlated with the quantity of antigen administered. Symptomatic relief was not great. The marginal response with respect to symptom severity may be attributed to either *1*) immunization with only one of the several antigens in ragweed pollen, *2*) an antigenic dose insufficient to induce enough antibody production or to change leukocyte sensitivity, or *3*) an inherent defect in the therapeutic concept.

Accumulation of Triglycerides in Heart and Kidney after Alcohol Ingestion. CHARLES S. LIEBER,* NORTON SPRITZ, AND LEONORE M. DECARLI, New York, N. Y.

Clinical evidence suggests that not only liver but also heart and kidney functions are affected by ethanol. We therefore extended the studies of hepatic triglyceride concentration and origin to hearts and kidneys of rats pair fed with nutritionally adequate liquid diets containing either ethanol (36% of calories) or isocaloric carbohydrate (controls). When the diet contained an olive-corn oil mixture (consisting of long chain triglycerides: LCT) to the extent of 41% of total calories, an amount of fat comparable to that of an average American diet, ingestion of ethanol over 24 days produced average rises in triglyceride concentrations of 700% in liver ($p < 0.001$), 300% in kidney ($p < 0.002$), and 200% in heart ($p < 0.01$). The role of dietary LCT was investigated by replacing them isocalorically with medium chain triglycerides (MCT) or carbohydrates. This resulted in an attenuation of the triglyceride rise from 700 to 300% in livers ($p < 0.02$) and from 300 to 50% in kidneys ($p < 0.01$), whereas in heart, the ethanol effect became nonsignificant. ¶ After feeding ethanol with either MCT or high carbohydrate diets, liver and kidney triglycerides had linoleate concentrations only one-third that of adipose tissue, indicating that, after elimination of dietary LCT, the largest fraction of triglyceride fatty acids originated from endogenous synthesis rather than depots. ¶ To assess possible effects of malnutrition, we fed rats ethanol with a carbohydrate-rich, choline- and amino acid-deficient diet. Contrasting with liver triglycerides, whose accumulation was increased three to five by the malnutrition, in hearts and kidneys the ethanol effect was not enhanced. ¶ In conclusion: *1*) Triglyceride accumulations in hearts and kidneys accompany hepatic steatosis produced by ethanol and LCT-containing diets. *2*) This can be reduced significantly by elimination of dietary LCT. *3*) Triglycerides then consist of endogenously synthesized fatty acids. *4*) Their accumulation in hearts and kidneys is not exaggerated by protein and lipotrope deficiencies.

Metabolic Changes in Diabetes, Cushing's Disease, and Acromegaly after Pituitary Suppressive Therapy. J. A. LINFOOT, J. A. GARCIA, J. L. BORN, C. A. TOBIAS, AND J. H. LAWRENCE,† Berkeley, Calif.

Metabolic activity of the normal pituitary gland (notably resistant to irradiation) has rarely been influenced by gamma rays or X rays. With externally delivered heavy particles it has been possible to suppress pituitary function safely and to produce dramatic effects on insulin sensitivity and glucose tolerance in patients with acromegaly and to alter insulin requirements in patients with diabetes. These effects appear to be primarily related to changes in pituitary growth hormone (HGH) secretion, which was measured with a sensitive radioimmunoassay employing ^{125}I-labeled HGH and double-antibody technique. Modified 30-minute insulin tolerance tests were performed in normal subjects and in patients undergoing pituitary irradiation, and glucose disappearance rates (k) and half-times ($t_{\frac{1}{2}}$) were calculated. Normal k values ranged from 2.5 to 5.9 mg per 100 ml per minute ($t_{\frac{1}{2}}$, 27.7 minutes); k values for untreated acromegalics and adult-type diabetics were lower (1.2 to 1.6), and $t_{\frac{1}{2}}$ were 47 to 56.8 minutes. After heavy particle exposure the k increased (for acromegalics mean k was 3.2; for diabetics, 4.1); this was associated with

a fall in circulating HGH in the acromegalics and a loss of response to hypoglycemia in the diabetics. Serial studies performed in a castrated male patient with mammary carcinoma, who had been receiving estrogens, and in a postmenopausal female patient with Cushing's disease complicated by diabetes mellitus showed progressive increases in k and decreases in $t_{\frac{1}{2}}$ after heavy particle irradiation. In the former patient the change was correlated with the fall in HGH response to hypoglycemia; in the latter it seemed more related to the fall in steroid secretion. These studies show that effects of heavy particles on the pituitary provide a unique opportunity to study the relation between trophic hormone production and other metabolic processes and to relate this simultaneously to therapeutic benefits many of these patients gain.

The Cellular Basis of Stress Ulcer Formation in the Stomach. MARTIN LIPKIN, ROBERT M. KERR, AND YOUNG-SHIK KIM, New York, N. Y. (introduced by Thomas P. Almy †).

To investigate the role that cell proliferation kinetics may have in the sequence of events leading to gastric ulceration, we produced stress ulcers of the stomach by placing mice in a wire mesh restraint cage for varying intervals. In the stomach, the rate of DNA synthesis was studied after injection of tritiated thymidine and microradioautography, the rate of protein synthesis after injection of tritiated leucine, and the production of mucin by histochemical staining procedures. Cell proliferation kinetics in stomach were also compared to those in duodenum and colon during stress. ¶ Although no differences in protein synthesis in epithelial, parietal, and zymogen cells were observed, a marked depression of epithelial cell proliferation was observed in stomach mucosa under stress. In control stomachs, the DNA synthesis (*S* phase) duration of the proliferative cycle was 8 hours, the metaphase duration 0.4 hour, and the replacement time of the epithelial mucosa 3 to 4 days. By contrast, at early intervals after stress, decreases were observed in the number of cells entering DNA synthesis ($0.02 > p > 0.01$) and mitosis ($0.02 > p > 0.01$). At later intervals, the fewer epithelial cells entering the proliferative cycle also developed a longer metaphase duration of 1.7 hours. The decrease in the number of cells entering DNA synthesis and mitosis was greater with a longer application of stress. These changes were also accompanied by a slight decrease in PAS staining, suggestive of some alteration in surface epithelial mucin production. At later intervals after the application of stress, surface erosions began to form as surface cells were extruded and replacement from below failed to occur. ¶ In conclusion, during the application of stress, a disparity between DNA synthesis and mitosis, and protein synthesis was observed. These changes in stomach, before the appearance of ulcerations, indicate the primary importance of a failure of cell replication in the mechanism of ulcer formation.

The Effect of Ethacrynic Acid and Furosemide on Sodium Transport and Ionic Permeability in the Toad Bladder. STEVEN LIPSON AND RICHARD M. HAYS,* New York, N. Y.

Ethacrynic acid and furosemide are unusually potent saluretic agents. Studies employing the toad bladder indicate that these drugs not only inhibit active sodium transport, but also decrease the ionic permeability of the bladder. Experiments were performed in a split chamber, in which one side of the bladder was a control for the other. Addition of ethacrynic acid or furosemide to the serosal medium (1.7×10^{-4} M) decreased short-circuit current significantly within 5 minutes; by 20 minutes, the current was 60 and 80% of control values, respectively. In addition, both agents produced a significant and sustained rise in the d-c resistance of the bladder, which was evident 5 to 10 minutes after their administration. The rise in resistance indicated that the permeability of the bladder to sodium or chloride or both (the two major ions in solution) had decreased. ^{36}Cl movement across the short-circuited bladder was not significantly changed by ethacrynic acid, indicating that the major effect of this drug is on a permeability barrier to sodium. The effect of furosemide on chloride movement is currently being studied. Additional studies with chlormerodrin and ouabain showed the following: Chlormerodrin (1.7×10^{-4} M) produced no change in resistance despite a significant decrease in short-circuit current; ouabain (3.5×10^{-5} M), the most potent inhibitor of short-circuit current employed, produced a rise in resistance that was smaller and appeared later than that seen with ethacrynic acid and furosemide. These studies provide evidence that ethacrynic acid and furosemide not only inhibit active sodium reabsorption by renal tubular cells, but decrease their ionic permeability. A comparable effect on permeability would not be predicted in the case of chlormerodrin. This distinctive action of ethacrynic acid and furosemide may contribute to their saluretic potency in man.

Urea Cycle Enzymes in Magnesium Deficiency. G. LIZARRALDE, V. E. MAZZOCCO, AND E. B. FLINK, † Morgantown, W. Va.

Induction of dietary magnesium deficiency in the rat results in a decreased growth rate and uremia. It has been suggested that the latter may occur due to a disturbance in the urea cycle. To examine this problem, we fed 200-g female Holtzman white rats a Mg-deficient diet (0.016 mEq Mg^{++} per day) for 24 days, at which time the animals were sacrificed, the livers removed and homogenized, and assays performed for carbamyl-phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinase, and arginase. When compared to control animals maintained on the same diet plus Mg supplement, the mean body weight of controls was 232 g and of deficients 210 g ($p < .05$); the mean liver weight of controls was 6.2 g and of deficients 7.8 g ($p > 0.05$). The milligrams of protein per gram of liver did not differ between the groups (controls, 9.76; deficients, 9.14;

$p > 0.6$). The enzymatic activity expressed as micromoles of product per hour per gram of liver showed significant differences only in ornithine transcarbamylase (controls, 20,240; deficients, 10,660; $p < 0.01$) and in argininosuccinase (controls, 434; deficients, 284; $p < 0.05$); when the results were expressed as micromoles per hour per total liver weight, only the former showed a significant difference. The activity of the other enzymes did not differ significantly. The results suggest that carbamyl-phosphate conversion to citrulline is reduced in Mg-deficient rats, and it is perhaps due to an increased shunting of this compound to the synthesis of carbamyl-aspartate, the precursor of pyrimidines. The increased blood urea nitrogen in deficient rats cannot be explained on the basis of altered urea cycle enzymes.

The Basis of Ventilatory Failure in Obstructive Lung Disease. RUY V. LOURENÇO, JOSEPH M. MIRANDA, EDWARD P. MUELLER, AND STEPHEN Y. K. CHUNG, Jersey City, N. J. (introduced by Gerard M. Turino*).

In the evolution of ventilatory failure in respiratory disease, the role of reduced nervous stimuli to ventilation, as opposed to augmented mechanical resistances, remains unclear. In this study, these factors were analyzed in 9 normal subjects and 11 patients with chronic obstructive lung disease, with and without chronic hypercapnia. Diaphragmatic electromyography (EMG) was used to assess the nervous stimuli to ventilation; previously we demonstrated, in animals, a direct correlation of diaphragmatic electrical activity with phrenic nerve impulses in states of airway obstruction and reduced ventilatory response. Diaphragmatic EMG, ventilation, alveolar P_{CO_2} (P_{ACO_2}), ventilatory mechanics, and arterial blood gases were measured simultaneously during graduated CO_2 response produced by continuous rebreathing of oxygenated expired air from a closed circuit. Diaphragmatic EMG was recorded with an esophageal bipolar lead, fed through a clipping circuit to minimize ECG interference, and integrated electrically for quantification. Incremental relationships of ventilation, EMG, and P_{ACO_2} during rebreathing were continuously recorded and analyzed by computer. Results demonstrate that the increment in ventilation per unit increment in P_{ACO_2} is abnormally low in all patients and results from a limited increment in tidal volume. The increment in EMG per increment in P_{ACO_2} was normal in patients with eucapnia, but markedly diminished in patients with hypercapnia. In the latter group, P_{ACO_2} and EMG showed a significant inverse linear correlation ($r = -0.845$, $p < 0.01$). It is concluded that in patients with obstructive lung disease, mechanical limitations to ventilation reduce the ventilatory response in the eucapneic state; however, with chronic hypercapnia, reduced ventilatory drive plays a major role in the reduced ventilatory response, becoming the predominant factor when marked CO_2 retention is present. This distinction remains of fundamental significance in the therapy of respiratory insufficiency.

Reduced PAH Extraction without Alteration in Distribution of Intrarenal Blood Flow during Renal Vasodilation in Man. JEROME LOWENSTEIN, RICHARD M. EFFROS, DAVID S. BALDWIN,* AND HERBERT CHASIS,† New York, N. Y.

Redistribution of intrarenal blood flow, with disproportionate increase in medullary or "noncortical" perfusion, has been postulated to explain the fall in extraction ratio of *p*-aminohippurate (E_{PAH}) that occurs when renal blood flow is increased by administration of acetylcholine. To test this hypothesis, we have recorded indicator-dilution curves (indocyanine green) across the renal vascular bed, before and immediately after renal arterial infusion of acetylcholine, 230 μg per minute in seven subjects. Renal blood flow, calculated from the clearance and extraction of PAH, increased from an average of 446 ml per minute to 767 ml per minute. E_{PAH} fell from an average value of 0.854 to 0.792 (the decrement in individual subjects ranged from 0.034 to 0.144). Indicator-dilution curves remained unimodal, whereas mean transit time decreased from 4.4 seconds to 3.1 seconds. The frequency distribution of specific blood flow (blood flow per unit renal blood volume) was derived from the dye curves as described by Gomez. Although mean specific blood flow increased from an average value of 0.227 to 0.322 ml per second per ml, the distribution of specific blood flows about the mean was unaltered. ¶ Direct measurements in discrete zones of the kidney have shown that the transit time through the medulla is longer than that through the cortex. With the present technique, medullary pathways would be represented predominantly in the later portions of the dye curve, and disproportionate increase in blood flow through these pathways would be expected to result in alteration in the shape of the indicator-dilution curve and in the distribution of specific blood flow. Our demonstration that the distribution of specific blood flow was unaltered during renal vasodilation indicates that a disproportionate increase in medullary flow did not occur. It is suggested that the fall in extraction of PAH during hyperfusion might be related to more rapid transit through the kidney.

Studies on Human Cardiac Myosin. ROBERT J. LUCHI, Philadelphia, Pa. (introduced by Hadley L. Conn, Jr.*).

Cardiac myosin was isolated from the papillary muscle of six patients undergoing prosthetic replacement of the mitral valve. All had a history of congestive failure but were free from clinical signs of congestive failure at the time of operation. Myosin was extracted and prepared by the LiCl-$(NH_4)_2SO_4$ technique. ¶ On ultracentrifugation, each protein preparation sedimented as a single sharp symmetrical peak. Mean values for the various biochemical determinations are as follows: Sedimentation coefficient, 6.2; intrinsic viscosity, 2.1 dl per g; absorbance indexes at 280 and 294 mμ, 4.50 and 4.95; tyrosine, 1.7×10^{-5} mole per g protein; tryptophan, 3.4×10^{-4} mole per g protein; tyrosine:tryptophan ratio, 5:1; molecular weight (Archibald technique), 513,000; 70% helix

(ORD); ATPase activity, 3.1 μmoles P per mg protein per 5′. ATPase activity was calcium activated, showed pH optima of 6.5 and 8.5, and was strongly inhibited by magnesium. Digitalis did not alter myosin ATPase activity. ¶ At this writing, ventricular muscle from patients without a history of heart failure has not been studied. Myosin from dogs with naturally occurring cardiac failure, or from dogs whose hearts have been subjected to anoxia, has biochemical properties similar to those described above for the human. Myosin from normal dogs sacrificed without hypoxia differs only in having a higher ATPase activity of 4.44 μmoles P per mg protein per 5′ ($p < 0.001$). Thus, reduced cardiac myosin ATPase activity, although not established as the prime pathogenetic defect in low output congestive heart failure, undoubtedly plays a role in the reduced contractility that characterizes this state.

Dissociation by Colchicine of Phagocytosis Per Se from Increased Oxygen Consumption in Human Leukocytes. STEPHEN E. MALAWISTA AND PHYLLIS BODEL, New Haven, Conn. (introduced by Elisha Atkins *).

The anti-inflammatory action of colchicine in acute gouty arthritis has been ascribed to effects on polymorphonuclear leukocytes. Colchicine interferes with locomotion in granulocytes and with the respiratory burst that accompanies phagocytosis. We did the present study to examine the effect of colchicine on phagocytosis per se in a sensitive bacterial system. ¶ Human blood leukocytes were incubated in a 12% serum-Krebs-Ringer phosphate buffer with and without colchicine at 37.5° C for 1 hour in a Warburg respirometer. Alpha-streptococci were then added to duplicate experimental and control flasks, and incubation was continued for another hour. Ratios of bacteria to granulocytes were adjusted from 1:15 to 9:1 in different experiments. After incubation, the flask contents were plated in agar, and live bacteria were subsequently estimated by colony counts. ¶ The increase in oxygen consumption that normally accompanies phagocytosis was consistently diminished in leukocytes incubated with colchicine in concentrations as low as 2.5×10^{-6} mole per L (1 μg per ml). Yet, there was no evidence of decreased phagocytosis with concentrations of colchicine as high as 2.5×10^{-4} mole per L (100 μg per ml). In time studies, the rate of disappearance of bacteria was comparable with and without colchicine. Appropriate controls ruled out killing of bacteria by colchicine or by products of leukocytes. ¶ A clue to the dissociation between oxygen consumption and phagocytosis was found in rapidly dried preparations of the incubated leukocytes. Ingested bacteria were present in both control and colchicine-treated granulocytes. In addition, control cells showed normal loss of granules (lysosomal particles) and resultant large digestive vacuoles. Colchicine-treated cells, however, showed less of such degranulation and vacuolization. Changes in granule-associated acid phosphatase activity after phagocytosis confirm the morphologic observations. These findings suggest that *1*) altered metabolic activity, occurring during phagocytosis and inhibited by colchicine, is related at least in part to degranulation, and *2*) incubation with colchicine results in less degranulation, not less phagocytosis.

Chronic Pesticide Exposure with Renal Tubular Dysfunction, Aminoacidemia, and Aminoaciduria. JOEL B. MANN, JOHN E. DAVIES, PAUL M. TOCCI, AND GEORGE A. REICH, Miami, Fla. (introduced by A. G. Hills †).

Evidence of multiple abnormalities of renal tubular function and of amino acid metabolism was uncovered in a total group of 70 spraymen and formulators occupationally exposed to various pesticides and was evaluated by one or more of the following tests: per cent tubular reabsorption of phosphate (TRP), urinary titratable acid (TA) and NH_4^+ excretion after NH_4Cl loading, concentrating ability and maximal free water reabsorption ($Tm^c_{H_2O}$), and paper and column chromatographic analysis of blood and urine for amino acids. ¶ Seven of 34 spraymen had TRP values < 0.87 as compared with none < 0.87 in 105 normal controls under age 40 ($p < 0.0001$). The regression of TRP values with the number of years employed reveals a significant ($p < .005$) decrease in TRP with duration of exposure. Only 1 of 5 hospitalized spraymen had normal tubular function. Two had cystinuria with excretion of arginine, ornithine, and lysine as well. One cystinuric had a TRP of 0.79 and a marked reduction in TA and NH_4^+ excretion, despite a drop in urine pH to 4.5. Another sprayman also had a low TRP (0.81) and subnormal TA and NH_4^+ excretion. The fourth had a TRP of 0.81 and a limitation of concentration of 456 mOsm per kg, with $Tm^c_{H_2O}$ of only 1 ml per minute. Some of the dysfunctions have persisted despite a marked decrease in or total lack of subsequent pesticide exposure. ¶ Twenty-three of 36 exposed persons had significant elevations of many serum amino acids. Those of leucine, isoleucine and valine, tyrosine, tryptophane, histidine, glutamine, and alanine were of particular significance. Elevations of urinary amino acids in general paralleled those of the blood, suggesting an overflow aminoaciduria in most, but not all, instances. ¶ This evidence suggests that chronic pesticide exposure produces widespread alterations of amino acid metabolism, as well as multiple potentially irreversible renal tubular dysfunctions, which increase with duration of exposure.

The Natriuretic Effect of Angiotensin Revealed after Autonomic Blockade. JOHN C. MCGIFF, Philadelphia, Pa. (introduced by René Wégria †).

Angiotensin (angiotensin II) produces sodium retention in man and dog, presumably due to renal vasoconstriction. In contrast, a natriuresis in response to angiotensin has been demonstrated when its vasoconstrictor effect is reduced, as in subjects with malignant hypertension. The vasoconstrictor action of angiotensin has been demonstrated in this laboratory to be reduced by certain adrenergic blocking agents. Experiments were designed to ascertain whether the natriuretic effect of angiotensin

could be expressed after attenuating its renal vasconstrictor effect by guanethidine. To demonstrate consistently the natriuresis elicited by angiotensin after guanethidine, it was necessary to eliminate the initial sympathomimetic effect of intravenous guanethidine with reserpine. In 12 reserpinized dogs, an angiotensin infusion (0.0375 μg per kg, iv) produced no significant change in GFR (C_{Cr}) 21 ± 3 (SEM) ml per minute, and in sodium excretion ($U_{Na}V$) 41 ± 15 and potassium excretion (U_KV) 21 ± 4 μEq per minute. (All values refer to a single kidney.) Renal blood flow (RBF, electromagnetic flowmeter) fell from 144 ± 15, to 81 ± 16 ml per minute. In the same dogs after guanethidine, GFR was unchanged by angiotensin (20 ± 2 and 20 ± 2 ml per minute), whereas $U_{Na}V$ was increased from 102 ± 44 to 220 ± 42 μEq per minute ($p < 0.01$), and U_KV was increased from 22 ± 4 to 36 ± 6 μEq per minute ($p < 0.05$). RBF was reduced from 121 ± 17 to 91 ± 16 ml per minute. After guanethidine, 2.8 to 14.6% of the filtered load of sodium was excreted. Reduction of the control GFR by 20 to 30% by renal arterial constriction failed to suppress the increased $U_{Na}V$ produced by angiotensin. These results demonstrate that the renal tubular activity of angiotensin may be expressed after autonomic blockade, and they indicate the interaction between catecholamines and angiotensin.

Systemic and Coronary Hemodynamic Effects of Beta-Adrenergic Blockade during Exercise. DAVID H. MCKENNA, SALVADOR SIALER, ROBERT J. CORLISS, AND GEORGE G. ROWE,* Madison, Wis.

Cardiac output (Fick principle) and coronary blood flow (nitrous oxide method) were determined in intact anesthetized dogs before and during exercise induced by electrical stimulation of the thigh muscles. While the same stimulation was continued, beta-adrenergic blockade was induced by 2 mg per kg of propranolol given intravenously, and repeat determinations were made of cardiac output and coronary blood flow. Thus, studies were made at rest, during exercise, and during exercise plus beta-adrenergic blockade. During exercise, the body oxygen consumption increased (+ 108%, $p < 0.001$) and decreased from this figure (− 12%, $p < 0.02$) after propranolol. Cardiac output increased 23% ($p < 0.02$) during exercise but returned nearly to control values after propranolol. Left ventricular work increased 22.5% ($p < 0.02$) during exercise and decreased below control levels during beta-adrenergic blockade. Coronary flow increased 17% with exercise and decreased to the control value during propranolol, but these changes were too variable to be statistically significant. ¶ These studies indicate that the circulatory response to exercise in the intact anesthetized dog can be reduced by beta-adrenergic blockade utilizing propranolol.

On the Mode of Action of Thyroid Stimulators. J. M. MCKENZIE,* Montreal, Canada.

Hormonal action through a primary effect on RNA or protein synthesis is frequently suggested. Inhibition by antibiotics of thyrotropin and the long-acting thyroid stimulator (LATS) has been offered as evidence that they also act by this means by Kriss and his colleagues. In mice, a single intravenous injection of thyrotropin increased *in vivo* amino acid-^{14}C incorporation into thyroid protein. Chronic administration of propylthiouracil produced greater incorporation and also measurable incorporation of labeled precursors into RNA. These phenomena were inhibited by, respectively, puromycin (4 mg) or cycloheximide (0.5 to 1.0 mg) and actinomycin D (8 to 16 μg) given ½ to 1 hour earlier. Release of thyroid ^{125}I after intravenous injection of thyrotropin or LATS was not inhibited unless the antibiotics were given 8 to 15 hours previously. In mice with thyroid iodine labeled with ^{125}I, uptake of ^{131}I and release of ^{125}I after 50 mU of thyrotropin were measured. Release was inhibited by cycloheximide before uptake was significantly lowered; this difference might, however, be influenced by reduction of renal iodide clearance. No clear qualitative differences in the effects of the two stimulators or their inhibition by antibiotics were seen, which may imply an action at the same site, or sites, in the thyroid. This also may be inferred from the finding that LATS given 2 hours before 0.2 to 0.8 mU thyrotropin almost completely prevented response (^{125}I release) to the latter (e.g., increment in blood ^{125}I due to 0.2 mU after control injection, 2,532 cpm per 0.1 ml; after LATS, 335 cpm per 0.1 ml), despite control mice that exhibited a greater total response to larger doses of either agent. Fresh protein or RNA synthesis thus does not appear necessary for acute effects of thyrotropin or LATS, but the short time interval mandatory for the inhibiting effect of antibiotics suggests that stimulation of thyroid iodine release is mediated by a protein with a rapid turnover rate.

Hypotensive Effect of Dopamine in Dogs and Hypertensive Patients after Phenoxybenzamine. J. L. MCNAY, K. L. MACCANNELL, M. B. MEYER, AND L. I. GOLDBERG,* Atlanta, Ga.

This study analyzes the modifying effects of phenoxybenzamine on the cardiovascular responses to dopamine infusions in dogs and hypertensive patients. In six pentobarbital-anesthetized dogs, renal and femoral blood flows were studied with an electromagnetic flowmeter. Dopamine, 3 μg per kg per minute, increased renal blood flow to an equal extent before and after phenoxybenzamine. Average mean blood pressure was not altered by dopamine before, but fell 20 mm Hg after, phenoxybenzamine. Femoral vascular resistance was not affected by dopamine before or after phenoxybenzamine. In five additional dogs, the effects of dopamine on cardiac output (dye dilution technique) and mean blood pressure were studied. Dopamine decreased mean blood pressure, increased cardiac output, and decreased total peripheral resistance. All changes were greater after phenoxybenzamine, but not significantly so. From these findings we concluded that dopamine consistently lowers canine mean blood pressure after phenoxybenzamine by selective vasodilatation. Dopamine was administered to six patients with severe

hypertension treated with bethanidine, guanethidine, or both. Dopamine infusion, 1 to 1.5 μg per kg per minute, without phenoxybenzamine raised mean blood pressure an average of 10 mm Hg. In five patients after phenoxybenzamine, 1 mg per kg, dopamine decreased recumbent mean blood pressure an average of 37 mm Hg (29%), increased cardiac output an average of 2.6 L per minute (44%), decreased total peripheral resistance 50%, and increased heart rate an average of 16 beats per minute (25%). Creatinine clearance did not diminish during the period of reduced blood presure. We concluded that prior administration of phenoxybenzamine enhances the vasodilating effect of dopamine in hypertensive human subjects.

Use of Air Comparison Pycnometry to Measure Bone Density, In Vitro. C. R. MELONI AND J. J. CANARY,* Washington, D. C.

We measured bone volume and calculated density on 15 autopsy and 5 biopsy specimens with an air comparison pycnometer. Sixth or seventh ribs were used in all cases. The bone was cleaned, flushed with water under pressure, opened and subjected to papain solution for 3 hours, extracted with ether, and allowed to equilibrate in a constant temperature and humidity atmosphere in which it was weighed and the volume measured. Day-to-day variations in temperature and humidity resulted in nonreproducibility on replicate measurements. Under the conditions mentioned, replicability was good. Marrow cavity fat contributed significantly to the density before ether extraction. Bone density did not vary significantly between right and left ribs or with age or disease. Neither osteoporotic nor osteopetrotic bone differed significantly in density. Calcium, phosphorus, and nitrogen content did not differ significantly. Use of this instrument permits chemical and densitometric analysis in the same specimen. The results indicate that density in normal, osteoporotic, and osteopetrotic bone is similar, and that the essential difference in these disorders is in total body bone mass.

Mixed Cryoglobulins Associated with Purpura, Arthralgia, and Acute Renal Failure. M. MELTZER, E. C. FRANKLIN,* K. ELIAS, R. T. MCCLUSKEY, N. COOPER, AND P. MIESCHER,* New York, N. Y.

Two-thirds of cryoglobulins from 35 patients with cryoglobulinemia were myeloma proteins or macroglobulins. However, almost one-third (11) were "mixed cryoglobulins," consisting of 60% IgM and 40% IgG molecules and possessing high titers of rheumatoid factor activity, best measured with HGG. Only two of the mixed cryoglobulins were found in patients with myeloma or macroglobulinemia. As shown by LoSpalluto, rheumatoid factor activity resided in the 19 S fraction, whereas cryoprecipitability required both IgM from the patient and IgG or Fc-fragments from human or rabbit IgG. Nine of these sera had broad γ-globulin peaks on electrophoresis; the isolated cryoglobulin in eight had a 7 S and 19 S peak; in one, 22 to 30 S complexes were seen. Six isolated 19 S fractions had both κ and λ chains, and one had only κ chains. Antinuclear antibodies were present in the supernatant fluids in four of seven, and serum complement (C'2) was low or absent in each of seven sera tested. ¶ Each of these nine patients had purpura, weakness, and arthralgias; hepatosplenomegaly and lymphadenopathy were present in the majority. Eight of the nine were females; two had had Sjögren's syndrome and three had thyroiditis. The most striking feature was the development of acute renal failure resulting in death in three. Postmortem examinations showed acute diffuse glomerulonephritis in all three, and necrotizing arteritis in two. IgM and IgG were localized in the glomeruli of one subject studied. Renal biopsies in two subjects without renal disease were normal. ¶ The possibility exists that these labile proteins, which resemble antigen-antibody complexes, can localize within glomeruli, producing glomerulonephritis, or in other small blood vessels, initiating a vasculitis in the skin and other organs analogous to that seen in experimental serum sickness.

The Cytotoxicity of Bilirubin: Protective Effects of Albumin for Brain Mitochondria *In Vitro*. MATTHEW MENKIN, JEANNE G. WAGGONER, AND NATHANIEL I. BERLIN,* Bethesda, Md.

Bilirubin is toxic for some mammalian cell cultures and tissue homogenates, and it uncouples oxidative phosphorylation in mitochondria prepared from liver and brain. Ernster demonstrated that albumin protected liver mitochondria from bilirubin-induced uncoupling. Since bilirubin is toxic *in vivo* primarily to the brain, the present studies were undertaken to determine whether a similar protective effect of albumin for brain mitochondria could be demonstrated. Mitochondria from rat brain, liver, and kidney were incubated under air for 15 minutes at 25° C in a medium containing 20 μmoles $MgCl_2$, 30 μmoles glutamate, 150 μmoles KCl, 10 to 30 μmoles Na orthophosphate, 2 μmoles ADP, 30 μmoles NaF, 100 μmoles Tris buffer, pH 7.3, 60 μmoles glucose, and 20 μg yeast hexokinase. Appropriate vessels contained bilirubin, 3.5×10^{-4} mole per L, and human serum albumin, 75 mg and 3.75 mg, calculated to bind 100% and 10% of the bilirubin present, respectively. Oxygen and inorganic phosphate consumptions were determined (P/O ratio). In the presence of unbound bilirubin, P/O ratios fell to less than 20% of base-line values in brain, liver, and kidney. The protective effect of albumin was demonstrated only for liver and kidney mitochondria, the P/O ratios returning to 75% of the control values. With brain mitochondria, however, the albumin effect was significantly less; the P/O ratios were only 36% of the control values even when the bilirubin was calculated to be completely bound to albumin. The present findings suggest that the selective toxicity of bilirubin for the central nervous system *in vivo* may be related to the less effective reversal of the toxic properties of the pigment by tissue proteins.

Effect of Diphenylhydantoin (Dilantin) on Left Ventricular Function in Dogs. D. S. MIERZWIAK, J. H. MITCHELL,* AND W. SHAPIRO, Dallas, Texas.

Most antiarrhythmic agents have a negative inotropic effect on the left ventricle. The inotropic effects of Dilantin, an antiarrhythmic agent of recent interest, are less well known. In open chest dogs, left ventricular performance was evaluated by relating end-diastolic pressure (LVEDP) to the maximal rate of rise of the left ventricular pressure (max dp/dt), stroke work (SW), and stroke power (SP) while aortic pressure, aortic flow, and heart rate were held constant. Intravenous injections of 5 mg per kg Dilantin were administered over 3 minutes. This is equivalent to the effective antiarrhythmic dose reported for humans and for dogs. The initial injection caused a transient increase in LVEDP from a control of 10 ± 2 to 17 ± 6 cm H_2O ($p < 0.01$) and decrease in max dp/dt from $2,170 \pm 593$ to $1,748 \pm 582$ mm Hg per second ($p < 0.01$) within 30 seconds after injection. Return to near control levels was usually observed within 3 minutes. A repeated injection at 30 minutes caused a more marked effect with less complete recovery. Ventricular function curves showed a decrease of SW and SP from any given LVEDP. Also, pulsus alternans developed in some instances after Dilantin administration. A similarly injected dose of 5 mg per kg quinidine base caused a slightly less marked response but of greater duration. Thus, in effective antiarrhythmic doses, Dilantin caused a transient negative inotropic effect on the left ventricle. Insofar as these data may be applied to clinical situations, one must weigh the possibility of these effects of Dilantin against its merits as an antiarrhythmic agent.

Relative Rates of Recovery of Parenchymal and Reticuloendothelial Function after Hepatectomy. TOHRU MIGITA, R. ROBINSON BAKER, AND HENRY N. WAGNER, JR.,* Baltimore, Md. (introduced by A. M. Harvey †).

Information is lacking about the return of function of hepatic polygonal and Kupffer cells after hepatectomy. The effect of subtotal hepatectomy (70% removed) was studied in dogs by serial measurement of the clearance half-times of labeled aggregated canine serum albumin (AA), colloidal gold, and rose bengal (RB). The rate of liver regeneration was measured quantitatively by Stern's method: colloid-^{198}Au was injected intravenously 24 hours before hepatectomy. The specific activity of both the resected and the regenerated portion of the liver was determined in 34 dogs sacrificed at 2- to 18-day intervals. The curve of the per cent regeneration versus time after operation proved to be exponential and approached a limiting value equal to approximately 85% of the original liver mass. The clearance of 5 mg per kg of AA-^{125}I decreased 20% from the control value, whereas the clearance of a 0.02 mg per kg dose showed almost no change. The clearance of 2 to 6 μg per kg colloidal gold-^{198}Au fell about 60% from the control value. The clearance half-time of 0.05 mg per kg of RB-^{125}I was several times longer than that of the normal control. In studies of the distribution of trace doses of AA-^{125}I and colloidal gold-^{198}Au, the per cent dose per unit weight of the liver after the third operative day was over 50% greater in the residual liver than in the liver of normal controls. Polygonal cell function measured by RB clearance was more severely affected by hepatectomy and remained abnormal for a longer period than did Kupffer cell function measured by the AA or colloidal gold clearance. The total capacity of the liver to clear rose bengal and particulate matter returned to normal before the liver had reached a stable level of regeneration.

Ionized Calcium in Normal Serum, Ultrafiltrates, and Whole Blood Directly Determined by Ion-Exchange Electrodes. EDWARD W. MOORE, Boston, Mass. (introduced by Joseph M. Hayman, Jr.†).

This report describes the first direct measurement of ionized calcium concentration [Ca^{++}] in biologic fluids with highly specific ion-exchange electrodes. Measurements were made in 66 fresh sera (56 healthy subjects) in a special Plexiglas chamber at 37° C. Both [Ca^{++}] and pH were simultaneously and continuously monitored. Sample pH was varied by alteration in chamber P_{CO_2}. [Ca^{++}] values below are those obtained at the original whole blood pH. ¶ Total serum calcium (EDTA method) varied from 2.12 mmoles per L to 3.37 mmoles per L [mean, 2.57 ± 0.26 (1 SD) mmoles per L]. Absolute variability in [Ca^{++}] was much less, 0.94 mmole per L to 1.21 mmoles per L (mean, 1.11 ± 0.06 mmoles per L). There was no correlation between ionized and total calcium concentrations. Repeat studies (9 subjects, 8 months) revealed little change in [Ca^{++}], mean Δ [Ca^{++}] = 0.04 mmole per L. ¶ Striking and consistent variation in [Ca^{++}] as a function of pH was observed. [Ca^{++}] increased curvilinearly with decreasing pH; average increase in [Ca^{++}] was 0.44 ± 0.10 mmole per L from pH 7.8 to pH 6.8. This change was not a simple protein-binding effect, since similar curves were obtained in protein-free ultrafiltrates. Heparinized whole blood [Ca^{++}] in 6 subjects was significantly less than that in corresponding sera (pH constant), with means of 1.05 and 1.10 mmoles per L, respectively ($p < 0.01$), apparently related to formation of a Ca-heparin complex. ¶[Ca^{++}] in ultrafiltrates (15 studies) was similar to that in corresponding sera (means, 1.14 and 1.13 mmoles per L, respectively). Mean total ultrafiltrate (diffusible) calcium was 1.49 ± 0.24 mmoles per L. Thus, only 76.5% of diffusible calcium was ionized. In these subjects, mean total serum calcium was 2.71 ± 0.32 mmoles per L, representing three fractions, *1*) nondiffusible, 1.22 ± 0.45 mmoles per L (45.0%), *2*) diffusible ionized, 1.13 ± 0.05 mmoles per L (41.7%), and *3*) diffusible complexed, 0.36 ± 0.22 mmole per L (13.3%). Total serum calcium was linearly related to nondiffusible calcium ($r = 0.85$). Thus, in normal subjects, serum ionized calcium was maintained within narrow limits; increased total calcium reflected primarily an increase in nondiffusible component. In sharp contrast, 3 hypercalcemic

patients showed striking elevations in ionized (41% increase) and complexed (142% increase) fractions with little change in nondiffusible component (7% increase).

Quantitation of Mitral Regurgitation With ^{133}Xenon. JOHN D. MORCH, H. JAMES SMITH, AND MAURICE MCGREGOR,* Montreal, Canada.

Previous attempts to measure mitral regurgitant flow ($\dot{Q}r$) by indicator dilution techniques have been only partially successful. This was for two reasons: *a*) After a bolus injection of indicator into the left ventricle (LV) the quantity of indicator that appears in the left atrium (LA) is influenced not only by cardiac output ($\dot{Q}$) and $\dot{Q}r$, but also by the volume of the LA. *b*) LA mixing is probably incomplete and cannot be assessed in the short period of observation. Constant infusion would be preferable, but most indicators cannot be employed because of recirculation. Xenon has a low partition coefficient and is largely cleared in the lungs. We have made constant infusion of ^{133}Xe in saline into the LV in 17 patients while withdrawing continuous samples from a systemic artery and either the LA or the pulmonary veins. In 7 subjects, there was no detectable xenon in pulmonary venous blood for 30 to 45 seconds, and concentration (C) increased thereafter at a rate of 2 to 9% arterial concentration (Ca) per minute. In 10 patients with mitral regurgitation, Ca and CLA increased to reach plateau values within 15 to 55 seconds of onset of LV infusion. $\dot{Q}$ and $\dot{Q}r$ could be estimated from these values. $\dot{Q}r$ ranged from 0.72 to 16.1 L per minute, and even when error due to increasing recirculation was ignored, these values remained almost constant from 60 to 120 seconds. There was good correlation between these values and clinical and angio-cardiographic assessment of severity. In 7 patients, once plateau values were established, the continuously sampling LA catheter was moved so as to estimate the adequacy of LA mixing. Error of estimation of $\dot{Q}r$ from this source ranged from 0 to 11%. These studies indicate the feasibility of measuring mitral regurgitation by constant infusion of ^{133}Xe into the LV at the time of cardiac catheterization. Results show that Ca and CLA can be determined from spot samples withdrawn at any time between 60 and 120 seconds without significant loss of accuracy.

Correlation of Surfactant Biosynthesis and Lung Stability during Fetal Lung Development. T. E. MORGAN, G. BRUMLEY, A. HODSON, V. CHERNICK, AND M. E. AVERY, Seattle, Wash., and Baltimore, Md. (introduced by Seymour J. Klebanoff *).

Previous work from our laboratory has suggested but not proved that surface-active lecithin (dipalmitoyl lecithin) may be synthesized by an uncommon pathway present in lung—methylation of preformed phosphatidyl ethanolamine with phosphatidyl dimethylethanolamine as an intermediate. Present studies on fetal lamb lung show a clear relationship among activity of this enzymatic pathway, appearance of surfactant phospholipid, and development of lung stability. ¶ In fetal lamb (term 147 days) the lungs can be first successfully aerated at about 120 to 130 days. Since upper lobes mature more rapidly than lower, there is an ideal opportunity to study maturation changes in the same animal. Fetal lambs of 60 to 145 days gestational age were exteriorized. After making compliance measurements on the intact lungs, we removed the lungs and divided them into upper and lower lobes. Saline extracts and homogenates of each were tested for surface activity and *N*-methyl transferase activity (incorporation of methyl-^{14}C from S-adenosyl methionine into lung phosphatidyl ethanolamine). Chloroform-methanol extracts of the homogenates were analyzed for phospholipid content, phospholipid distribution, and lecithin fatty acid distribution. ¶ An increase in distensibility toward term was found with stable pressure-volume relationships appearing between 120 to 130 days. At the same time surfactant became demonstrable on surface-film balance measurements of lung extract, phospholipids increased progressively to 500 μg per g weight, and saturated fatty acids on lecithin rose from 48 to 68%. Most significantly, *N*-methyl transferase activity was highest (203 mμmoles methyl-^{14}C per g per hour) at 100 to 110 days before the appearance of surfactant. At all times before day 110 upper lobe enzyme activity was greater than lower lobe; then lower lobe activity also rose. The higher levels of enzyme activity in the upper lobes preceding the increase of phospholipid and saturated lecithin and the appearance of surfactant strongly suggest that synthesis of surfactant does proceed via methylation of phosphatidyl ethanolamine.

Evidence for an Acidification Defect of the Proximal Renal Tubule in Experimental and Clinical Renal Disease. R. CURTIS MORRIS, San Francisco, Calif. (introduced by Lloyd H. Smith, Jr.*).

The proximal renal tubule is generally considered uninvolved in clinical acidification defects because the measured Tm HCO_3^- of patients affected has not been subnormal. A defect in the acidification function of the proximal tubule has been suggested but not demonstrated to underlie the renal tubular acidosis (RTA) associated with the Fanconi syndrome. Demonstration of a Tm HCO_3^- of 75 to 85% of normal or less would presumably implicate the proximal tubule, since it accounts for 85 to 90% of the renal reabsorption of HCO_3^- in the rat and 75% in the dog. In two unrelated patients with hereditary fructose intolerance, intravenous administration of fructose resulted in a decrease in Tm HCO_3^- from a normal value of 2.7 to 2.8 mEq per 100 ml glomerular filtrate to 1.9 to 2.1 and occurrence of the Fanconi syndrome. In a 2-year-old girl with cystinosis, Fanconi syndrome, and RTA, Tm HCO_3^- was 2.0 mEq per 100 ml glomerular filtrate. In a 3-week-old nonazotemic premature girl with hyperchloremic acidosis, the renal threshold for HCO_3^- was 2.0, and the Tm HCO_3^-, 2.3, mEq per 100 ml glomerular filtrate. In a 14-year-old boy with medullary cystic disease, serum creatinine of 4 mg per 100 ml, and hyperchloremic acidosis, Tm HCO_3^- was 1.6 mEq per 100 ml glomerular

filtrate. In the infant and patients with experimental and clinical Fanconi syndrome, a urinary pH < 5.5 during marked metabolic acidosis indicated capacity of the distal nephron to generate a steep lumen-paratubular H^+ gradient. A normal Tm HCO_3^- was demonstrated in a 7-year-old girl and two adults with RTA. The Tm HCO_3^- data allow separation of acidification defects into functional types. A 20% or greater reduction of Tm HCO_3^- is tentatively suggested as identifying "proximal RTA," and normal Tm HCO_3^-, "distal RTA."

Hexokinase and Glucokinase Activities in Bile Duct Epithelium and Hepatic Cells of the Normal Human and Rat Liver Lobule. GEORGE R. MORRISON, St. Louis, Mo. (introduced by ROBERT E. SHANK †).

Recent work by several investigators has disclosed that the liver of mammals contains a glucose-adenosine triphosphate phosphotransferase (glucokinase), which differs from the hitherto described conventional hexokinases in having a low affinity for glucose, no end-product inhibition by glucose 6-P, and a decrease in activity after fasting or the production of alloxan diabetes. ¶ Weinhouse and his associates have suggested that the two enzymes, hexokinase and glucokinase, are derived from different cells within the liver, because the hexokinase activity increased as the glucokinase activity correspondingly decreased in livers in which bile duct proliferation was induced by 3'-methyldimethylaminoazobenzene. They suggested that the hexokinase activity in homogenates of the liver is derived from bile duct cells and the glucokinase activity from parenchymal cells. ¶ In the present investigation, hepatic cells of the liver lobule were separated from bile duct epithelium by microdissection of lyophilized sections of normal human and normal rat livers. After weighing on a quartz beam balance, 0.35- to 3.5-μg segments of tissue were assayed for the activities of hexokinase and glucokinase by incubation in 1.5 to 15 μl of reaction mixture (containing $NADP^+$ and glucose 6-P dehydrogenase) and by determining the amount of $NADPH_2$ generated fluorometrically. The results indicate that hepatic parenchymal cells contain both hexokinase and glucokinase, whereas bile duct epithelium contains hexokinase with an activity exceeding that in parenchymal cells.

***In Vivo* Transmission of Antibiotic Resistance between Strains of *Staphylococcus aureus*.** STEPHEN I. MORSE * AND RICHARD P. NOVICK, New York, N. Y.

The widespread prevalence of antibiotic resistant staphylococci derives mainly from the selective pressure exerted by the use of the antimicrobials themselves. However, antibiotic resistance can be transferred between strains by conventional transduction procedures. Moreover, certain resistant *Staphylococcus aureus* strains are also lysogenic, and after spontaneous lysis the mature bacteriophage particles can in some cases transfer resistance. In the present studies, *in vivo* transmission of antibiotic resistance by such lysogenic strains was demonstrated. ¶ The donor strain utilized in the majority of experiments was an erythromycin resistant and streptomycin sensitive group III strain of *S. aureus*. The recipient was of the same phage type but was erythromycin sensitive and streptomycin resistant. The organisms were injected intravenously into mice, and at appropriate intervals the number and antibiotic susceptibility of organisms in the kidneys were assayed. ¶ When the strains were injected 4 hours apart, erythromycin and streptomycin resistant transductants were found in the kidneys of 33 to 50% of the mice 4 to 6 days later, irrespective of the order of injection. The kidneys of 40% of mice injected with the recipient strain 24 hours after the donor also contained many transductants. Transductants were rarely noted 24 hours after initiation of double infection and were not found in animals injected with either strain alone. Cotransductants resistant to penicillin and erythromycin were detected when another donor strain resistant to both antibiotics was employed. ¶ Thus, spontaneous infectious transmission of antimicrobial resistance between strains of *S. aureus* can occur *in vivo*, and these findings indicate a supplementary mechanism to account for the dissemination of antibiotic resistance in *S. aureus* besides the primary one of selection. Infectious transfer might also account for the spread of drug resistance before the general use of antibiotics.

Stimulation of Chloroquin of the Esterification of Tryptophan with Transfer RNA. KARL H. MUENCH, Miami, Fla. (introduced by W. J. Harrington *).

Chloroquin, 7-chloro-4-(4-diethylamino-1-methyl butylamino) quinoline, has recently been shown to bind to helical DNA and to inhibit DNA polymerase and RNA polymerase reactions, apparently as a direct consequence of its interaction with the DNA primer. Others also have described a complete block in protein synthesis effected by the drug in sensitive bacteria. Because of the partially helical nature of transfer RNA (t-RNA), the interaction of t-RNA and chloroquin was studied. Difference spectroscopy revealed that chloroquin does interact with t-RNA. The effects of this interaction on aminoacylation of t-RNA were then examined. Chloroquin produces a concentration dependent fourfold increase in the extent and a 2.5-fold increase in the rate of enzymatic esterification of tryptophan to t-RNA. Both the basal and the chloroquin-stimulated reactions are mediated by tryptophan-RNA-synthetase, as shown by a constant ratio of chloroquin-stimulated to unstimulated activity through a 130-fold purification of the enzyme from *Escherichia coli* B. The site of esterification is sensitive to periodate oxidation. Because the phenomenon could not be explained by simple equilibrium changes, attempts were made to find a chloroquin-induced error in specificity of t-RNA. Hydroxylapatite chromatography revealed a single but heterogeneous tryptophanyl t-RNA

peak responsive to chloroquin. Gradient partition chromatography resolved tryptophanyl t-RNA into two peaks, one responding and one not responding to chloroquin. There is no evidence from chromatography or other studies that chloroquin alters the specificity of t-RNA. We have obtained electrophoretic evidence that with or without chloroquin the enzyme esterifies tryptophan to the 2′(3′) hydroxyl group of ATP in addition to its charging of the two tryptophanyl t-RNA. The stimulation of aminoacylation of t-RNA by a drug is an unusual effect; its mechanism and its relation to the pharmacologic action of chloroquin remain to be elucidated.

The Circulatory Effects of Changes in Blood Viscosity: Comparison of Methemoglobinemia and Anemia. JOHN F. MURRAY* AND EDGARDO ESCOBAR, San Francisco, Calif.

Cardiac output rises equally when oxygen-carrying capacity of blood is lowered by either a reduction in hemoglobin concentration (anemia) or formation of inactive hemoglobin compounds (e.g., methemoglobin). These observations have led to conclusions that whole blood viscosity, which differed in the two studies, is not an important determinant of the circulatory changes in anemia. However, in the presence of methemoglobin (MetHgb) the oxygen dissociation curve shifts to the left; this causes greater changes in tissue oxygenation, so the stimuli are not comparable in the two conditions. The present studies compared the circulatory effects of reduced oxygen-carrying capacity in anemia and MetHgb in experiments designed so that no shift in oxyhemoglobin dissociation occurred and the only variable was blood viscosity. ¶ Cardiac output was measured by the indicator dilution technique and end-diastolic and end-systolic volumes by aortic thermodilution. Blood in which all hemoglobin had been converted to MetHgb by NO_2 was exchanged for equal volumes of whole blood in six dogs. There was no change in hematocrit and MetHgb concentrations were 27% (first infusion) and 47% (second infusion). Comparable exchanges with plasma were performed in five dogs that reduced hematocrit to 27% and 19%. Arterial oxygen content was reduced equally by the two procedures. Cardiac output did not change with MetHgb but increased significantly in animals given plasma; this difference occurred despite a greater fall in mixed venous P_{O_2} with MetHgb. MetHgb caused a significant and progressive diminution of end-diastolic volume and end-systolic force, possibly related to changes in myocardial oxygen tension. We conclude that a reduction in oxygen-carrying capacity of up to 50% by MetHgb, unaccompanied by changes in viscosity, does not increase cardiac output; moreover, a decrease in blood viscosity contributes to the circulatory responses in anemia.

Controlled Induction of Vasodepressor Syncope by Hemospasia (Lower Body Negative Pressure). RAYMOND H. MURRAY, LEONARD J. THOMPSON, JOHN A. BOWERS, AND CHARLES D. ALBRIGHT, Indianapolis, Ind. (introduced by John B. Hickam †).

To study the hemodynamic changes associated with vasodepressor syncope without the untoward effects of orthostasis, bleeding, or drugs, we exposed seven subjects in a special chamber to graded degrees of negative pressure applied to the lower half of the body until severe presyncopal symptoms were induced; symptoms began at chamber pressures 30 to 70 mm Hg below ambient levels. Arterial and central venous catheters, electrocardiogram, and arm and leg mercury in Silastic girth gauges permitted serial hemodynamic measurements and blood sampling. Through the presymptomatic period, as chamber pressure fell, mean heart rate ($\overline{HR}$) rose 52%, cardiac output ($\overline{CO}$), central blood volume ($\overline{CBV}$), and pulse pressure ($\overline{PP}$) decreased 30%, stroke volume fell 53%, and systemic vascular resistance ($\overline{SVR}$) rose 41%; central venous pressure ($\overline{CVP}$) fell gradually 6.9 mm Hg, rising 1 mm before symptoms; estimated plasma water ($\overline{EPW}$) fell 7%; forearm volume ($\overline{AV}$) did not change significantly, but leg volume ($\overline{LV}$) rose and arm blood flow ($\overline{ABF}$) rose slightly. With the onset of severe presyncopal symptoms, $\overline{HR}$ and arterial blood pressures fell abruptly and markedly, whereas $\overline{CVP}$ rose 2.3 mm. $\overline{CO}$ and $\overline{CBV}$ fell another 15% from control values and $\overline{EPW}$ fell 1%, whereas $\overline{SV}$, $\overline{SVR}$, $\overline{AV}$, $\overline{LV}$, and $\overline{ABF}$ remained generally unchanged. After the return to ambient chamber pressure, these factors returned to near control values, but $\overline{CO}$, $\overline{CBV}$, and $\overline{SV}$ rose to levels 14% above control, $\overline{CVP}$ rose 2.4 mm above control, and $\overline{LV}$ and $\overline{ABF}$ remained elevated and $\overline{EPW}$ low. These studies brought out several unexpected findings: the presyncopal state persisted long enough for adequate testing; late venous pressure rose with falling effective blood volume; there was absence of significant changes in $\overline{SVR}$, $\overline{AV}$, and $\overline{ABF}$ with the onset of presyncope; $\overline{CO}$, $\overline{SV}$, $\overline{CBV}$, and $\overline{CVP}$ rose above control values after stress.

Platelet Thrombosthenin—Function and Subcellular Localization. RALPH L. NACHMAN AND AARON J. MARCUS,* New York, N. Y.

Thrombosthenin, a platelet protein with contractile properties analogous to muscle, was extracted from human platelets and purified. After threefold reprecipitation in a low ionic medium, the material was fractionated by polyacrylamide gel chromatography. The molecular weight was greater than 300,000, and calcium-dependent ATPase activity was demonstrated. Addition of low concentrations of ATP resulted in a marked decrease in viscosity of the protein. Immunologic analysis revealed no reactivity with human smooth and striated muscle antisera, indicating that thrombosthenin was antigenically a unique contractile protein. Immunoelectrophoresis with thrombosthenin antiserum revealed two precipitin bands, one of which was identical to fibrinogen. Clot retraction of recalcified platelet-rich plasma was

completely inhibited by thrombosthenin antiserum absorbed with fibrinogen, but no inhibition was noted when antifibrinogen, antialbumin, antigamma-globulin, anti-whole serum, antistriated muscle, and antismooth muscle antisera were used. Studies on the subcellular localization of thrombosthenin were carried out with the use of antisera prepared against isolated platelet granules and membranes. These antisera inhibited clot retraction of recalcified platelet-rich plasma, a property that disappeared after absorption with thrombosthenin. We concluded that thrombosthenin mediated clot retraction by virtue of its presence in platelet granules as well as membranes. It is possible that at least some of the activity in the granules represented mitochondrial contractile protein.

Effect of Administration of ACTH in Patients with Generalized Myasthenia Gravis. TATSUJI NAMBA, JOSEPH N. SILVERSTEIN, AND DAVID GROB,† Brooklyn, N. Y.

The effect of a short intensive course of ACTH (100 mg iv daily for 10 days) was studied in 16 courses administered to 8 patients with severe generalized myasthenia gravis. In most instances, as previously described by Von Reis, Liljestrand, and Matell, the patients became weaker during administration of ACTH. Shortly after cessation of ACTH, there occurred, in those patients who had become weaker, improvement in strength, muscle response to nerve stimulation, and response to acetylcholine and anticholinesterase compounds significantly above the pre-ACTH level and lasting for weeks to months. The increase in plasma level of 17-hydroxycorticosteroids and urinary excretion of 17-hydroxycorticosteroids, 17-ketogenic steroids, and tetrahydrocortisone during ACTH administration was greater than in normal subjects. There was no change in the serum level of muscle striation-binding globulin. ¶ Exacerbation of myasthenia gravis occurring spontaneously or during infection was followed by sustained remission in 7 of 60 patients studied during exacerbation. The increase in plasma and urinary steroid levels during exacerbation was greater than in normal subjects under comparable stress, suggesting that increased endogenous ACTH production may play a role in the change in clinical state. ¶ The effects of ACTH administration on the course of myasthenia gravis could not be reproduced by the daily administration of prednisone or by the daily intravenous or oral administration of methopyrapone, which normally increases endogenous ACTH production by inhibiting cortisol synthesis. Methopyrapone produced less increase in plasma 17-hydroxycorticosteroids and urinary 17-hydroxycorticosteroids or 17-ketogenic steroids than in normal subjects. Intra-arterial administration of hydrocortisone improved neuromuscular transmission in myasthenic patients and had no effect in normal subjects. ¶ The observations indicate that ACTH produces a unique effect on the course of some patients with myasthenia gravis and suggests a relationship between steroid biosynthesis and neuromuscular transmission in this disease.

Postimmersion Oxygen Consumption in Man. JOHN NAUGHTON AND STEWART WOLF,† Oklahoma City, Okla.

It has been shown in several animal species that an O_2-conserving reflex is elicited during immersion of the face in water. The reflex, mediated at the level of the brain stem, is characterized by bradycardia, reduction of blood flow to viscera and extremities, and evidences of anaerobic metabolism. Robin and Murdaugh reported last year that in diving seals the O_2 consumption during the immediate postimmersion period was less than expected if the metabolism were calculated on the basis of the immediate pre-dive control period. The current report concerns 16 immersion experiments on healthy human subjects aged 24 to 61. Respiratory gas exchange was measured during a 5-minute period at rest before immersion of the face and during three separate 1-minute collections immediately after breath holding in water. In none of the experiments was there any significant change in RQ. The change in respiratory rate was very slight, although tidal volume increased considerably in all tests (mean increase 525 ml). Oxygen consumption was from 25 to 50% less than would be expected if O_2 utilization were equal to that during the "basal" period before immersion. Similarly, and in contrast to Robin and Murdaugh's data in seals, CO_2 dissipation during the postimmersion period was 15 to 35% less than estimated from the preimmersion state, thus supporting the findings of Scholander in animals that metabolic rate during diving is reduced.

Hemoglobin Synthesis and Normoblast Maturation. THOMAS F. NECHELES, Boston, Mass. (introduced by William H. Crosby †).

Insight into the relationship between cellular maturation and protein synthesis may be obtained from the study of erythroid precursors. As the normoblast matures, morphological changes occur in the nucleus with eventual loss of all nuclear material as the penultimate stage in normal erythroid maturation. Concurrently, there is a progressive increase in the cellular hemoglobin content, as evidenced by the staining characteristics of the cytoplasm. Changes in the normal relationship between nuclear and cytoplasmic maturation are accepted as prima facie evidence of pathology. The rate of hemoglobin synthesis was measured in suspensions of adult human bone marrow and compared with evidence of cellular proliferation and maturation. Hemoglobin synthesis was measured from the incorporation of glycine-^{14}C into globin. Normoblast proliferation and maturation were estimated from cell counts, radioautography using pulse labeling with thymidine-^{3}H, and mitotic indexes in the presence of colchicine. Agents that inhibit protein synthesis, including actinomycin D and puromycin, led to a marked inhibition of normoblast maturation. How-

ever, proliferation appeared to be unaffected. In some respects this experimental situation appears to be analogous to the thalassemias, where a primary inhibition of hemoglobin synthesis leads to an accumulation of nucleated hypochromic erythroblasts, some of which are eventually released into the circulation. On the other hand, inhibition of nucleic acid synthesis led to both a decreased rate of cellular proliferation and a decrease in the rate of hemoglobin synthesis. Hemoglobin synthesis appeared to continue for a longer period of time, and the eventual total quantity of hemoglobin synthesized per cell approached normal levels. These experiments suggest that normal erythroid maturation is related not only to nucleic acid synthesis but also, in an as yet undefined manner, to a normal rate of hemoglobin synthesis.

The Role of Adrenal RNA in the Regulation of Protein Synthesis and the Rapid Steroidogenic Response to ACTH. R. L. NEY, W. W. DAVIS, AND L. D. GARREN, Nashville, Tenn., and Bethesda, Md. (introduced by E. V. Newman †).

We have previously shown that the stimulation of corticosterone secretion that occurs within 10 minutes of the administration of ACTH to rats is mediated by the synthesis of protein that regulates steroid biosynthesis prior to Δ^5-pregnenolone. We have found this protein to have a rapid turnover rate of about 20 minutes. Because the concentration of ribonucleic acids (RNA) is an important factor in determining the rate of protein synthesis, the effect of inhibitors of RNA synthesis on over-all adrenal protein synthesis and on the rapid steroidogenic response to ACTH was studied. In these experiments, after the administration of 1 mg actinomycin D to rats, RNA synthesis was almost completely inhibited, as shown by measuring incorporation of intravenously injected uridine-^{14}C into adrenal RNA. However, the increase in adrenal vein corticosterone in response to 2 mU ACTH remained normal during an 8-hour period in which adrenal RNA synthesis was inhibited by actinomycin D, indicating that the rapid steroidogenic response to ACTH does not require newly synthesized RNA. The response to ACTH also remained intact over an 8-hour period after the administration of 30 mg 5-fluorouracil, which has been shown to enter newly synthesized RNA and thereby modify its function in regulating protein synthesis. In contrast, over-all adrenal protein synthesis, measured by the incorporation of intravenously injected algal protein hydrolyzate-^{14}C into adrenal protein, decreased to 51% of control 4 hours after the administration of actinomycin D. ¶ We reached the following conclusions: *1*) The stimulation by ACTH of protein that mediates a rapid steroidogenic response does not require newly synthesized RNA but appears to result from modification in activity of RNA that is stable for at least 8 hours. *2*) The experiments indicate that the RNA that regulates a large fraction of adrenal protein synthesis turns over at a more rapid rate (about 4 hours) than the RNA that is involved in a major biological function of the adrenal, the acute stimulation of steroidogenesis.

Induction of Metabolic Changes in Normal Bone Cells by Medullary Neoplasia. GEORGE NICHOLS, JR.,* Boston, Mass.

Multiple myeloma was selected for study of the origins of the widely variable skeletal response to neoplastic invasion because: *1*) bone lesions ranging from widespread osteolysis to local osteosclerosis occur in 90% of cases; and *2*) the tumor develops diffusely in marrow, permitting the myeloma cells and bone cells in a biopsy sample to be separated for simultaneous *in vitro* study. The contributions of each to over-all tissue metabolism can thus be distinguished. ¶ Twelve patients were biopsied. No evidence was found to support the view that tumor cells destroy host tissue by consuming all available substrate. Indeed, the relative affinity for glucose and proline of bone and myeloma cells was closely similar in three of four tests, whereas in one, myeloma cells had only one-half the affinity for glucose. However, metabolic patterns of bone cells were changed. Q_{O_2} was doubled while lactate production remained normal. Incorporation of labeled proline into bone cells was increased 100 to 200% without change in bone collagen labeling, suggesting that the cells were stimulated to make some other protein. Similar but more variable changes were noted in glucose incorporation. Changes in myelomatous marrow were different in pattern, more variable, and rarely correlated with changes in bone cells from the same sample rendering marrow contamination unlikely as a cause of the bone cell changes observed. Finally, collagenase activity was decreased in bone cells and was negligible in myelomatous marrow and two plasmacytomas—the former fits with a change in the nature of the proteins being made in bone cells, and the latter rules out bone lysis due to tumor production of lytic enzymes. The suggestion (and its implications) that normal bone cell biosynthetic activity is both stimulated and redirected in multiple myeloma will be discussed.

Some Aspects of the Physiology of Human Luteinizing Hormone (HLH) as Determined by Radioimmunoassay. WILLIAM D. ODELL AND GRIFF T. ROSS, Bethesda, Md. (introduced by Mortimer B. Lipsett *).

When existing bioassays are utilized, it is not feasible to quantitate gonadotropins in the blood of normal men or women. Because of this, many facets of the physiology of gonadotropins have remained unclarified. A sensitive and precise radioimmunoassay capable of quantitating HLH in plasma has been developed by using a selected antiserum directed against human chorionic gonadotropin and a purified preparation of HLH (Hartree). The dose response curves of *1*) purified HLH, *2*) Pergonal PR-1958, *3*) acetone-dried pituitary powder, and *4*) plasma from postmenopausal women were all similar. Seven preparations have been simultaneously immuno-

assayed and bioassayed in terms of a common standard from human sources. The index of discrimination was approximately unity in each case. Plasma from 14 postmenopausal women contained an average of 7.3 mμg of the purified HLH per ml (range, 5.0 to 12.0), or 3.7 mg of Pergonal PR-1958 per L by immunoassay. This may be compared to values of 2.4 mg per L (McArthur) and 5.4 mg per L (Apostolakis) obtained by bioassay of plasma pools. Highly purified human follicle-stimulating hormone (Reichert) had a similar dose response curve but with one two-hundredth the potency of the purified HLH in the assay, probably explained by ½% contamination of the FSH with LH. Plasma from a variety of sources has been examined: *1*) 30 normal men and 12 normal women (210 determinations at other than midcycle) averaged 1.5 mμg per ml (range, 0.8 to 3.0); *2*) 8 children less than 8 years old, 1.2 mμg per ml (0.5 to 2.0); *3*) 12 children aged 9 to 14, 1.7 mμg per ml (0.6 to 2.4). Plasma from hypophysectomized rats did not contain detectable HLH (less than 0.15 mμg per ml), and plasma from adult men and women could be suppressed to below that of prepubertal children with either androgen or estrogen treatment. Blood levels of HLH in normal women were quite constant throughout the menstrual cycle except at midcycle, when a sharp peak rising to over 10 mμg per ml and lasting less than 24 hours occurred. Treatment of normal men with Clomiphene resulted in elevation of HLH values. Preliminary estimates indicate that the half-time of disappearance of HLH is substantially longer than that for growth hormone, ACTH, and TSH.

Some Submicroscopic Characteristics of Epithelial Regeneration in Experimental Human Skin Wounds. GEORGE F. ODLAND AND RUSSELL ROSS, Seattle, Wash. (introduced by Robert G. Petersdorf*).

Recent increments in knowledge of the biochemistry of healing skin wounds and the ultrastructure of connective tissue repair emphasize the need for equivalent information concerning the submicroscopic features of epidermal regeneration. For this reason, series of standard incised superficial skin wounds were made in the forearms of human volunteers. For electron microscopic analysis, skin biopsies were done at 1, 2, 3, 5, and 7 days, providing samples at intervals encompassing the major early events of clot formation, inflammation, and epithelial closure. ¶ New observations, particularly concerning the early epidermal regeneration, have shown the following: *1*) Cytological changes at the advancing edge of the epidermis characterized by increased cell volume and increase in free ribosomal complexes. *2*) Apparent phagocytosis by the epidermal cell of extravasated proteins in the environment of the clot. *3*) Persistence of intercellular adhesion by retention of desmosomes. *4*) Sequential modifications of the epidermal cell surface in the inflammatory exudate. At first, the surface membrane of the epidermal cell maintains direct contact with the extravasated proteins of the early inflammatory exudate. Subsequently, and concomitant with the evolution of a fibrin matrix, hemidesmosomes and a basal lamina appear at the epidermal cell surface. Morphologically, these structures suggest an adhesion interrelationship with the temporary fibrous architecture. *5*) Modification of the internal surface of the clot achieved in the presence of neutrophils, macrophages, and the advancing epithelial margin. This occurs in the presence of extracellular neutrophil granules as well as dense bodies, possibly lysosomal, in the macrophages and epidermal cells. ¶ The study has identified several new structural features that characterize the epidermal cells before wound closure. The presence of dense bodies in epidermal cells, neutrophils, and macrophages suggests possible sites of action of hydrolytic enzyme systems that occur in the early stages of wound healing.

The Role of Erythropoietin in Establishing a Hematopoietic Graft. LOIS F. O'GRADY, JERRY P. LEWIS, ROBERT D. LANGE, AND FRANK E. TROBAUGH, JR., Chicago, Ill. (introduced by Theodore B. Schwartz†).

Hematopoietic tissue administered to heavily irradiated mice seeds and grows in the spleen as discrete colonies, most of which are composed of only one cell type, whether it be erythroid, granulocytic, or megakaryocytic. Each colony presumably develops from a single stem cell. We have used this observation to study the influence of erythropoietin on the differentiation of transplanted hematopoietic tissue. ¶ CAF_1 female mice were used throughout. To observe the effect of irradiation on endogenous erythropoietin, we irradiated 700 mice with 750 r, and daily for 10 days 70 mice were exsanguinated, and the erythropoietic activity of their plasma was determined. The effect of erythropoietin on the growth and development of hematopoietic colonies was determined by two studies. To examine the effect of reduced endogenous erythropoietin, we produced graded levels of plethora in host animals by hypertransfusion. To examine the effect of exogenous erythropoietin, we daily injected mice, hypertransfused sufficiently to block formation of erythroid colonies, with graded doses of erythropoietin. In both studies, mice were irradiated with 750 r and injected with 15,000 isogeneic marrow cells. Eight days later, their spleens were removed, fixed, and the cellular components of hematopoietic colonies determined in serial sections. ¶ We obtained the following results: *1*) There was no detectable level of endogenous erythropoietin in lethally irradiated mice until the seventh day postirradiation, several days after the hematopoietic graft had been established. *2*) As the degree of plethora was increased, there was first a decrease in size, and then in number, of erythroid colonies. Moderate plethora eliminated all colonies containing erythroid elements, and marked plethora even reduced the number of colonies containing granulocytes and megakaryocytes. *3*) In highly plethoric mice, the inoculation of erythropoietin resulted in the formation of erythroid colonies, the number and size of which were related to the dose of erythropoietin administered.

Determination and Kinetic Significance of the Exchangeable Intracellular Thyroxine Pool in Man. JACK H. OPPENHEIMER, GERALD BERNSTEIN, JULIAN HASEN, AND CARL H. SUTTON, New York, N. Y. (introduced by Louis Leiter †).

Analysis of the acute plasma disappearance curves of simultaneously injected thyroxine-^{131}I and albumin-^{125}I allows serial calculation of the fraction of administered thyroxine contained within the cellular compartment. Four hours after injection of the isotopes, the intracellular thyroxine fraction reached a plateau (I_{max}). In six normal subjects the average I_{max} was 0.52 (range, 0.48 to 0.55). At this time, the exchangeable intracellular thyroxine pool can be calculated to be 296 μg. The characteristics of the intracellular accumulation curve indicate that the interchange between cellular and extracellular thyroxine can be represented by a closed two-compartmental system. This provides a convenient theoretical framework for a comprehensive analysis of factors responsible for the partition of thyroxine between cells and plasma protein binding sites. Consonant with known alterations in thyroxine binding by plasma proteins, I_{max} was decreased in four estrogen-treated patients (Av. 0.38), decreased in two hypothyroid patients (Av. 0.44), and increased in two hyperthyroid patients (Av. 0.67). Acute infusion of diphenylhydantoin in two patients resulted in an immediate increase of I_{max}. Simultaneous hepatic uptake curves in four patients obtained by external radioactive monitoring closely approximated intracellular accumulation curves. This finding, in conjunction with direct radioactive assay of hepatic biopsies obtained during surgery, indicates that the liver is an important component of the intracellular compartment. In agreement with this formulation, I_{max} was reduced in five patients with severe liver disease (Av. 0.26). Fractional transfer constants between compartments can also be calculated and interpreted in terms of tissue- and plasma-binding factors. The average normal unidirectional cellular thyroxine clearance rate was 39 ml per minute, in contrast to an average normal metabolic clearance rate of 0.7 ml per minute. Analogous studies in a variety of experimental animals suggest that tissues other than liver may also bind thyroxine. The intracellular localization of thyroxine has been demonstrated by radioautographic studies employing rabbit liver. These findings emphasize the importance of tissue binding in determining the concentration of circulating free and bound thyroxine.

Urinary Excretion of a Cationic Protein with Lysozyme (Muramidase) Properties in Monocytic Leukemia. ELLIOTT F. OSSERMAN * AND DOLORES P. LAWLOR, New York, N. Y.

An abnormal, exceptionally cationic protein (CP) has been found in the urine of nine patients with monocytic leukemia. CP migrates electrophoretically (pH 8.6) as a homogeneous peak far beyond the cathodal boundary of the gamma globulins. It constituted 16 to 40% (0.6 to 1.4 g per day) of the total proteinuria in these nine patients. When isolated by salt precipitation and DEAE chromatography (0.1 M NaOH:glycine, pH 9.5), CP showed the following properties: electrophoretic mobility $+2.5 \times 10^{-5}$ cm^2 v^{-1} second^{-1}; isoelectric point (pI) $\cong$ 10.5; sedimentation constant ($S_{20,w}$) 1.8 to 2.0 S; estimated molecular weight 13,000 to 15,000; homogeneous on ultracentrifugation and starch gel and acrylamide electrophoresis; not dissociated by mercaptans or 6 M urea; limited solubility in water and dilute salt solutions; soluble in 0.1 M HCl; nitrogen 18% of dry weight. Amino acid analyses of three samples of CP were identical within methodologic limits. Tryptic digestion yielded 19 peptides, and peptide maps of three CP samples were indistinguishable. ¶ Antiserum to one CP (anti-CP) gave reactions of identity with all others. Anti-CP revealed low concentrations of CP not detectable electrophoretically in serum and sonicated buffy coat preparations from monocytic leukemics, in sonicated normal leukocytes, and in the urine of occasional patients with sarcoidosis and pyelonephritis (concentration less than one-hundredth that of monocytic leukemics). CP could not be demonstrated in normal urine or serum, or in acute or chronic lymphatic or myelogenous leukemics. ¶ Because of the presence of hydrolases in monocytes, CP was assayed for enzymatic activity. All CP exhibited lysozyme (muramidase) activity (*M. lysodeikticus*) 2 to 3 times greater than twice-crystallized egg white lysozyme. Phosphatases, cathepsin, and β-glucuronidase were absent. Lysozyme activity of CP was completely inhibited by anti-CP. ¶ These data indicate that CP is a lysozyme elaborated by monocytes. The finding of large quantities of CP in all nine patients with monocytic leukemia studied to date and its absence in other types of leukemia indicate its specificity and possible diagnostic value.

Binding of Thyrotrophin to Thyroid Slices: A Reversible Step in Hormone Action. IRA PASTAN, Bethesda, Md. (introduced by Jacob Robbins *).

Polypeptide hormones produce specific effects on a limited range of tissues. Most previous work in this field has concentrated on the metabolic responses of tissues to hormones. This study concerns the initial interaction of thyrotrophin (TSH) with the thyroid. To delineate the initial interaction of TSH, we exposed thyroid slices to TSH under various conditions and subsequently incubated them at 37° for measurement of glucose-1-^{14}C oxidation. TSH produces a prompt, specific, readily quantitated increase in glucose-1-^{14}C oxidation to $^{14}CO_2$. When slices were exposed briefly to TSH at 2°, washed thoroughly, and incubated at 37°, the effect of TSH persisted despite its absence from the medium. When slices were similarly exposed to TSH, washed, and then exposed to anti-TSH antiserum, the persistent effect of TSH was obliterated. The magnitude of the persistent TSH effect is *1)* proportional to TSH concentration over the range of 1 to 10 mμ per ml, *2)* proportional to the duration of the preincubation for the first 10 minutes only, and *3)* unaffected by vigorous washing for 60 minutes. The TSH effect is completely reversed when antibody is added to

slices preincubated with TSH for up to 15 minutes at 2°. Reversibility by antibody decreases progressively as the preincubation is extended so that at 60 minutes the TSH effect is only 30% reversed. Reversibility is also decreased after preincubation at 24° or 37°. The observation of a persistent TSH effect in washed slices, analogous to the persistent insulin effect in muscle reported by Stadie, in itself is difficult to interpret. However, obliteration by antibody indicates that the persistent effect after washing is due to relatively intact hormone bound firmly to a superficial cell site. Thus, by the use of antibody, the phenomenon of hormone binding to tissue has been separated from subsequent effects on target tissue metabolism.

Cyclophosphamide Inhibition of an Autoimmune Disease, Allergic Encephalomyelitis, in Rats. PHILIP Y. PATERSON,* EDWARD W. GERNER, FRANK M. STEELE, AND MARGARET A. HANSON, Chicago, Ill., and New York, N. Y.

Cyclophosphamide, an alkylating agent, exerts a marked suppressive effect on hapten sensitization and antibody production against defined antigens in animals. It has been used in man, not only as a potent antitumor agent, but as a means for prolonging survival of allogeneic kidney transplants. Cyclophosphamide, thus, might be a useful immunosuppressive agent in systemic diseases where immune mechanisms have been implicated. We have approached this question by examining the effect of cyclophosphamide on allergic encephalomyelitis (AE), a prototypic experimental autoimmune disease. ¶ Wistar rats were sensitized to spinal cord plus adjuvant and immediately started on cyclophosphamide. Doses of 10, 12, or 15 mg per kg were given daily or 5 days per week and continued through the 17th or 18th day after sensitization. AE was completely inhibited. None of the rats developed clinical neurological signs; none had microscopic lesions of AE. Production of complement-fixing antibrain antibodies was suppressed in parallel with inhibition of disease. Serious drug toxicity (weight loss and leucopenia) was noted only with the 15-mg dose. Control rats, similarly sensitized but not drug treated, developed paralysis, and virtually all had disseminated lesions of AE within 18 days. ¶ Of special interest, and in contrast to reported effects of other immunosuppressive agents on AE, rats sensitized and treated with cyclophosphamide did not develop AE even after the drug was stopped. The animals remained clinically well, gained weight, and had no lesions of AE when examined 2 or 3 weeks after the last dose of cyclophosphamide.

The Relation of Differential Cellular Recognition of Antigen-Antibody Complexes to Species Origin of Antibody. ROY PATTERSON * AND IRENA M. SUSZKO, Chicago, Ill.

Antigen-antibody complexes are phagocytized differently by cells when the species source of antiserum is varied. Precipitating antisera against bovine serum albumin (BSA) were prepared by immunization of chickens and rabbits. These antisera were standardized by direct precipitation and ammonium sulfate coprecipitation reactions with ^{131}I-labeled BSA. The ability of these antisera to produce passive immune elimination of BSA-^{131}I in normal rabbits, guinea pigs, ducks, and chickens was determined. BSA-^{131}I was injected intravenously, and circulating levels of the antigen were determined in 10 minutes. The test animals were then injected with rabbit or chicken antisera in amounts that would react with the antigen at equivalence or in slight antigen excess. Control animals received BSA-^{131}I followed by normal rabbit or chicken sera. The rate of elimination of BSA-^{131}I from the circulation was observed for 5 hours. Rabbit anti-BSA resulted in rapid immune elimination of BSA-^{131}I from the blood of rabbits, guinea pigs, ducks, and chickens. Chicken anti-BSA resulted in rapid immune elimination of BSA-^{131}I from the circulation of chickens and ducks but not from that of rabbits or guinea pigs. The chicken anti-BSA resulted in actual retention of BSA-^{131}I in the circulation of rabbits and guinea pigs compared with BSA-^{131}I levels in control animals. This differential disappearance of complexes was confirmed by intravenous injection of soluble chicken or rabbit complexes prepared *in vitro* with the same antigen-antibody reactants and injected in the same four species. When we used a system of *in vitro* ingestion of soluble antigen-antibody complexes of guinea pig and chicken spleen cells, we obtained results confirming the *in vivo* studies. To explain these results, which demonstrate a specific difference in phagocytosis of complexes, it is postulated that a specific recognition system of immunoglobulins by receptor sites on cell membranes may exist.

Responses of Scorbutic Bone Cells to Ascorbic Acid *In Vitro*. WILLIAM A. PECK AND STANLEY J. BIRGE, JR., Rochester, N. Y., and Bethesda, Md. (introduced by Seymour Reichlin *).

Recent observations indicating that ascorbic acid stimulates collagen synthesis by isolated bone cells in primary culture have been extended to define the characteristics of ascorbic acid action. Bone cells were dispersed enzymatically from rat calvaria and cultured in medium of known composition. Collagen synthesis was estimated by the incorporation of L-proline-U-^{14}C into labeled hydroxyproline of water soluble protein containing newly synthesized collagen and residue protein containing collagen from the extracellular matrix. Ascorbic acid stimulation of proline hydroxylation was apparent in both fractions within 30 minutes and increased progressively with time during the first 2 hours. The degree of stimulation was directly proportional to the log of the ascorbic acid concentration over the physiologic range (5 to 50 μg per ml), and higher concentrations of ascorbic acid provided no further stimulation. The stimulation of collagen synthesis was eliminated within 24 hours of ascorbic acid withdrawal. When ascorbic acid was added to stationary phase cultures 9 to 10 days old, increased collagen synthesis was not accompanied by increases in

noncollagen protein synthesis, radioactivity of the tissue pool of free proline, or DNA content. When ascorbic acid was added to proliferating cultures 2 to 3 days old for a period of 6 to 7 days, increased collagen synthesis, noncollagen protein synthesis, and DNA accumulation resulted. These results indicate that the initial effect of ascorbic acid is a rapid, reversible, and specific stimulation of proline hydroxylation during the early stages of collagen formation. When added to proliferating cell cultures for prolonged periods of time, ascorbic acid can also promote cell proliferation and noncollagen protein synthesis.

Abnormal Thyroid Hormone Binding in Obesity. I. B. PERLSTEIN, B. N. PREMACHANDRA, AND H. T. BLUMENTHAL, Louisville, Ky., and St. Louis, Mo. (introduced by J. Murray Kinsman†).

When guinea pigs were immunized with thyroglobulin, thyroid hormone was shown to be bound by thyroid antibodies with concomitant effects on the peripheral metabolism of thyroid hormone. Furthermore, some degree of hypothyroidism was evident in the immunized animals, as shown by, among other things, increase in body weight. To study the effect of thyroid autoantibodies on thyroid hormone transport in human obesity, we carried out paper electrophoresis of sera from 300 obese individuals (30 or more pounds over ideal weight) by conventional techniques. All the sera were examined for the presence of agglutinating and precipitating antibodies. In 15% of the sera of obese individuals, varying titers of thyroid autoantibodies were present. In 5% of the 300 sera from obese individuals tested, abnormal thyroid-binding protein was found with abnormal radioactivity distribution in the electrophoretograms. Thyroid hormone was bound by an abnormal protein with an electrophoretic mobility akin to either γ-globulin or between α_2- and β-globulin. The thyrobinding indexes in obese individuals with thyroid autoantibodies were in the hypothyroid range. When these individuals were treated with triiodothyronine (T_3) for several weeks, there was an apparent disappearance of abnormal thyroxine-binding protein, and the radioactivity distribution in the electrophoretograms of such sera was normal. The T_3-treated patients felt considerably better and lost unusual amounts of weight, averaging 20 pounds per month. ¶ In addition, proliferative vascular lesions with basement membrane thickening were found in skin biopsy specimens of obese individuals in whom thyroid antibodies were found in the circulation.

Comparison of Electrolyte Transport in Relation to Intraluminal Concentrations in the Human Jejunum and Ileum. SIDNEY F. PHILLIPS AND W. H. J. SUMMERSKILL, Rochester, Minn. (introduced by C. F. Code†).

Relationships between intraluminal concentrations and mucosal transport of sodium, potassium, bicarbonate, and chloride in the small intestine were studied on 67 occasions by perfusion of 20-cm segments of ileum or jejunum distal to an occluding balloon in healthy volunteers. All perfusing solutions were isotonic and contained reciprocal concentrations of both cation and anion, isotopes of sodium and potassium, 0.1% glucose, and a marker. ¶ Net ileal transport of sodium ($r = 0.94$) and potassium ($r = 0.79$) was directly related to their luminal concentrations; absorption occurred with perfusates containing 110 and 5 mEq per L, respectively. Unidirectional movement to blood was also concentration dependent ($r = 0.97$, $r = 0.91$), although that from blood was not. Similar characteristics of cation transport were found in the jejunum. ¶ Net movement of bicarbonate was related to luminal concentrations in both jejunum ($r = 0.91$) and ileum ($r = 0.62$); absorption commenced at 5 mEq per L in the jejunum, but at only 45 mEq per L in the ileum. No close relationship was found between intraluminal concentrations and net movement of chloride. ¶ Changes in luminal electrolyte concentrations during perfusion were related to their respective initial concentrations. The composition of perfusates containing 6 mEq potassium, 145 mEq sodium, 6 mEq bicarbonate, and 138 mEq chloride per L was unaltered in the jejunum; no change occurred in the ileum with 5 mEq potassium, 138 mEq sodium, 40 mEq bicarbonate, and 105 mEq chloride per L. ¶ We concluded that net movements of sodium, potassium, and bicarbonate in the small intestine are determined largely by their luminal concentrations. In both jejunum and ileum, sodium moves against concentration gradients, whereas transport of potassium is consistent with a passive process. Bicarbonate is absorbed in the jejunum yet secreted in the ileum against concentration gradients. Thus, different mechanisms of anion transport in the jejunum and ileum contribute to maintenance of isotonicity of the gut contents.

Age-related Decrease in Desmosine and Isodesmosine of Human Lung Elastin. JOHN A. PIERCE* AND ROBERT J. KORDSMEIER, Little Rock, Ark.

The connective tissues of the lung are of special interest because of the frequency with which a deformity of lung structure, pulmonary emphysema, occurs in older men. Whereas conventional amino acid analyses have not demonstrated any alterations in the composition of elastin, the total quantity of elastin in the adult human lung has been found to increase with advancing age. For this reason, it seemed important to examine the elastin from human lungs for age-related changes. The purpose of the present study was to measure certain covalent cross links in elastins from subjects of various ages. ¶ Elastin was prepared by the Lowry alkaline extraction method from the lungs of nine adults between 19 and 83 years of age. The protein was hydrolyzed in 6 N HCl at 110° C for 72 hours. Desmosine and isodesmosine were analyzed, as described by Partridge, by partial isolation with alkali-pretreated alumina and separation on sulfonated polystyrene 8% divinylbenzene resin in citrate buffer at pH 4.25. The mean desmosine concentration was $0.63 \pm 0.08\%$, and the mean isodesmosine concentration was $0.52 \pm 0.10\%$ taken from the lungs of four young

subjects (mean age, 24 years). In contrast, desmosine was $0.45 \pm 0.08\%$ and isodesmosine $0.39 \pm 0.08\%$ taken from the lungs of five old subjects (mean age, 75 years). ¶ This result was not expected, since these recently described amino acids, desmosine and isodesmosine, consist of a central pyridine nucleus with four side chain groups that enter covalent linkages with at least two peptide chains. We propose that with advanced age, elastin polypeptide chains become more closely packed and achieve lateral stability without such frequent covalent cross-linkages.

The Humoral Mechanism of Hyperadipokinesis of Starvation in Normal and Obese Men. The Role of β-Adrenergic Receptors. E. J. PINTER AND C. J. PATTEE, Montreal, Canada (introduced by J. S. L. Browne †).

In 20 of 26 obese subjects, increment of FFA levels during starvation was less than in 14 normals. This could be attributed to defect in FFA production, primary increment in FFA utilization during starvation, or increased plasma volume. To investigate this, we gave successive measured injections of labeled palmitate to 7 obese subjects, 6 of these from the 20 hyporeactors, and 5 normals before and during starvation. Less increase of FFA production and FFA pool was found in obese than in normal subjects at >40 hours of starvation. In most of the obese subjects, the base-line FFA production rates and FFA pool were higher than normal. ¶ VMA excretion was unchanged in the obese subjects, either at rest or during starvation for up to 20 days. A β-receptor blocker, Inderal, suppressed FFA production rates only moderately during starvation in both normal and obese subjects, as measured with tracer injections. In contrast, iv infusion of 400 mg nicotinic acid at >50 hours of starvation suppressed FFA production rates below the prefasting values in all subjects. The fall in the FFA levels and production rates was accompanied by simultaneous significant increases in blood sugar levels. ¶ In 20 of 25 obese patients, the *in vitro* reactivity of adipose tissue to exogenous epinephrine was unimpaired, as measured by both FFA levels and determinations of FFA production rates and FFA pool. There was no correlation between the response to exogenous epinephrine and to starvation in terms of *in vivo* reactivity of adipose tissue of the obese subjects. The epinephrine-induced FFA overproduction was almost completely abolished by Inderal. ¶ We concluded that in the starving obese subjects, the humoral mechanism of adipokinesis, rather than the adipose tissue itself, is at fault. The defect seems to involve agents other than catecholamines.

Selective CNS Lactic Acidosis in Response to Hypocapnia. FRED PLUM * AND JEROME B. POSNER, New York, N. Y.

Hyperventilation-hypocapnia profoundly affects the brain by reducing the cerebral blood flow, slowing the EEG, and inducing confusion and seizures in man. Because the P_{O_2} of exposed brain and jugular blood falls, anoxia has been incriminated in causing the hypocapnia effects. Other data contradict the anoxic hypothesis, since neither a fall in cerebral oxygen consumption nor a substantial rise in the arterial-sagittal blood lactate difference has been demonstrated during hypocapnia. However, lactate only slowly passes the blood-brain barrier. ¶ We measured the pH, P_{CO_2}, HCO_3^-, and lactate of the CSF, arterial, and sagittal sinus venous blood in anesthetized dogs at rest and passively hyperventilated for 3½ to 6½ hours. The Pa_{CO_2} fell to 15 to 20 mm Hg in 3 dogs and to 7 to 10 mm Hg in 5; 4 dogs were concurrently exposed to mild hypoxia ($Pa_{O_2} < 60$ mm Hg). Arterial pH levels as high as 7.75 were maintained throughout the 6-hour experiment, with no tendency to fall. Mean blood lactate levels rose from a control of 1.5 to a peak of 5.2 mmoles per L at 2 hours, but thereafter declined to approach normal. The CSF pH rose rapidly to 7.44 to 7.67, but after 2 hours it gradually declined, reflecting a progressive rise of CSF lactate from a control of 2.8 mmoles per L to a 6-hour mean of 8.2 mmoles per L in eupoxic animals and 12.1 mmoles per L in hypoxic animals. Final CSF pH values ranged from 7.47 to 7.59 in the eupoxic and 7.25 to 7.38 in the hypoxic dogs. ¶ Thus, profound hyperventilation-hypocapnia, with or without hypoxia, transiently increased blood lactate but failed to produce systemic acidosis. The same hypocapnia progressively increased the CSF lactate and, when coupled with mild hypoxia, produced actual CSF acidosis. The results are consistent with the hypothesis that selective cerebral hypoxia causes the CNS effects of hypocapnia.

Mechanisms for the Regulation of Serum Insulin Levels by Catecholamines in Man. DANIEL PORTE, JR., Seattle, Wash. (introduced by Robert H. Williams †).

Recent studies in man have shown epinephrine (epi) infusions to inhibit an expected rise in serum immunoreactive insulin (IRI) when given alone or with glucose, glucagon, or tolbutamide. To elucidate the nature of this inhibition, the effect of selective α- or β-receptor adrenergic blockade and β-receptor stimulation was investigated. Normal young adult men and women without a family history of diabetes were studied after an overnight fast. Epi, 6 μg per minute, was infused during α-receptor blockade with phentolamine, β-receptor blockade with propranolol or butoxamine, and lipolytic blockade with nicotinic acid. Nicotinic acid lowered free fatty acids FFA to 25% of control values but had no effect on IRI or glucose. When epi was added, the usual rise in FFA associated with epi was prevented, but IRI and glucose responses were unchanged. β-Receptor blockade with butoxamine lowered basal FFA and partially inhibited the lipolytic effect of epi. Epi plus butoxamine depressed IRI to 50% of control levels and produced bradycardia and hypertension, rather than tachycardia and widened pulse pressure. β-Adrenergic blockade by propranolol, which also caused hypertension and bradycardia during

epi, completely blocked lipolysis by epi, and similarly exaggerated the inhibition of IRI by epi. α-Adrenergic blockade by phentolamine completely reversed epi inhibition of insulin release without effect on the lipolytic or cardiovascular response to epi. The pure β-receptor stimulator isopropylnorepinephrine (Isuprel, 2 μg per minute) caused significant elevation of IRI without hyperglycemia. It is concluded that *a*) IRI inhibition by epi involves α-adrenergic receptors, *b*) stimulation of β-adrenergic receptors elevates IRI, and *c*) FFA levels are unrelated to these responses and apparently do not regulate IRI or IRI responses to epi or glucose.

Distribution of Blood Flow to the Maternal and Fetal Portions of the Sheep Placenta Using Macroaggregates. GORDON G. POWER, LAWRENCE D. LONGO, HENRY N. WAGNER, JR.,* DAVID E. KUHL, AND ROBERT E. FORSTER,† Philadelphia, Pa., and Baltimore, Md.

Previous studies have suggested that placental O_2 exchange may be partially limited by uneven distribution of maternal blood flow relative to fetal blood flow. We sought to measure blood flow to various regions by injecting macroaggregated albumin particles (MAA*) labeled with ^{125}I or ^{131}I in five pregnant sheep. One isotope was injected into the left ventricle of the ewe while the other isotope was injected simultaneously into the umbilical vein of the fetal lamb *in utero*. Since sheep have a capillary bed on both sides of the placenta with vessels small enough to trap MAA*, the distribution of radioactive particles indicated the apparent distribution of blood flow. Over-all distribution for each isotope was estimated by scanning the opened uterus and attached placenta. Relative particle distribution between various cotyledons and in the surrounding uterine wall was measured by well counting of ten to eighty 0.5- to 2-g tissue samples. Microscopic distribution was examined by radioautographs. Our results of particle distribution indicate that apparent maternal and fetal blood flow per gram of tissue ranged from one-tenth to five times the mean value. Approximately 50% of maternal and fetal portions of the placenta received about the average blood flow, whereas 40% of the placenta received a flow less than one-half the mean, and 10% of the placenta received greater than twice the mean flow. In addition, the ratios of maternal to fetal flow in a single cotyledon varied up to tenfold. We concluded that the maternal and fetal blood flows are unevenly distributed to their respective placental capillaries, and the ratio of maternal to fetal capillary flow is also nonuniform. This nonuniformity may be important in limiting placental O_2 exchange in sheep.

Changes in Tissue Enzymes and Trace Metals in Zinc Deficiency. ANANDA S. PRASAD, DONALD OBERLEAS, PAUL WOLF, AND J. HORWITZ, Detroit, Mich. (introduced by Richard J. Bing †).

Deficiency of zinc produces growth retardation and testicular atrophy in animals and man. Vallee and his associates have uncovered a pattern of zinc occurring as an essential component among the nicotinamide dinucleotide dependent dehydrogenases. These observations suggest that cellular zinc concentration controls the physiological processes through the activity of zinc sensitive enzymes. To test this hypothesis, we made rats zinc deficient by feeding a high calcium soybean protein diet for 6 weeks, and then sacrificed them. The tissues from zinc deficient and normal rats were analyzed for trace metals and their enzyme activities determined histochemically. The following enzymes were studied: malic dehydrogenase (MDH), lactic dehydrogenase (LDH), nicotinamide adenine dinucleotide (NADH) diaphorase, alcohol dehydrogenase (ADH), and alkaline phosphatase (AP). Zinc, iron, copper, and magnesium were determined by atomic absorption spectrophotometry. In two deficient and two control rats, calcium, cobalt, manganese, chromium, and vanadium were also determined by spectrographic analysis. In the deficient rats, zinc content, LDH, MDH, NADH diaphorase, and ADH activities were significantly reduced in the testis and zinc concentration; ADH and AP activities were significantly decreased in the bones. The liver showed no changes in the zinc concentration, but NADH diaphorase activity was reduced. Other trace elements showed no significant changes except for iron, which was increased in the testis of the deficient rats. Presuming that the above enzymes are zinc sensitive, the hypothesis that zinc level in tissues controls the physiological processes through the activity of zinc enzymes seems very likely. In conclusion, under the conditions of our experiments, two end organs that exhibit clinical effects of zinc depletion showed a decrease in their zinc content and reduced activities of several enzymes. This is the first demonstration of changes occurring in enzymes and trace metals in the tissues of animals made zinc deficient.

Decreased Bactericidal Activity of Polymorphonuclear Leukocytes in Children with Chronic Granulomatous Disease. PAUL G. QUIE, JAMES G. WHITE, BEULAH HOLMES, AND ROBERT A. GOOD,* Minneapolis, Minn.

Chronic granulomatous disease is a rare sex-linked syndrome involving recurrent sepsis and chronic granulomas. Although the bacteria involved are often of low virulence, prior efforts to define an immunologic defect have been unsuccessful; however, little attention has been given to the phagocytic and bactericidal activity of polymorphonuclear leukocytes. ¶ Polymorphonuclear leukocytes (PMN) from patients with chronic bacterial diseases were compared with PMN from normal subjects by incubating with excess serum opsonins and a standard ratio of bacteria to cells, using Hirsh's adaptation of the Maaløe technique. Striking and reproducible diminution of bactericidal activity against both gram-positive and gram-negative organisms was demonstrated in three patients with chronic granulomatous disease. Less than ½ log of bacteria were killed at 120 minutes by these

patients' cells, compared with a rapid 1.5- to 2-log decrease in simultaneously run controls. Other patients with increased susceptibility to infection did not show lack of bactericidal activity of PMN. That intracellular survival of bacteria rather than lack of engulfment was involved was demonstrated by: *1*) surviving bacteria in the PMN fraction after differential centrifugation of the phagocytic mixture and plating of resuspended lysed PMN; *2*) intact bacteria in PMN fixed and stained after incubation; and *3*) viability of bacteria after incubation of PMN with antibiotics after phagocytosis. Concurrent electron micrographs confirmed the intracellular location of bacteria and showed intact bacteria in phagocytic vacuoles surrounded by intact cytoplasmic granules. ¶ Thus, by using methods for determining both PMN engulfment and killing of organisms, it has been possible to distinguish a new category of immune defect in a defined clinical disease. Study of these patients should offer insights into normal cellular mechanisms for destroying microorganisms and processing antigens of different types for activation of specific cellular or humoral adaptive processes.

Inhibition of Active Sodium Transport by Purified Staphylococcal Alpha-Toxin. JAMES J. RAHAL, JR., MARTIN E. PLAUT, HERMAN ROSEN, AND LOUIS WEINSTEIN,† Boston, Mass.

The effect of purified staphylococcal alpha-toxin (alpha-toxin) on active sodium transport and oxidative metabolism was studied in the isolated toad bladder. Alpha-toxin (2.5 to 4.0 μg), when applied to the serosal surface of the bladder, rapidly inhibited short circuit current and transmembrane potential as measured by a voltage clamp apparatus. Addition of the toxin to the mucosal surface of the bladder produced no effect. Alpha-toxin was added in comparable amounts to a suspension of toad bladder in buffered succinate by using the Warburg technique. We observed a significant stimulation of oxygen consumption. These effects were neutralized by equivalent amounts of staphylococcal antiserum. ¶ These findings are similar to those produced by 2, 4, dinitrophenol (DNP) in the same experimental system, suggesting that alpha-toxin uncouples oxidative phosphorylation. However, a comparison of the effects of alpha-toxin and DNP on oxygen consumption by bladder suspensions incubated with DNP showed dissimilar effects, suggesting that their mechanisms of action are not identical. ¶ The administration of vasopressin after alpha-toxin caused little or no stimulation of transport. Alpha-toxin completely inhibited transport in bladders previously stimulated by vasopressin. ¶ These results indicate that alpha-toxin acts upon a membrane component of the isolated toad bladder very likely located at the serosal surface of the epithelial cell layer. The inhibition of active cell membrane function by the toxin suggests that this mechanism may be responsible for its lethal action in the rabbit and toad.

Early Effects of Parathyroid Hormone on Bone in Tissue Culture. LAWRENCE G. RAISZ* AND INGRID NIEMANN, Rochester, N. Y.

The early events associated with stimulation of bone resorption by parathyroid hormone were analyzed by sequential measurement of calcium release and RNA synthesis in paired cultures of embryonic bone shafts. Nineteen day rat embryo bones were obtained from mothers that had been injected with ^{45}Ca. After preliminary culture for 18 to 24 hours to release exchangeable calcium, bones were transferred to media with or without parathyroid hormone (1 μg per ml). After ½, 3, 6, or 9 hours, the bones were transferred again to media containing uridine-5-^{3}H with or without hormone for a final 2½- or 3-hour period during which ^{45}Ca release and RNA synthesis were measured. Parathyroid hormone caused an early significant increase in ^{45}Ca release, with mean treated to control ratios of 1.18 for the ½- to 3-hour period, 1.23 for 3 to 6 hours, 1.37 for 6 to 9 hours, and 1.57 for 9 to 12 hours. The treated to control ratios for relative specific activity of RNA decreased significantly to 0.87 for the 3- to 6-hour period and increased to 1.18 for 6 to 9 hours. There were no significant changes in RNA synthesis at ½ to 3 or 9 to 12 hours. Crude rat thyrocalcitonin (1.7 μg per ml) inhibited the parathyroid effect on ^{45}Ca release during each period with no consistent effect on RNA synthesis. Actinomycin D, in a dose that partially inhibited RNA synthesis (0.04 μg per ml), did not affect early ^{45}Ca release but did inhibit the increment seen during the 6- to 9- and 9- to 12-hour periods. These data suggest that parathyroid hormone stimulates bone resorption in two phases: an initial phase that is probably independent of RNA, and a later RNA dependent phase. The observed changes in RNA synthesis could result in the characteristic inhibition of osteoblasts and stimulation of fibroblasts and osteoclasts that are seen in parathyroid-treated cultures.

Rabbit Factor V: Differences in Activation by Russell's Viper Venom and Thrombin with Resultant Errors in Assay. SAMUEL I. RAPAPORT,* PETER F. HJORT, AND MARY JANE PATCH, Los Angeles, Calif., and Oslo, Norway.

Two one stage Factor V assays work well for human plasma. One utilizes tissue thromboplastin (TT); the other utilizes Russell's viper venom (RVV). The former measures the original activity state of Factor V in the test sample. In the latter, RVV activates Factor V (to Factor V_r) before recalcification. Thrombin also activates Factor V (to Factor V_t), and human Factor V_r and V_t have been considered identical activities. However, striking differences have been found between rabbit Factor V activity as measured by the TT and RVV techniques that are understandable if: *1*) Rabbit Factor V_r is less active than human Factor V_r. Factor V in rabbit plasma was assayed by the TT and RVV

methods, and percentages were calculated from rabbit and human plasma reference curves. The former gave percentages that agreed for the two assays. The latter gave percentages for the TT assay 10 times greater than those for the RVV assay. Yet, venom does activate rabbit Factor V; adding the test sample to the RVV incubation mixture gave shorter clotting times than adding the test sample just before recalcification (e.g., 31 vs. 40 seconds). *2)* Rabbit Factor V_v is less active than rabbit Factor V_t, allowing the RVV assay to detect traces of rabbit Factor V_t. Ear and heart plasma from the same animals was assayed by the two methods, and percentages were calculated from rabbit plasma reference curves. Means for the ear and heart plasma in the TT assay were 183 and 115% and in the RVV assay, 364 and 119%. These differences probably stemmed from traces of Factor V_t formed during collection of ear blood, for incubation of heart plasma with 0.1 U thrombin per ml increased its Factor V activity to only 150% in the TT assay but to nearly 500% in the RVV assay.

Distribution of Immunoreactive Insulin (IRI) and Insulin-like Activity (ILA) in Vascular and Interstitial Fluid of Man. E. A. RASIO, C. L. HAMPERS, J. S. SOELDNER, AND G. F. CAHILL, JR.,* Boston, Mass.

Six patients with bilateral nephrectomy and thoracic duct cannulation were studied while maintained in adequate nutritional and metabolic balance by hemodialysis. After overnight fast, a rapid iv glucose tolerance test (0.5 g per kg) was performed. On the following day, pork *ins*ulin (0.1 U per kg) was injected rapidly iv. With each test, inulin was also injected. After *ins*ulin, IRI and inulin appeared simultaneously in lymph at 2 minutes and peaked at 10 minutes, when concentrations in serum and lymph were equal (IRI over 100 μU per ml). The $t_{\frac{1}{2}}$ disappearance of IRI was 7 minutes in serum and 13 minutes in lymph. After iv glucose, serum IRI peaked at 1 to 2 minutes (60 μU per ml). Diffusion of endogenous IRI together with inulin into lymph was similar to that observed after *ins*ulin. Subsequent values of serum IRI suggested continued *ins*ulin secretion and did not allow $t_{\frac{1}{2}}$ estimation. Fasting lymph ILA (rat adipose tissue bioassay) was low or undetectable. After *ins*ulin, serum and lymph ILA increased, and the increment approximated the high serum and lymph IRI at 10 minutes. After glucose, no significant increase in lymph ILA occurred; however, maximal serum IRI levels achieved were substantially lower than after iv *ins*ulin. We concluded that endogenous and exogenous IRI diffuse rapidly and equally into the extravascular space. Significant elevations in lymph ILA occur after iv *ins*ulin when high levels of serum IRI are produced, but not after glucose when lower IRI levels are achieved. The magnitude of lymph ILA rise after *ins*ulin is comparable to the rise in lymph IRI.

Evidence for Passive Reabsorption of NaCl in Proximal Tubule of Rat Kidney. FLOYD C. RECTOR, JR.,* MANUEL MARTINEZ-MALDONADO, FELIX P. BRUNNER, AND DONALD W. SELDIN,* Dallas, Texas.

Sodium reabsorption in proximal convolutions (PC) is regarded as active, without significant passive transport. The present studies were designed to characterize the magnitude and nature of passive Na^+ reabsorption. The fraction of NaCl reabsorption (R_{NaCl}) attributable to passive movement can be calculated from the reflection coefficient for NaCl (σ_{NaCl}) by the equation:

$$R_{NaCl} = (\overline{TF/P})_{Cl}(1-\sigma_{NaCl})\left[\frac{(TF/P)_{inulin}-1}{(TF/P)_{inulin}-(TF/P)_{Cl}}\right].$$

During free flow, $(TF/P)_{In}$ was 2.01 ± 0.44 and $(TF/P)_{Cl}$ was 1.18 ± 0.04 at end of PC. The average $(\overline{TF/P})_{Cl}$ between glomerulus and end of PC was 1.12. To determine σ_{NaCl}, we perfused single proximal tubules at a constant rate with the following hypertonic solutions: *1)* 200 mM sucrose + 110 mM NaCl; *2)* 210 mM NaCl; *3)* 100 mM $NaHCO_3$ + 110 mM NaCl. Respective osmotic flows of H_2O into the tubule were: *1)* 0.0043 mμ per second per mm tubule length per mOsm gradient; *2)* 0.00158 mμl per second per mm per mOsm; *3)* 0.00294 mμl per second per mm per mOsm. σ_{NaCl} and σ_{NaHCO_3}, calculated by dividing their osmotic flows by that of hypertonic sucrose, were 0.36 and 0.67, respectively. Hypertonic sucrose pulled a dilute solution of NaCl (70 mEq per L) into the tubule, providing an independent estimate of σ_{NaCl} (0.42). Substituting these values into the equation discloses that 85% of NaCl reabsorption can be attributed to passive movement of solution. ¶To determine whether $NaHCO_3$ and glucose could furnish the osmotic driving force for passive transport of NaCl solution, we examined the effect of varying plasma $[glucose]_P$ and $[HCO_3^-]_P$ on reabsorption of isotonic saline from single tubules using the shrinking-drop technique. Raising $[glucose]_P$ by 20 mmoles per L increased the reabsorption (0.095 ± 0.010 sec^{-1} vs. control of 0.062 ± 0.004 sec^{-1}), whereas lowering $[HCO_3^-]_P$ to 15 mEq per L decreased it (0.043 ± 0.004 sec^{-1}). In addition, in free flow experiments metabolic acidosis decreased proximal $(TF/P)_{In}$ from a control value of 2.01 to 1.45, indicating marked reduction in proximal reabsorption. ¶The following model for proximal reabsorption of NaCl is proposed: *1)* Virtually all NaCl reabsorption is passive. *2)* The mechanism mediating this passive transport is bulk movement of solution. *3)* The driving force is active reabsorption of $NaHCO_3$ (via H^+ secretion) and glucose.

The Physicochemical Properties and Specificity of Human Antibrucella Globulins. JACK L. REDDIN, SOLOMON J. ZAK, AND ARLENE LANG, Minneapolis, Minn. (introduced by Wesley W. Spink†).

The diagnostic significance of agglutinating and complement-fixing antibody was evaluated on sera of 47 human subjects with and without bacteriologically proved

brucellosis. Sera were obtained from patients with acute and chronic brucellosis and from asymptomatic individuals previously exposed to brucellosis. Samples were chromatographed on Sephadex G-200, and 7 S and 19 S gamma globulin fractions were isolated. Antibrucella agglutinating and complement-fixing antibody titers were determined on the whole serum and on globulin fractions with standard techniques. ¶ Elevated titers of agglutinating and complement-fixing antibody were found in the whole serum of 20 patients with acute brucellosis. Agglutinating antibody was recovered from both 7 S and 19 S fractions, but complement-fixing antibody was localized only in the 7 S fraction. Serum obtained during convalescence demonstrated little or no complement-fixing antibody, and agglutinating antibody, when present, was in the 19 S fraction. ¶ Sera from 7 patients with chronic suppurative brucellosis revealed elevated agglutinating and complement-fixing titers. Chromatography showed both types of antibody confined to the 7 S fraction with none in the 19 S fraction, differing from the results in acute disease. Low titers of agglutinating antibody were present only in the 19 S fraction of sera from 20 asymptomatic subjects. No complement-fixing antibody was detected in sera from individuals with inactive disease. Anticomplementary activity was encountered in 10 of the 47 sera, a property detected in the gamma globulin fractions intermediate between 19 S and 7 S fractions. Analytical ultracentrifugation studies suggest that these fractions are 11 S gamma globulin. Further identification is being continued. ¶ These experiments demonstrate the following two significant findings: *1*) both acute and chronic brucellosis may be distinguished from inactive disease by the predominance of antibody activity in the 7 S gamma globulin fraction, and *2*) with human antibrucella complement-fixing antibody confined principally to the 7 S globulin, a specific serological test for active infection is available.

Insulin Release Induced by Glucagon and Sulfonylureas in Organ Cultures of Fetal Rat Pancreas. ALBERT E. RENOLD,* DANIELE VECCHIO, ALFRED LUYCKX, AND GASTON R. ZAHND, Geneva, Switzerland.

Organ cultures of fetal rat pancreas obtained 3 days before term have been routinely maintained in Eagle's Hela medium supplemented with 16 mM glucose, 5% pooled rat serum, and 5% rat embryo extract. After 4 days, the previously undifferentiated tissue exhibited clear-cut differentiation into exocrine and endocrine cells, with predominance of beta cells among the latter. Insulin content increased from measurable amounts to 4 to 5 mU per mg. Identifiable alpha cells were rarely seen. The extent of differentiation and endocrine cell growth was influenced by the concentration of glucose in the medium (optimal concentration 16 mmoles per L) and also by the presence or absence of sodium tolbutamide (81 μg per ml). The explants were then washed and transferred to bicarbonate buffer containing 0.5% albumin for periods varying from 15 to 210 minutes. The preparation has proven highly suitable for studies of insulin release, using a double antibody immunoassay procedure and a crystalline rat insulin standard. Base-line insulin release was small (3.5 ± 0.7 μU per mg) both in the presence and absence of glucose, but it was markedly stimulated in the presence of glucose and glucagon (19.4 ± 6.1, 56.3 ± 9.9, 92.7 ± 11.0 μU per mg with 0.2, 0.8, and 3.2 μg glucagon per ml, respectively). These responses follow a straight line when plotted against the logarithm of the dose. Sodium tolbutamide also stimulated insulin release, but less markedly so (maximal tolbutamide-induced release, 35.0 ± 5.8; minimal effective dose, 9 μg per ml). The preparation may be particularly well suited for *in vitro* studies, in that the 4-day period of organ culture and differentiation yields a preparation that has been exposed to a more standard environment before acute studies of insulin secretion and release *in vitro*.

Radiological, Physiological, and Structural Estimations of the Total Lung Capacity in Normal and Emphysematous Subjects. A. D. RENZETTI, JR., T. M. NICKLAUS, S. WATANABE, AND M. M. MITCHELL, Salt Lake City, Utah (introduced by F. H. Tyler †).

The purpose of this study was to evaluate a radiological method for the determination of the total lung capacity (TLC) as a means of diagnosing pulmonary emphysema and quantitating its severity. TLC was measured by the roentgenographic method of Barnhard and associates, and the results were compared to those of the closed-circuit helium and body plethysmograph methods in 20 normal subjects and 20 patients with emphysema of varying severity. In addition, the radiological TLC was determined from premortem X rays in 73 patients and compared to the degree of anatomic emphysema measured from a single whole lung macrosection prepared from an inflated lung obtained at autopsy. In the normal living subjects, no significant difference was found in the mean TLC among the three methods. In the living emphysematous patients, there was no significant difference in the mean TLC between the radiological and body plethysmographic methods, but these were significantly ($p < 0.01$) greater than the TLC obtained by the helium dilution technique. The 73 deceased individuals consisted of 19 with normal lungs and 54 with emphysema of varying degrees of severity from very mild to very severe. The mean radiological TLC was normal in those with normal lungs and of progressively larger size in those with emphysema. For the total deceased group, the radiological TLC correlated well with the grade of emphysema ($r = 0.69$, $p < 0.001$). ¶ We concluded that the radiological method of TLC estimation is as accurate in normal and emphysematous subjects as the best physiological methods currently in use, and that its simplicity recommends it for widespread application.

Increased Capacity for Urate Excretion by Residual Nephrons of the Chronically Diseased Human Kidney. RICHARD E. RIESELBACH AND THOMAS H. STEELE, St. Louis, Mo., and Madison, Wis. (introduced by Robert F. Schilling †).

In chronic renal disease (CRD), elevation of plasma urate occurs later and to a much lesser degree than BUN. It has been unclear whether intestinal uricolysis is the sole basis for urate homeostasis in CRD. The present studies in 45 patients with varying degrees of CRD were designed to measure and characterize urate excretion by residual nephrons as their number decreases. In 17 patients with a C_{Inulin} of < 12 ml per minute, the mean UV_{urate}/C_{In}, and C_{urate}/C_{In} exceeded control values obtained in 9 normals by eightfold and fivefold, respectively. Increased excretion per nephron was also present in the remaining patients with CRD, but to a lesser degree. The unilateral chronically diseased dog kidney excretes the same quantity of urate per nephron as a contralateral control organ; thus, the pattern observed in CRD in man may not result from a specific tubular lesion producing deficient reabsorption. In normal man, UV_{urate}/C_{In}, and C_{urate}/C_{In} are elevated by urate loading, which ostensibly increases tubular secretion (TS_{Ur}). If residual nephrons in CRD also respond to an increased requirement for urate excretion by adaptively increasing their TS_{Ur}, a potent inhibitor of TS_{Ur} (pyrazinamide) should produce a greater decrease in UV_{urate}/C_{In} in CRD than in normals. After pyrazinamide (3 g orally or 20 mg per kg iv), the mean decrease in TS_{Ur} (μg per minute per C_{In}) was 3.6 in normal vs. 7.5 in patients with a C_{In} of 12 to 90 ml per minute. Although patients with a C_{In} of < 12 ml per minute had a threefold greater baseline UV_{urate}/C_{In} than the less severely diseased group, TS_{Ur} was only 6.6. Thus, enhanced TS_{Ur} by residual nephrons in CRD appears to be the major homeostatic mechanism until severe disease supervenes. Then, other physiologic factors that might alter tubular reabsorption appear to exert an additional effect.

On the Mechanism of the Splay in the Glucose Titration Curve in Chronic Uremia. ALAN M. ROBSON, STEWART W. SHANKEL, AND NEAL S. BRICKER,* St. Louis, Mo.

The glucose titration test is believed to permit reliable definition of nephron homogeneity. Normally, as arterial glucose concentration rises, filtered glucose is reabsorbed completely until filtered load approaches Tm. After load exceeds Tm, reabsorption remains constant. Glucose excreted before Tm produces "splay" in the titration curve. The greater the splay, the greater the apparent heterogeneity of nephrons. We previously applied this technique to dogs with one diseased and one normal kidney. Splay was minimal and equal bilaterally. Moreover, in patients with chronic bilateral glomerulonephritis or pyelonephritis no increase in splay was observed despite GFR reductions of up to 80%. However, at GFR levels below 15 ml per minute, splay was increased, and at levels below 10, splay was marked. To clarify the mechanisms of splay, glucose titration curves were obtained in rats with unilateral pyelonephritis. There was minimal splay bilaterally. When control organs were removed and uremia evolved, $Tm_{glucose}$ increased, GFR increased proportionately more, and the same diseased kidneys exhibited splay. The smaller the apparent nephron population, the greater the splay. To evaluate the possibility that structural abnormalities initiated asymmetric hypertrophy of glomeruli and tubules, we reduced the nephron population unilaterally in rats by ligating arteries supplying 75 to 90% of the parenchyma. Surviving nephrons were free of pathologic changes. With a control kidney present, minimal splay was observed. After removing control kidneys and with uremia, splay evolved in anatomically normal residual nephrons. Again GFR increased more than $Tm_{glucose}$, and splay was inversely proportional to the residual nephron population. ¶ Thus, splay emerges with reduction of nephrons and the development of uremia. It is accompanied by an increase in $Tm_{glucose}$ and a greater increase in GFR. However, it is not dependent upon intrinsic renal disease. Presumably, some factor or factors associated with adaptation or uremia either alter glucose transport or influence homogeneity of glomerulotubular balance.

Valve Closure as the Mechanism of the "Opening" Snap and Diastolic Murmur in Mitral Stenosis. SIMON RODBARD AND ALBERT J. LIBANOFF, Duarte, Calif. (introduced by Ernest Beutler *).

The "opening" snap is generally considered to be generated at the moment that stiffened or agglutinated valve leaflets are forced apart by the increasing atrioventricular pressure difference of early diastole. Studies in man demonstrate that atrioventricular flow begins before the snap. Catheterization data at ventricular end-systole show that atrial pressure drops as soon as ventricular pressure falls below atrial pressure; the rate of fall of ventricular pressure decreases, and the pressure then rises, suggesting that filling is taking place. The "opening" snap follows onset of the atrial pressure fall by about 0.1 second. Immediately after the snap, a transitory rise of atrial pressure is synchronous with a ventricular pressure fall; these effects are consistent with sudden mitral valve closure. The diastolic murmur is associated with a succession of oscillations in which atrial and ventricular pressures are 180° out of phase. These findings are consistent with recurrent closure of the mitral leaflets during diastole. The pressure on the atrial surface of the leaflets falls as a function of stream velocity until the leaflets approach each other and close the orifice, producing a closing snap and the resulting atrial and ventricular pressure oscillations. A succession of such closures generates a coarse murmur together with pressure oscillations in the two chambers that are out of phase. The snap and murmurs may thus represent recurrent mitral closures.

Megakaryocyte Kinetics in Experimentally Induced Immune Thrombocytopenia. ZORAN ROLOVIC, MARIO BALDINI, AND WILLIAM DAMESHEK,† Boston, Mass., and Providence, R. I.

It has often been suggested that in immune thrombocytopenias the antiplatelets antibody may not only cause destruction of the circulating platelets but also exert an injurious effect on the megakaryocytes. This hypothesis was explored by studying the pattern of megakaryocyte maturation in rats during prolonged experimental heteroimmune thrombocytopenia, using radioautography with tritiated thymidine. A thrombocytopenic "steady state" (10,000 to 40,000 mm^3) was produced in 30 female Sprague-Dawley rats by properly tritiated intraperitoneal injections of a potent rabbit antirat platelet serum. Tritiated thymidine (0.75 mc per g body weight) was injected in a single dose on the fourth day after the thrombocytopenia was established. For each animal 500 megakaryocytes were examined. Control studies were done in 50 normal rats. Morphologic studies showed that in the thrombocytopenic rats megakaryocytes were more numerous than normal, with a preponderance of immature forms. Many megakaryocytes displayed altered morphology, which included hyaline appearance and lack of cytoplasmic granules. Measurement of megakaryocyte kinetics revealed that in the thrombocytopenic rats the total megakaryocyte transit time was prolonged to 66 hours (60 to 72), whereas it was 54 hours (48 to 60) in the controls. The rise in labeling index of mature megakaryocytes was delayed 6 hours (24 hours instead of 18 hours) and never reached a level above 60% at 48 hours (normal 65 to 90%). These data indicated that in experimentally induced immune thrombocytopenia, *1)* kinetics of megakaryocytes was severely impaired, and *2)* the increase in number of these cells and the changes in the proportional distribution in their maturation groups were at least in part due to depression in their maturation rate.

Hereditary Deficiency of the Second Component of Complement in Man. FRED S. ROSEN, JESUS KUMATE, MARTIN R. KLEMPERER, AND K. FRANK AUSTEN,* Boston, Mass., and Mexico, D. F.

A hereditary deficiency of the second component of complement has been identified in three kindreds. Six individuals homozygous for the deficiency have been studied with regard to host resistance and various hypersensitivity reactions. ¶ The abnormality is defined by markedly reduced serum hemolytic complement titers of < 1 $C'H_{50}$ U (normal, 32 to 45 $C'H_{50}$ U per ml) and C'2 titers of < 4 U by the reagent titration (normal, 128 U), or by a stoichiometric titration with $EAC'_{1,4}$ cells (normal, 325 to 670 U). The deficiency is further manifested by a reduced immune adherence complement titer of 32 to 128 U (normal $C'I\text{-}A_{50}$, 760 to 1,400 U per ml), diminished serum bactericidal activity of 1:3 against *Salmonella typhosa* O_{901} (normal, 1:25), and a markedly reduced rate of conversion of β_{1c}-globulin in the presence of a preformed antigen-antibody aggregate. ¶ Titrations of C'1, C'4, and C'3 by the reagent method and levels of serum β_{1c}-, β_{1e}-, and β_{1f}-globulins and C'1a esterase inhibitor by immunodiffusion study were normal, indicating a selective deficiency in C'2. Addition of highly purified human C'2 restored all complement dependent reactions to normal, i.e., the whole complement titer, the conversion of β_{1c}, the immune adherence titer, and the serum bactericidal assay. ¶ The defect appears to be transmitted as an autosomal recessive characteristic. In two of the three kindreds, individuals heterozygous for the defect cannot be detected; in the third family, heterozygotes are easily detectable since their sera have approximately one-half the normal whole complement and C'2 titers. In this kindred 15 heterozygotes have been recognized. Because no gene product of the abnormal allele has been recognized by competition studies, codominance of the alleles has not been established.

Distribution of Blood Flow within the Normal and Transplanted Human Kidney. S. M. ROSEN, N. K. HOLLENBERG, J. B. DEALY, JR., AND J. P. MERRILL,† Boston, Mass. (introduced by Kendall Emerson, Jr.†).

The importance of changes in the distribution of renal blood flow between cortex and juxtamedullary region has been repeatedly suggested. However, no reports are available in man. Intrarenal distribution of blood flow was determined with ^{133}Xe in man according to the method of Barger and co-workers, which demonstrated in the dog that renal blood flow could be divided into four major compartments (I, II, III, and IV), which correspond, respectively, to cortex, juxtamedullary region, medulla, and renal fat. The mean of 10 measurements in 10 normal human kidneys showed that compartments I, II, III, and IV received, respectively, $79.0 \pm 1.0\%$ (SE), $16.5 \pm 6.2\%$, $2.6 \pm 0.2\%$, and $3.4 \pm 0.5\%$ of total renal blood flow. The blood flow in milliliters per minute per 100 g tissue of the respective compartments was 358 ± 33, 63 ± 10, 15 ± 2, and 9 ± 1. The ratio of mass of perfused cortex to mass of perfused juxtamedullary region calculated from these data was 0.47 ± 0.06 (SE). Seventeen measurements were made on 2 isotransplanted kidneys with normal creatinine clearances and 12 homotransplanted kidneys that had creatinine clearances ranging from 100 to 38 ml per minute due to various degrees of rejection. The percentage of total blood flow supplied to compartment I decreased with diminishing creatinine clearance ($p < 0.01$), but flow per 100 g tissue of this compartment remained within normal limits. These findings are best explained by areas of normally perfused cortex coexisting with areas of ischemic cortex during rejection. We concluded that this method yields results with a narrow range of variation in a normal human population, and that it can be used to determine the role of alterations in the intrarenal distribution of blood flow in renal pathophysiology.

Racial Difference in the Incidence of Lactase Deficiency. N. S. ROSENSWEIG AND T. M. BAYLESS, Baltimore, Md. (introduced by T. R. Hendrix *).

The incidence of milk intolerance and lactase deficiency in a healthy nonpatient adult population is not known, and its etiology (acquired vs. inherited) remains unclear. In this study, 40 consecutive healthy nonhospitalized prison volunteers, including 20 Negroes and 20 Caucasians, were interviewed to determine the incidence of symptoms after milk ingestion. The incidence of *1*) "flat" lactose tolerance tests (dose, 50 g per m^2), *2*) symptoms from the tests, and *3*) lactase deficiency in jejunal biopsy specimens, were determined. ¶ Twenty-one of the 40 gave a history that milk caused abdominal bloating, cramps, or diarrhea. Of these 21 with milk intolerance, 19 were Negro. Thus, 19 of 20 Negroes gave this history. The lactose tolerance test produced similar symptoms in 20 individuals, all milk-intolerant by history. Lactose tolerance tests were flat (maximal blood sugar rise less than 25 mg per 100 ml) in 16 of the intolerant group. Mean rise was 16.3 mg per 100 ml. By contrast, this test produced no symptoms and a blood sugar rise greater than 25 mg per 100 ml in 18 of 19 milk-tolerant subjects. Mean value was 41.6 mg per 100 ml. Glucose and galactose tolerance tests, performed to rule out monosaccharide malabsorption, produced no symptoms and were normal in all patients. Mean of milk-intolerant group was 40.1 mg per 100 ml, and mean of tolerant group was 38.4 mg per 100 ml. Intestinal lactase levels in 15 of the 21 milk-intolerant subjects were lower than the lowest value in the milk-tolerant group. Mean value in the milk-intolerant group was 1.9 U (range, .1 to 6.7), whereas mean level of the milk-tolerant group was 7.1 U (range, 2.2 to 17.7). ¶ These findings show that milk intolerance secondary to lactase deficiency is very common in certain segments of an otherwise healthy adult population, and the difference in incidence between Negroes and Caucasians strongly suggests that intestinal lactase activity is genetically controlled.

Vasopressin Antibodies and a Sensitive Radioimmunoassay. JESSE ROTH, LESTER A. KLEIN, AND MARTIN J. PETERSEN, Bethesda, Md. (introduced by J. E. Rall *).

Rabbits, hogs, and chickens, injected repeatedly with pure bovine arginine vasopressin octapeptide (AVP) in adjuvant, produced antibodies to vasopressin. Vasopressin antibody was also detected in one patient who had been treated with commercial vasopressin. AVP was labeled with ^{131}I by modification of the chloramine-T method. After brief purification, AVP-^{131}I showed single spots on paper chromatography and paper chromatoelectrophoresis. The specific activity of AVP-^{131}I was increased markedly by passage through Sephadex G-25, since dextran gels loosely adsorb iodinated vasopressin and separate it from unlabeled hormone. The binding of AVP-^{131}I to antibody was demonstrated by equilibrium dialysis, immunodiffusion in agar, precipitation with antibody to gamma globulin in buffer, chromatoelectrophoresis, conventional paper electrophoresis, and distribution on dextran gels. AVP-^{131}I was readily displaced from antibody by unlabeled AVP. Some of the antisera bound lysine vasopressin (LVP) equally well, whereas other sera bound AVP much more strongly than LVP. Oxytocin was bound very weakly. When the unlabeled hormone was assayed as biological activity in toads and radioactivity counted simultaneously, the unlabeled and labeled AVP behaved identically with respect to dialysis, antibody binding, and subsequent dissociation from antibody. These studies show that vasopressin is clearly antigenic, despite a molecular weight of less than 1,100 and despite the similarity or, in the case of rabbits, apparent identity of the injected and endogenous hormone. At present, radioimmunoassay is sensitive to AVP at a concentration of 50 $\mu\mu$g per ml with an absolute sensitivity of 10 $\mu\mu$g of hormone. Thus, by appropriate modification, the range of the radioimmunoassay method has been extended to include even the smallest polypeptide hormones.

Thermal Stress and Albumin Metabolism. MARCUS A. ROTHSCHILD,* MURRAY ORATZ, CARL S. WALKER, AND SIDNEY S. SCHREIBER, New York, N. Y.

Whereas changes in environmental temperature have been associated with alterations in serum protein levels, data are not available on associated changes in protein metabolism. The present report describes the measurement of albumin metabolism in rabbits exposed to different temperatures. After an injection of albumin-^{131}I and an appropriate control period at room temperature, six female rabbits were maintained at 34 to 36° C for 16 to 20 days and observations continued. While the animals remained in the incubator, a second injection of albumin-^{131}I was given to remeasure the albumin pool. During the experimental periods the rabbits' food intake decreased, and a third study period was obtained in these and in three other rabbits at room temperature but on an equivalent low ration diet. Similar studies were conducted in four rabbits before and during exposure to 1° C for 16 days; in these rabbits food intake was not reduced. ¶ After exposure to 36° C, serum albumin fell significantly from 3.61 to 2.55 g per 100 ml. Albumin degradation decreased from 226 to 169 mg per kg per day, exchangeable albumin from 3.32 to 2.52 g per kg, and albumin synthesis from 226 to 87 mg per kg per day. Upon removal from the high temperature, but retained on the limited diet, albumin synthesis rose to 164 mg per kg per day, with no change in albumin distribution during heat exposure. The animals maintained on an equivalent low ration diet from the start demonstrated only a 13% decrease in albumin degradation and a 19% decrease in albumin synthesis. In the "cold" no significant change in albumin metabolism occurred, though plasma volume and extracellular space (sucrose-^{14}C) decreased. These results indicate that high environmental temperature significantly depresses albumin production. This change is only partially explained by a decreased nitrogen intake.

Exposure to cold does not result in any alteration in albumin metabolism.

Renal Reabsorption of Protein and Compensatory Renal Hyperplasia. PAUL C. ROYCE, New York, N. Y. (introduced by Quentin B. Deming).

This report presents evidence that hyperplasia of remaining rat kidney after unilateral nephrectomy may be initiated by increased renal reabsorption of low molecular weight plasma proteins. Bilateral nephrectomy is followed by a gradual rise in activity of two endogenous plasma enzymes, ribonuclease and muramidase, and in the concentration of other proteins demonstrated on disc electrophoresis. A significant but less pronounced rise in plasma ribonuclease and muramidase activities occurs after unilateral nephrectomy and is accompanied by increased activity of the two enzymes in the remaining kidney. Within the kidney, ribonuclease and muramidase are found almost exclusively with the subcellular particulate fractions of the cortex. Activity of both enzymes is substantially increased by subjecting homogenates to conditions (e.g., hypotonic media, repeated freezing and thawing) that disrupt biological membranes. These findings indicate that ribonuclease and muramidase are sequestered within membrane-bound vesicles of the kidney cortex and suggest that renal uptake of both enzymes occurs by pinocytosis from the glomerular filtrate. The fate of the membrane-bound enzymes is unresolved. In the case of ribonuclease only slight loss of enzyme activity occurs after prolonged incubation of kidney homogenates. Increased renal reabsorption of protein appears to be a sufficient condition for initiating renal hyperplasia. Incorporation of ^{32}P into renal DNA is accelerated after intraperitoneal injection of egg white muramidase, a low molecular weight (17,500) protein that is filtered, reabsorbed, and sequestered by the kidney. We propose that increased concentrations of several plasma proteins occur after renal mass reduction, and that increased reabsorption of these proteins by the remaining kidney provides the stimulus for initiating compensatory renal hyperplasia.

Anatomical and Physiological Studies of Heterotopic Intestinal Epithelium. WALTER RUBIN, LEONARD L. ROSS, EMMANUEL THEODOR, GRAHAM H. JEFFRIES, AND MARVIN H. SLEISENGER,* New York, N. Y. (introduced by Roy C. Swan†).

The fine structure, ontogenesis, and physiologic capabilities of aberrant intestinal epithelium in gastric mucosa ("intestinal heterotopia" or "metaplasia") have not been previously determined. In the present study, electron microscopic examination of aberrant intestinal epithelium in atrophic gastric mucosae of pernicious anemia patients demonstrated its histologic arrangement and fine structure to be identical to those of normal human jejunum. Aberrant intestinal epithelium contained a full complement of small intestinal epithelial cells—differentiated villus, undifferentiated crypt, goblet, Paneth, and argentaffin. Cells often observed in mitosis and identical to jejunal undifferentiated crypt cells line the crypts of aberrant intestinal epithelium and probably serve as its undifferentiated stem cell. The crypts of aberrant intestinal epithelium contained only intestinal-type epithelial cells; no undifferentiated stem cells common to both gastric and intestinal types of epithelium were found. Gastric and intestinal type epithelial cells met only at or near the mucosal surface where heterotopic differentiated villus cells could be observed adjacent to gastric surface mucus cells. These observations support the hypothesis that aberrant intestinal epithelium represents a congenital heterotopia rather than a metaplasia. ¶ Oil red O staining of gastric biopsies from fasting pernicious anemia patients demonstrated lipid within the lamina propria beneath surface heterotopic intestinal epithelium but not beneath gastric surface mucus cells. Gastric biopsies obtained sequentially before and after infusion of micellar lipid into a patient's stomach were examined by electron and light microscopy after lipid staining. Heterotopic intestinal surface epithelium readily incorporated lipid and transported it to the adjacent lamina propria. Lipid was not observed within gastric types of epithelial cells or in their adjacent lamina propria. Heterotopic intestinal epithelium, therefore, like normal jejunal epithelium, has the physiologic capacity to absorb dietary and micellar lipid and transport it to the gastric lamina propria, where appreciable amounts are not detected histologically in normal stomachs.

Fate of the Hydrogen Ion during Mobilization of Free Fatty Acids from Adipose Tissue. DANIEL RUDMAN* AND PAUL W. SHANK, New York, N. Y.

Lipolytic hormones stimulate fat cells to convert stored triglyceride into glycerol and FFA, which are transported from adipose tissue as the albumin complex of ionized FFA ($RCOO^-$). To investigate the fate of H^+ arising by dissociation of RCOOH newly formed through lipolysis, we incubated adipose tissue slices from rat, hamster, or rabbit for 3 hours in albumin-free or albumin-containing Krebs-Ringer phosphate medium with and without epinephrine or ACTH (10 μg per ml). In the absence of albumin, FFA produced by hormone-stimulated lipolysis were not released into medium but accumulated instead in the tissue's aqueous insoluble triglyceride rich fraction, whence they could be extracted as RCOOH by heptane without prior acidification; H^+ did not accumulate detectably in tissue or medium. In the presence of albumin, RCOOH accumulated in the stimulated tissue, whereas $RCOO^-$ and H^+ were continuously released in molar-equivalent quantity into the medium, whose pH declined progressively from 7.40 to 7.00–7.10. ¶ *In vivo* acidosis after hormonal stimulation of lipolysis was also demonstrated. Two mg ACTH injected in fed rabbits caused, during the following 3 hours, six- to tenfold increase in plasma FFA and reductions in blood pH from 7.40 to 7.50 to 7.20 to 7.30 (despite marked hyperventilation), in plasma HCO_3^- from 19 to 22 to 8 to 12 mEq per L, and in plasma P_{CO_2} from 27 to 33 to 10 to 17 mm Hg. Plasma ketone bodies were absent.

¶ Conclusions: *a*) FFA formed by lipolysis are stored within fat cells as RCOOH dissolved in triglyceride; dissociation to $RCOO^-$ and H^+ occurs only when RCOOH interacts with extracellular albumin. *b*) Release of H^+ from fat cells during lipolysis, though thus regulated by availability of albumin binding sites for $RCOO^-$, can occur rapidly enough *in vivo* to cause severe acute nonketotic metabolic acidosis.

Cardiac Output and Organ Blood Flow of the Fetus *In Utero*. ABRAHAM M. RUDOLPH * AND MICHAEL HEYMANN, New York, N. Y.

Hitherto no information has been available regarding distribution of blood flow in the undisturbed fetus. We have developed technics for insertion of small vinyl catheters into an umbilical artery (UA) and vein (UV) and into forelimb and hindlimb vessels in the sheep or goat fetus through small uterine incisions, under spinal analgesia. The catheters are exteriorized and the fetus studied in its normal intrauterine environment. Blood-gas status of fetus and mother are repeatedly assessed. Plastic microspheres (50 μ diameter) labeled with various nuclides were injected into forelimb, hindlimb, and UV. These spheres do not recirculate and are distributed in the pattern of blood flow. By measuring the amount of activity of each nuclide in each organ, the proportions of flow derived from superior and inferior venae cavae can be calculated. ¶ During constant infusion of 4-amino-antipyrine into a fetal limb vein, UA and UV concentrations were measured after equilibration, and placental blood flow could be determined by the Fick method. By relating the proportion of nuclide in each fetal organ to that in placenta, actual organic flow and total cardiac output were calculated. ¶ In eight animals with gestational ages of 95 to 130 days and normal blood-gas status, placental blood flow was 123 to 360 ml per kg fetal weight per minute. Cardiac output was 298 to 919 ml per kg per minute. Percentages of total cardiac output distributed to various organs and the actual flow per gram of organ weight were: placenta, 40 to 60% (0.62 to 2.2 ml per g); kidney, 1.4 to 2.8% (0.6 to 2.0 ml per g); spleen, 0.3 to 2.9% (1.8 to 8.0 ml per g); intestine, 2.6 to 6.2% (0.2 to 1.7 ml per g); brain, 2.0 to 8.8% (0.2 to 2.4 ml per g); heart, 1.6 to 10.4% (0.7 to 12.7 ml per g); and lungs, 1.4 to 24.5% (0.6 to 0.8 ml per g). ¶ It is possible to study distribution of blood flow under varying physiologic situations by successive injection of microspheres with different nuclide labels. The technic should have application to study of blood flow distribution in many physiologic and pathophysiologic states.

Preferential Localization of Palmitate-^{3}H Incorporation in the Large Alveolar Cell of Dog Lung: A Radioautographic Study. SAMI I. SAID,* WILLIAM R. HARLAN, JR., LAURA W. NORRELL, AND YVONNE T. MADDOX, Richmond, Va.

Evidence is mounting that mammalian lung is active in phospholipid synthesis and probably related to the production of a surface active alveolar lining. To localize the cellular site of this synthesis, we prepared radioautographs of lung *in vivo* and of alveolar macrophages *in vitro*. Tritium-labeled sodium palmitate, complexed to serum albumin, was injected intravenously in normal dogs (1 mc per kg), and the lung was sampled ½, 1, and 2 hours later. Fresh frozen sections (4 to 6 μ) were cut in cryostat, fixed in formalin, coated with Kodak NTB2 nuclear track emulsion, and exposed for 2 to 3 months. Silver granules were concentrated predominantly in the large alveolar cells (type II, pneumonocytes), less intensely in the remainder of the alveolar wall, and almost not at all in the walls of bronchi or blood vessels. Incorporation was most marked in the 1-hour sample. For better definition of the time course of uptake, we prepared radioautographs of smears taken from saline suspension of alveolar macrophages (Myrvik and associates) a few minutes to 6 hours after adding a dose of palmitate-^{3}H. Concomitant samples of suspension were centrifuged, and cells and cell-free medium were separately extracted with chloroform-methanol. Thin layer chromatography and subsequent assay of radioactivity in lipid fractions showed incorporation by cells of labeled palmitate into phospholipids. We conclude that the large alveolar cell (possibly alveolar macrophage) is a major site of palmitate-^{3}H incorporation by dog lung, but other alveolar cells apparently contribute.

The Metabolism of Deoxycorticosterone (DOC) in Humans. AVERY A. SANDBERG,* EDWARD CHANG, AND W. ROY SLAUNWHITE, JR., Buffalo, N. Y.

Deoxycorticosterone has been demonstrated to be a probable secretory product of the adrenal cortex under abnormal conditions, and the possibility exists that DOC may be a product of the normal gland. In addition, for many years DOC has constituted one of the major therapeutic mainstays in adrenal insufficiency. With the above as background, we undertook studies to determine the metabolism of ^{14}C-labeled DOC given intravenously to subjects with and without bile fistulas. The free steroid was cleared from the plasma at a rapid exponential rate with a half-life of 30 minutes, followed by a slower exponential clearance with a half-life of 90 minutes. The glucuronidated metabolites of the steroid reached their highest plasma levels within 15 to 30 minutes after injection and declined rapidly within 4 hours. About 50 to 60% of the radioactivity was excreted in the urine over a period of 5 days. Substantial radioactivity appeared in the stools (15% or more). In the subjects with biliary fistulas approximately 25% of the radioactivity appeared in the bile, and these findings indicate that the metabolites of DOC are not involved in an extensive hepatoenteric circulation. Of interest was the identification of DOC in the bile and urine and of progesterone in the bile, even though both constituted a small percentage of the radioactivity in the fluids. The tetra- and hexahydrogenated metabolites of DOC comprised the bulk of the hydrolyzable conjugated compounds in the urine and bile. In

many respects the metabolism of DOC in human subjects resembles that of progesterone and differs from that of other neutral steroids, e.g., cortisol.

Improvement in Cardiac Efficiency of Catecholamine-induced Positive Inotropy by Partial Beta-Adrenergic Blockade. CHARLES A. SANDERS, RICHARD K. MYLER, AND HOWARD B. CORNING, Boston, Mass. (introduced by Stephen M. Krane*).

Induction of positive inotropy by catecholamines is usually accompanied by an increase in myocardial O_2 consumption (MVo_2) and a decrease in mechanical efficiency (ME). Recent evidence suggests the augmented MVo_2 results from increased velocity of contraction (Vel). Since efficiency of skeletal muscle contraction diminishes as Vel increases beyond an optimal load and velocity, the decline in myocardial efficiency may be due to excessive increments in Vel. In this milieu reduction in Vel should lead to improvement in ME. To explore this possibility studies were performed in 16 closed-chest dogs to determine the effect of partial beta-adrenergic blockade (PBB) on the positive inotropy produced by the beta-mimetic agent, isoproterenol (Ip). With heart rate held constant, measurements of cardiac output (CO), coronary flow (CF), MVo_2, and intracardiac pressures were taken during three periods: *a*) control, *b*) during Ip infusion, and *c*) during PBB with continuing Ip infusion. CO increased $51.9 \pm 31.4\%$ ($p < 0.001$) with Ip and remained $45.6 \pm 28.4\%$ ($p < 0.001$) above control after PBB. CF and MVo_2 rose $47.8 \pm 25.6\%$ ($p < 0.001$) and $44.1 \pm 17.6\%$ ($p < 0.001$), respectively, with Ip but fell to $3.3 \pm 15.6\%$ and $-11.8 \pm 10.6\%$ of control after PBB. Increments in rate of pressure rise and systolic ejection rate of the left ventricle were $84.1 \pm 64.5\%$ ($p < 0.001$) and $71.1 \pm 35.6\%$ ($p < 0.001$), respectively, with Ip but only $29.3 \pm 11.9\%$ ($p < 0.001$) and $39.1 \pm 27.7\%$ ($p < 0.001$) of control with PBB. ME fell in 9 dogs and rose slightly in 7 with Ip producing no significant change over control values. With PBB, ME rose $68.3 \pm 60.4\%$ ($p < 0.001$) above control and $70.1 \pm 49.6\%$ ($p < 0.001$) over Ip. These data indicate that PBB can effect marked changes in Vel and MVo_2 without significantly influencing isoproterenol-induced increments in CO. Since heart rate was held constant, the lack of change in CO suggests the increments in fiber shortening were also little affected by PBB. These studies demonstrate that Vel and extent of fiber shortening are dissociable and that the "O_2-wasting" effects of the catecholamines can be reversed and ME markedly improved by reducing velocity of contraction.

Aldosterone Metabolism in Anephric Humans. LOUIS L. SANDERS, WILLIAM J. FLANIGAN, THOMAS E. WILSON, SIDNEY L. DALE, AND JAMES C. MELBY,* Little Rock, Ark., and Boston, Mass.

Dietary sodium restriction in healthy subjects is associated with a progressive rise in plasma renin activity and aldosterone secretion. The reciprocal relationship of sodium intake and activation of the renin-angiotensin system is regarded as a prime stimulus of aldosterone secretion. To examine the role of the renin-angiotensin system in the regulation of aldosterone secretory response to sodium restriction, we injected aldosterone-1,2-^{3}H intravenously into five patients who had undergone bilateral nephrectomy from 7 days to 9 weeks previously and were awaiting transplantation of kidneys from healthy donors. Peritoneal dialysis was carried out for 24 hours after injection of labeled aldosterone and dialyses scheduled at 5- to 7-day intervals. Sodium restriction, both dietary and in the dialyzing solution, and sodium loading were carried out in three patients. Endogenous corticotropin was suppressed by simultaneous administration of dexamethasone. The metabolic clearance rate of aldosterone-1,2-^{3}H was determined in the course of these experiments. Twenty-seven to 60% of injected radioactivity was recovered from dialyzates within 24 hours. Pervaporation, hydrolysis, extraction, chromatographic isolation, counting, and quantification of acid labile conjugate and tetrahydro-3-glucuronide metabolites of aldosterone were carried out, and "production rates" of aldosterone were roughly estimated. Excretion of the tetrahydro-3-glucuronide metabolite was more constant. The metabolic clearance rate of aldosterone was unaffected by alterations in sodium intake in anephric subjects. Though imprecise, estimated "aldosterone production" was four- to fivefold greater during sodium restriction than during sodium loading. We concluded that activation of the renin-angiotensin system is not essential for the aldosterone secretory response to sodium restriction.

Gluconeogenesis as a Source of Glyceride-Glycerol. RICHARD SANDLER AND NORBERT FREINKEL,* Boston, Mass.

Hepatic glycogen is low and there is net production of glucose in starvation, diabetes, and other conditions in which long chain acyl CoA accumulate within the liver. Under such circumstances, glycerophosphate for esterification has been attributed to exogenous glycerol, since formation via glycolysis would be negligible. We instituted experiments to assess the hitherto neglected possibility that significant glyceride-glycerol could originate from triosephosphates arising in the course of gluconeogenesis. ¶ Liver slices from fasted animals were incubated with 1.0 mM alanine-U-^{14}C in KRB; medium glucose-^{14}C and tissue glyceride-glycerol-^{14}C (glyc-^{14}C) were measured after 60 to 90 minutes. The ratio glucose-^{14}C/glyc-^{14}C was 5.9 ± 0.9, 12.6 ± 1.9, 18.7 ± 7.5, and 30.7 ± 8.8 with rabbit, rat, guinea pig, and mouse liver, respectively, thus indicating modest but definite esterification of glycerophosphate derived during gluconeogenesis. Factors influencing the partition of triosephosphates between glucose and glyceride-glycerol were evaluated by adding triamcinolone, carnitine, or ethanol *in vitro*. Triamcinolone produced parallel increases in glucose-^{14}C and glyceride-glycerol-^{14}C from alanine-^{14}C, so that the ratio was unaffected. In contrast, carnitine, which also

increased glucose-^{14}C, did not effect commensurate increases of glyceride-glycerol-^{14}C, so that glucose-^{14}C/glyc-^{14}C rose 71.2% in rat liver ($p < 0.01$). Inhibition of gluconeogenesis with ethanol was accompanied by relative preservation of glyceride-glycerol-^{14}C, so that glucose-^{14}C/glyc-^{14}C fell 64% in rabbit liver ($p < 0.01$). ¶ These experiences with carnitine, which promotes fatty acid oxidation, suggest that availability of long chain acyl CoA as esterification "traps" for glycerophosphate may influence relative partitioning of triosephosphates. The results with alcohol, which generates DPNH during its oxidation, indicate that cytoplasmic-reducing environment may also be determinative. ¶ The demonstration that triosephosphates of gluconeogenic origin can supply appreciable glyceride-glycerol may have relevance for the fatty liver and/or triglyceridemia that can occur when gluconeogenesis is activated (e.g., starvation, diabetes, steroid therapy) or when glycerologenesis is favored selectively (e.g., alcoholism).

The Role of Bile Salts in the Renal Excretion of Bilirubin. JOHN SANDSON* AND MILFORD FULOP, Bronx, N. Y.

Conjugated bilirubin (CB) is excreted in the urine mainly by glomerular filtration of the small percentage of plasma CB that is ultrafilterable and dialyzable. Plasma-unconjugated bilirubin (UB) is not dialyzable and is not excreted in the urine. The present studies are concerned with the basis for the dialyzability of plasma CB and the nature of the dialyzable CB. ¶ *Dialysis.* The addition of the bile salts glycocholate, taurocholate, or glycodeoxycholate to conjugated hyperbilirubinemic plasma enhanced the dialyzability of CB, presumably by displacing it from plasma protein. The addition of bile salts to unconjugated hyperbilirubinemic plasma did not cause perceptible dialysis of UB. ¶ *Electrophoresis.* Previous studies of urinary CB and the dialyzable portion of plasma CB suggested that they might be combined with another substance. The addition of glycocholate or glycodeoxycholate to conjugated hyperbilirubinemic plasma altered the mobility of CB on paper electrophoresis so that some of it migrated in the alpha globulin zone, where a portion of the added bile salt also migrated. This was very similar to the migration of CB in urine. The association of bile salts and CB on electrophoresis suggests that the bile salts may actually be complexed with the dialyzable portion of plasma CB. ¶ Plasma bile salt concentrations are usually elevated in clinical states associated with conjugated hyperbilirubinemia. The ability of bile salts to enhance the dialyzability of plasma CB suggests that the endogenous bile salts in conjugated hyperbilirubinemic plasma may be responsible for the dialyzability of CB from the native plasma, and hence for the renal excretion of CB. The failure of UB to be excreted in the urine may be related to the absence of elevated concentrations of plasma bile salts in clinical states with predominantly unconjugated hyperbilirubinemia.

Glucagon Regulation in Normal and Obese Diabetic Subjects. DON S. SCHALCH, Rochester, N. Y. (introduced by John R. Jaenike*).

Factors that influence the secretion of glucagon have been studied in humans by the double antibody radioimmunoassay method. The mean overnight fasting level in eight normal subjects was 3.4 ± 0.2 mμg per ml and was not significantly different from that in six obese diabetics (3.6 ± 0.2 mμg per ml). There was no significant increase in plasma glucagon in eight maturity-onset diabetics during a 10-day period of total starvation. After a 100-g glucose load, plasma glucagon levels rose significantly and to the same degree in both normals and diabetics (from 3.4 to 4.5 and from 3.6 to 4.9 mμg per ml, respectively). The peak glucagon level occurred within 90 minutes after the ingestion of glucose in most instances, and in some there was a subsequent drop in glucagon concentration followed by a second peak at 4 to 5 hours unassociated with hypoglycemia. Tolbutamide administered intravenously led to a significant fall ($p < 0.001$) in plasma glucagon both in two normal and six diabetic subjects (from 3.7 to 2.4 mμg per ml). This drop in glucagon occurred usually in the first 15 minutes and at the time of maximal plasma insulin rise. Plasma glucagon was unaffected by insulin-induced hypoglycemia in five other subjects. The effect of increased plasma growth hormone (GH) concentration on glucagon secretion was evaluated in two acromegalics and two normal subjects given 5 mg of human GH iv. Plasma glucagon levels in these subjects were not significantly different from those of normal individuals. ¶ From the finding that hyperglycemia raises and that hypoglycemia does not affect systemic glucagon levels, it is apparent that blood glucose does not control glucagon secretion by a simple negative feedback mechanism. In maturity-onset diabetics, these regulatory changes are similar to normals, indicating that such patients do not have an abnormality in glucagon secretion. From an analysis of the glucagon response to tolbutamide, the suggestion is made that inhibition of glucagon secretion may be in part responsible for the hypoglycemia that this drug induces.

Studies on Intracerebral Toxicity of Ammonia (NH_3). S. SCHENKER, D. MCCANDLESS, E. BROPHY, AND M. LEWIS, Dallas, Texas (introduced by B. Combes*).

Interference with cerebral energy metabolism due to excess NH_3 has been postulated as a cause of hepatic encephalopathy. Accordingly, the three major sources of intracerebral energy, ATP (luciferin-luciferase), phosphocreatine (PC) (Ennor-Rosenberg assay), and glucose (glucose oxidase), were assayed in cortex and medullopontine area (base) of rats given ammonium acetate (NH_4Ac, 60 mg per 100 g ip), with resultant drowsiness at 5 minutes and subsequent coma lasting at least 30 minutes. ¶ Cortical ATP and PC remained unaltered during coma. By contrast, basilar ATP, initially 1.28 ± 0.15 μmoles per g, was unchanged at 2.5 minutes but fell by 28, 27.3, and 26.2% ($p < 0.001$) at 5, 15, and 30

minutes after NH_4Ac. At comparable times, basilar PC fell more strikingly by 62.2, 96, 77.1, and 71.2% ($p < 0.001$) from control levels, 13.4 ± 5.0 mg per g. These basilar changes could not be induced by anesthesia, excitement, or moderate hypoxia and were not due to increased NH_3 localization in the base. ¶ Other studies indicate: *1)* Basilar respiration and blood flow (estimated indirectly with iodoantipyrine-^{131}I) are normal in NH_3-intoxicated rats; *2)* NH_3 does not uncouple cerebral oxidative phosphorylation; *3)* glucose falls comparably in both cortex and base by 31.3 and 60.4% ($p < 0.01$) from control level of 28.4 mg per 100 ml at 2.5 and 5 minutes after NH_4Ac, whereas blood sugar remains normal. Pretreatment of rats with glucose prevents brain glucose decline but neither NH_3-induced coma nor basilar ATP fall. *4)* Base removed from experimental and control animals has comparable ATPase activity *in vitro*. These data suggest that increased basilar utilization (through NH_3-stimulated metabolic/electrical activity), rather than decreased synthesis, is mainly responsible for the selective basilar depletion of ATP and PC. ¶ Coma, hypernea, rigidity, and "asterixis," features of hepatic encephalopathy, are believed to be caused by malfunction of basilar structures. Accordingly, this demonstration of NH_3-induced selective basilar ATP and PC depletion suggests that impaired basilar energy metabolism may account for many of the findings of hepatic coma.

Splanchnic Lactic Acid Production during Hyperventilation and Shock. JAMES SCHEUER AND MICHAEL N. BERRY, Pittsburgh, Pa. (introduced by Jack D. Myers†).

In view of the great capacity of the liver to metabolize lactic acid, it seems unlikely that sustained hyperlactatemia would occur unless liver function were impaired. To test this hypothesis we anesthetized dogs, and they respired artificially. After a control period, hyperlactatemia was produced by hyperventilation, shock, or bicarbonate infusion. Blood was sampled through catheters placed at the junction of the femoral veins (V), in the aorta (A), and in a hepatic vein (H). Samples were analyzed for pH, P_{CO_2}, P_{O_2}, lactate, pyruvate, and glucose. Hyperventilation with air caused elevated arterial lactate levels (mean = 6.95 mmoles per L), outpouring of lactate into the hepatic vein (mean, H-A = 3.64 mmoles per L), lactate uptake by peripheral tissues (mean, A-V = 0.85 mmole per L), and glucose output into the hepatic vein (H-A up to 7 mmoles per L). During hyperventilation, pH rose initially and then fell. Splanchnic lactate extraction began soon after cessation of hyperventilation, or if the hyperventilation mixture was changed to 5% CO_2 in air. Shock also caused release of lactate into the hepatic vein that was reversed during recovery. During hyperventilation, lactate to pyruvate ratios were elevated in the hepatic vein (range, 35:1 to 290:1), and arterial blood (range, 18:1 to 46:1), and hepatic venous P_{O_2} fell. BSP infusions demonstrated a two- to threefold increase in per cent hepatic extraction during hyperventilation. Bicarbonate infusion (1 mole per L) caused mild hyperlactatemia (3.7 mmoles per L), not associated with splanchnic production of lactic acid. ¶ These experiments suggest that splanchnic production of lactic acid may play a role in the pathogenesis of some types of lactic acidosis. The known similarity of fasting lactate levels in arterial and portal venous blood and the simultaneous output of glucose and lactate into the hepatic vein suggest that the lactic acid was produced by the liver. Increased hepatic glycogenolysis and glycolysis associated with decreased hepatic blood flow and hepatic hypoxia are the most likely factors responsible for the changes observed in splanchnic lactate balance.

Treatment and Study of Chronic Lymphocytic Leukemia (CLL) and Chronic Myelocytic Leukemia (CML) with Extracorporeal Irradiation of the Blood (ECIB). L. M. SCHIFFER, H. ATKINS, A. D. CHANANA, E. P. CRONKITE,† M. L. GREENBERG, D. JOEL, AND P. A. STRYCKMANS, Upton, N. Y.

Normal lymphocytes recirculate through lymph nodes and can be depleted by ECIB. Similar depletion of leukemic cells and tissues might also occur. Five patients with CLL and four with CML were treated with ECIB. Each segment of blood routed past a ^{137}cesium source received 250 to 500 r, 4 to 5 blood volumes being irradiated in 4-hour sessions. ¶ Lymphocytes of all CLL patients decreased after ECIB but at different rates. In one patient, lymphocytes plummeted exponentially from 400,000 to 8,000 per mm^3 within 9 days, a rate of 40% per day. The lymphocytes of three patients fell 5% per day; a fifth fell 1 to 2% per day. Those with the slowest response had been treated with considerable chemotherapy and external radiation. Coincident with the decrease in lymphocytes were diminished lymph node sizes, measured by caliper and lymphangiogram, in four of the five patients. The spleen size by ^{99m}Tc scan became smaller in one patient. After cessation of ECIB, lymph node size increased before any significant rise in blood lymphocytes. ¶ In one patient, the specific activity (cpm per cell) of methionine-^{75}Se-labeled lymphocytes decreased and then rose higher than pre-ECIB levels despite a 96% decrease in total blood lymphocytes. These and other data suggest a significant sequestration and eventual release from lymph nodes of circulating lymphocytes. Other possibilities, such as extremely radioresistant lymphocytes and deutilization of label, seem unlikely under these conditions. ¶ All four patients with CML had greater decreases in immature granulocyte precursors than in morphologically normal granulocytes. Two patients, after initial increase in spleen size, had marked reduction of splenomegaly and clinical improvement. ¶ These preliminary results indicate that ECIB uniformly depletes the peripheral blood, and sometimes tissues, of leukemic cells and may be useful therapy for untreated cases of CLL and CML, reserving chemotherapy, with attendant dangers, for a later time.

The Immunochemical Relationship between Delayed and Immediate Hypersensitivity Reactions. STUART F. SCHLOSSMAN, Boston, Mass. (introduced by Irving H. Goldberg *).

Studies of the immunochemical relationships between delayed and immediate hypersensitivity reactions in guinea pigs sensitized to a chemically defined series of α-DNP, oligo-L-lysyl peptides, have led to the following observations: *1*) Small nonimmunogenic members of this homologous series of peptides elicit only the immediate skin reaction. *2*) Larger members that are immunogenic elicit both immediate and delayed reactions. *3*) Serum antibodies from these animals have permitted studies of quantitative inhibition of precipitation; the peptides show increasing efficiency of inhibition, on a molar basis, with increasing chain length, up to the heptamer. *4*) The upper limit of antibody-combining site size in this system appears complementary to the heptamer. *5*) Although the hexamer contributes approximately 97% of the binding energy of the heptamer with conventional antibody, the heptamer, but not the hexamer, elicits a delayed reaction. To invoke a circulating high affinity antibody as the mediator of the delayed response would require that the seventh lysyl residue of the heptamer supply a critical addition in binding energy. Rather, these results suggest that the combining sites on the antibodies mediating immediate and delayed reactions are probably similar in size, but that the antibody involved in the delayed response has additional unrelated sites used for tissue binding. Furthermore, since only immunogenic peptides provoke the delayed skin reaction in this system, the delayed response may depend on local biosynthesis of sufficient antibody (cell bound or free) to form antigen-antibody complexes capable of producing tissue damage. The delayed response may thus be analogous to a local secondary response wherein immunogenic peptides stimulate sensitized lymphocytes to produce antibody. In contrast, nonimmunogenic peptides, e.g., the hexamer, react only with preformed circulating antibody to provoke an immediate skin response.

Comparison between Responses of Forearm Capacitance and Resistance Vessels during Intra-arterial Infusions of Sympathomimetic Amines. PHILLIP G. SCHMID, JOHN W. ECKSTEIN,* AND FRANÇOIS M. ABBOUD,* Iowa City, Iowa.

Two doses of norepinephrine, metaraminol, and methoxamine were infused into the brachial artery of each of six subjects. Forearm blood flow (FBF) and forearm venous volume at 30 mm Hg distending pressure (VV_{30}) were measured with a water plethysmograph before and during each infusion. The six possible permutations of drug administration were assigned randomly. Drugs were given in doses (norepinephrine, 0.0375 and 0.075; metaraminol, 0.6 and 1.2; methoxamine, 2.4 and 4.8 μg per minute) that, in preliminary experiments, caused approximately equivalent reductions in FBF. FBF averaged 3.7, 2.9, and 2.7 ml per minute per 100 ml forearm during control periods and low and high doses of norepinephrine, respectively. Corresponding values were 3.6, 3.2, and 2.4 for metaraminol and 3.8, 3.6, and 3.3 for methoxamine. Since blood pressure was unaltered, flow changes reflect changes in tone of resistance vessels. A parallel line bioassay revealed the potency of metaraminol, with respect to effect on resistance vessels, to be 0.0738 (95% fiducial limits, 0.0223 to 0.2899) and the potency of methoxamine to be 0.0018 (limits, 0.00005 to 0.0060) that of norepinephrine. VV_{30} averaged 3.8, 3.6, and 3.0 ml per 100 ml forearm during control periods and low and high doses of norepinephrine, respectively. Corresponding values were 3.9, 3.8, and 3.2 for metaraminol and 3.8, 3.7, and 3.4 for methoxamine. At constant distending pressure, changes in venous volume reflect changes in tone of capacitance vessels. Bioassay showed the potency of metaraminol, with respect to effect on capacitance vessels, to be 0.0362 (limits, 0.0194 to 0.0609) and the potency of methoxamine to be 0.0072 (limits, 0.0037 to 0.0122) that of norepinephrine. The order of potency, with respect to both resistance and capacitance vessels, was norepinephrine, metaraminol, and methoxamine. When these drugs were infused in doses that produced equivalent effects on resistance vessels, they also produced equivalent effects on capacitance vessels.

Separation of Human Adipose Tissue Neutral and Alkaline Lipolytic Activities. J. DAVID SCHNATZ AND JEAN A. CORTNER, Buffalo, N. Y. (introduced by Evan Calkins *).

The importance of lipolysis in the metabolism of triglycerides prompted investigation of lipolytic activity in human adipose tissue. Our initial studies had demonstrated the presence of two lipolytic activities other than lipoprotein lipase. The present paper describes separation of these neutral and alkaline lipolytic activities (NLA and ALA). ¶ Cell-free preparations representing 3 to 10 g of tissue were subjected to $(NH_4)_2SO_4$ precipitation at 4° C. Fifty per cent saturation produced a precipitate, which contained 65% of original NLA and 14% of original ALA. The supernatant contained 14% of NLA and 53% of ALA. ¶ The equivalent of 25 g of tissue was subjected to gel filtration by elution with 0.008 M phosphate-citrate buffer, pH 7.0, from eight columns containing 10 g of Sephadex G-200 and measuring 2.5 × 55 to 60 cm. The maximal concentration in a 5-ml fraction occurred at the following average elution volumes: NLA, 105 ml; ALA, two peaks at 140 and 180 ml; and protein, two peaks at 105 and 180 ml. For comparison, dextran blue, gamma globulin, albumin, and hemoglobin were placed on the column separately and eluted. Peak concentrations occurred at an average of 95, 135, 165, and 200 ml, respectively. ¶ In addition to having different elution volumes, the NLA and ALA recovered from Sephadex gel filtration differed in pH optima, inhibition by 0.01 M NaF, and electrophoretic migration of lipolytic activity on starch gel. Based on elution volumes, NLA is associated with a molecule that is larger than gamma globulin and ALA with two molecules of smaller size. ¶ Separa-

tion of these activities should prove valuable in exploring their physiological roles, which are presumed to be related to lipolysis.

Bile Acids and Cholesterol in the Bile of Guinea Pigs with Induced Gallstones. LESLIE J. SCHOENFIELD AND JAN SJOVALL, Rochester, Minn., and Stockholm, Sweden (introduced by Hugh R. Butt †).

Since cholesterol is held in solution in bile primarily by the bile salts, a lowering of the bile acid to cholesterol ratio might promote cholesterol gallstone formation. Cholestyramine, an anion exchange resin, interferes with the reabsorption of bile acids and might therefore, by removing bile acids from the pool, create a lithogenic biliary milieu. ¶ In two series of experiments (I and II), 86% of 28 guinea pigs that were fed 1% cholestyramine for 1 month developed gallstones comprised of about 50% cholesterol. Concrements were not found among control animals fed the same diet without added cholestyramine. The animals in both series lost weight. In two subsequent series (III and IV), a diet with hay supplement was given, weight gain was normal, and gallstones did not form. The gallbladder biles were analyzed by gas-liquid chromatography for bile acids and cholesterol. ¶ In all four series, in animals fed cholestyramine the bile acid concentration was 34 to 55% lower than in the respective control animals ($p < 0.01$). A lowered bile acid concentration, a high cholesterol concentration (about 10 times higher in series I and II than in series III and IV), and a low ratio of chenodeoxycholic acid to 7-ketolithocholic acid (CDC/7 − KL = 0.7) were associated with gallstone formation. The dietary cause of the high mean cholesterol concentration (0.20 to 0.28 mg per ml) among the groups in the first two series of experiments was not established. Hay added to the diet resulted in a lower cecal pH and favored the preponderance in bile and intestinal contents of chenodeoxycholic acid over 7-ketolithocholic acid (CDC/7 − KL = 3.0 to 4.6). There were no differences among the groups in the cholesterol contents of the livers (mean, 2.37 mg per g fresh tissue ± 0.56 SD) and no inflammation of the gallbladders. The findings provide a mechanistic explanation for cholesterol gallstone formation in these experiments.

Studies of Extracellular and Tissue Fatty Acid Pools and Glucose Metabolism in Striated Muscle. GUSTAV SCHONFELD AND DAVID M. KIPNIS,* St. Louis, Mo.

To clarify the metabolic interactions of glucose and fatty acids in striated muscle, we have examined extracellular and tissue FFA pool relationships *in vivo* and *in vitro* in the rat diaphragm and correlated them with concomitant measurements of sugar transport and phosphorylation and insulin responsiveness. ¶ Acute elevations of plasma FFA (1,250 to 1,525 μEq per L) after injection of anti-insulin serum did not affect phosphorylating capacity or tissue FFA (120 ± 20 mμEq per g). At comparable plasma FFA (1,200 to 1,650 μEq per L) in 48-hour alloxan diabetic rats, phosphorylating capacity was depressed, and tissue FA increased (340 ± 36 mμEq per g). In these animals, insulin rapidly reduced the plasma FFA but did not immediately restore to normal the phosphorylating capacity or tissue FFA pool. Starvation (4 days) preduced an initial rise and then fall in plasma FFA, but impaired phosphorylating capacity and increased tissue FFA (310 ± 18 mμEq per g) persisted. ¶ 2-Deoxyglucose transport and phosphorylation in the rat diaphragm in the presence and absence of insulin were not affected by added palmitate, < 30 to 2,500 μEq per L (FA to alb molar ratio = 0.5 to 7.0). The tissue FFA pool (190 ± 15 mμEq per g) was not increased by 90 minutes incubation in added palmitate, 250 to 1,200 μEq per L. At these concentrations, palmitate-1-^{14}C distribution between tissue and medium FFA rapidly attained a steady state with specific activity ratios of 0.03 to 0.17 at low and high external [palmitate]. Palmitate-1-^{14}C esterification rates were linear throughout incubation and increased as the external [palmitate] increased. ¶ These data indicate that *1)* the tissue FFA pool is not significantly influenced by the external FFA level or FFA/albumin molar ratio, *2)* the tissue FFA pool is functionally heterogeneous, and *3)* changes in glucose metabolism and insulin responsiveness are not causally related to the external FFA level but correlate well with the tissue FFA pool size.

Variable Mechanisms of Phosphate Transfer across Erythrocyte Membranes. STANLEY L. SCHRIER, LYDIE DOAK, AND BETSY CHAPMAN, Palo Alto, Calif. (introduced by David A. Rytand †).

The transfer of inorganic phosphate (Pi) into erythrocytes has been reported to occur via a variety of mechanisms. Therefore, Pi transfer was systematically studied using erythrocyte membranes, which are essentially free of hemoglobin, intracellular enzymes, and substrates, and which retain membrane-bound enzymes and carriers. Membranes into which known amounts of substrates were introduced by hemolysis reversal were incubated at 37° C in isotonic solutions containing varying amounts of isotopically labeled Pi. After 5 minutes, membranes were separated, and Pi was determined chemically and isotopically in trichloroacetic acid filtrates. ¶ At Pi concentrations from 0.05 to 2.00 mmoles per L, Pi uptake was greater into membranes containing triose phosphate, ADP, and DPN than membranes containing uridine diphosphate instead of ADP. From 4 to 20 mM Pi there is a linear relationship between Pi uptake and Pi concentration. Pi uptake from 30 to 60 mM Pi shows saturation, and the points form a straight line when plotted in the manner of Lineweaver and Burke. From 60 to 85 mM Pi, there is a surprising increase in Pi uptake as well as accelerated loss of phosphorylated substrates from membranes. ¶ We interpret the data as showing that there are several concentration dependent mechanisms of Pi transfer across erythrocyte membranes. At lowest levels of Pi there may be substrate dependent transport, since membranes containing appropriate sub-

strates took up more Pi than did controls. The linear phase of Pi transfer is compatible either with simple diffusion or a carrier mechanism that is far from saturation, whereas the saturable phase of transfer conforms to carrier-mediated transport. Finally, the accelerated transfer is probably due to a Pi-induced membrane alteration, since there were excessive exchanges in both directions.

Paradoxical Effects of Starvation and Cortisone Administration on Free Thyroxine Concentration and Thyroxine Metabolism. GEORGE C. SCHUSSLER, Buffalo, N. Y. (introduced by David K. Miller †).

The low PBI of euthyroid sick patients has been associated with a decreased concentration of thyroxine-binding proteins and an apparently compensatory increase in the ratio of free to bound thyroxine so that serum free thyroxine concentration is normal. The relationship between free thyroxine concentration and thyroxine turnover was investigated in starved rats. The ratio of free to bound thyroxine (FTF) was determined by ultrafiltration. Fractional degradation of thyroxine (K) was determined by following the disappearance of thyroxine-^{131}I from the blood. The starved animals manifested a decreased metabolic rate, decreased PBI (60% control), and an increased FTF (160%). The latter changes were compensatory, so that the product PBI × FTF was unchanged. Therefore, barring an alteration in the proportion of thyroxine iodine in the PBI, free thyroxine concentration was normal in these animals. Despite the increased FTF, K was diminished (36%). Whole body counting of rats and the determination of activity in excreta eliminated the possibility that the slowed disappearance of thyroxine-^{131}I from the blood resulted from a continuous contraction of the thyroxine distribution space. Since the starved animals had decreased oxygen consumption and presumably decreased protein catabolism, it was of interest to determine the effect of a protein catabolic agent. In contrast to the effect of starvation, cortisone administration increased FTF slightly (117%) while increasing K (135%). These findings do not support the hypothesis that free thyroxine concentration regulates the metabolism of extrathyroidal thyroxine or that free thyroxine concentration is an index of thyroxine turnover. The results are consistent with a direct relation between thyroxine turnover and the catabolism of carrier protein, with secondary effects on FTF.

Comparative Studies of Acquired Inactivators of Factor VIII (Antihemophilic Globulin). SANDOR S. SHAPIRO, Philadelphia, Pa. (introduced by Allan J. Erslev †).

Inactivators of Factor VIII are recognized to occur in some persons with classical hemophilia, in occasional postpartum females, in association with drug reactions or connective tissue-related diseases, as well as in otherwise normal individuals. The nature of such inhibitors is still uncertain. Do they possess a common pathogenesis and mode of action? To answer this question we undertook detailed physicochemical and kinetic studies of three severe inhibitors, one from each of the first three clinical categories (hemophilia A, postpartum female, ulcerative colitis). ¶ As determined by salt fractionation and Sephadex G-200 filtration, all three inhibitors have molecular weights in the range 150,000 to 200,000. On starch block electrophoresis two possess gamma mobility, whereas the third (from the patient with ulcerative colitis) was clearly separable from the bulk of the gamma globulin and migrated in the beta range. Kinetic studies clearly indicated stoichiometric inactivation of Factor VIII by all three inhibitors and were inconsistent with an enzymatic mechanism of action. Over a wide range of Factor VIII concentrations, both the initial rate of inactivation and the equilibrium Factor VIII concentration were proportional to the concentration of inhibitor. Temperature dependence of these reactions permitted calculation of Arrhenius energies, and equilibrium measurements allowed an assessment of the avidity of the inhibitors for Factor VIII. The most avid inhibitor was from the patient with ulcerative colitis ($E_a = 1{,}600$ calories per mole), the least avid from the postpartum female ($E_a = 9{,}600$ calories per mole). ¶ Thus, it appears likely that acquired Factor VIII inhibitors form a heterogeneous group of stoichiometric inactivitors, possessing widely varying avidity for Factor VIII, and differing as well in molecular charge.

The Relative Importance of Calcium and Phosphate in the Secretion of Parathyroid Hormone.. LOUIS M. SHERWOOD, G. P. MAYER, CHARLES F. RAMBERG, JR., DAVID S. KRONFELD, JOHN T. POTTS, JR., AND G. D. AURBACH,* Bethesda, Md., and Kennett Square, Pa.

We have previously reported that the concentration of parathyroid hormone (PTH) in bovine plasma measured by a sensitive radioimmunoassay (method of Berson and co-workers) varied inversely with changes in plasma calcium. The present studies define the rapidity and extent of physiological changes in hormone concentration, determine the endogenous and exogenous half-life of PTH in plasma, and evaluate the relative importance of calcium and phosphate in regulating hormone secretion. In nine cows that received infusions of EDTA, as well as in animals with spontaneous hypocalcemia, concentrations of PTH increased rapidly to three to eight times the normal value of 0.4 to 1.8 mμg per ml. Hormone concentration increased within minutes after the fall in plasma calcium and varied directly with the degree of hypocalcemia. Simultaneous administration of calcium and EDTA completely prevented the stimulation noted with EDTA alone. When animals with high concentrations of hormone due to spontaneous or EDTA-induced hypocalcemia were infused with calcium, PTH disappeared rapidly from plasma, with a half-life that varied from 10 to 20 minutes. This compared favorably with a half-life of 18 minutes measured after the intravenous

administration of unlabeled bovine PTH. Infusions of phosphate were of interest in terms of the view that high phosphate is the primary stimulus to parathyroid hyperplasia in chronic renal disease. When sodium phosphate was given intravenously, hormone concentration increased only during the hypocalcemia that followed the rapid rise in plasma phosphate. When calcium was maintained in the normal range by a supplemental infusion of calcium, no increase in hormone concentration was noted. These studies show that blood calcium is the primary determinant of PTH secretion, that the half-life of hormone in plasma is short, and that phosphate has no direct effect on hormone secretion, only secondarily stimulating PTH by lowering plasma calcium.

Deletion of the Cholesterol Negative Feedback System in Precancerous Liver. MARVIN D. SIPERSTEIN,* Dallas, Texas.

Previous studies have demonstrated consistent loss of the cholesterol feedback system in one spontaneous mouse hepatoma, two human hepatomas, and 12 induced minimal deviation rat hepatomas. Since this lesion is the sole example of feedback deletion in cancer tissue, experiments were designed to determine whether loss of the cholesterol feedback system may actually precede the development of spontaneous and induced liver tumors. Mouse strains $A^{y}a$ and aa with high hepatoma incidence and BALB/C, C-57, and Cb × Db with low hepatoma incidence were examined for feedback activity. The precancerous livers of both $A^{y}a$ and aa strains showed no detectable cholesterol feedback system—normal diet, 0.800 mμmole acetate-2-^{14}C converted to cholesterol per hour; high cholesterol diet, 0.768 mμmole per hour. Low hepatoma strains showed, with normal diet, 0.329 mμmole per hour; with high cholesterol diet, 0.014 mμmole per hour. ¶ To determine whether deletion of the cholesterol feedback system represents a primary event in the development of induced hepatomas, we examined feedback control in 3-year-old rainbow trout fed aflatoxin, 10 parts per billion, since birth. Eighty to 100% of rainbow trout develop hepatomas on this dose of aflatoxin. Cholesterol feedback is consistently deleted in such precancerous livers; cholesterol synthesis with normal diet is 30.5 mμmoles per hour, with cholesterol diet, 57.5 mμmoles per hour. To determine the time required for aflatoxin to delete feedback control, 60 μg of aflatoxin was administered ip to ten rainbow trout and cholesterol feedback studied 5 days later. In contrast to normal trout, feedback control was absent in all treated fish. Finally, rats fed 10 parts per million of aflatoxin for only 2 days showed deletion of feedback regulation by the 15th day. ¶ We concluded that deletion of the cholesterol feedback system has been demonstrated in precancerous livers preceding both spontaneous and induced hepatoma formation. The consistent absence of this feedback control suggests that this lesion may represent an early and perhaps primary event in the development of hepatomas.

Hemorrhage in Normal Man: Effect on Renin, Aldosterone, and Cortisol. J. J. SKILLMAN, D. P. LAULER, R. B. HICKLER, J. H. LYONS, J. E. OLSON, AND F. D. MOORE,† Boston, Mass.

Hemorrhage without other injury is a stimulus to the secretion of hormones of renal and adrenal origin. The present investigations correlate hemodynamic changes with the secretion of renin, cortisol, and aldosterone after hemorrhage in normal man. ¶ Eleven healthy young male volunteers on a constant intake of sodium, potassium, fluid, and calories were studied during a 7-day hospitalization. Intra-arterial blood pressure, cardiac output (dye-dilution technique), and central venous pressure were measured. The average hemorrhage was 15% of the blood volume. Plasma cortisol, renin, and aldosterone were measured at successive hemorrhage levels of 250, 500, and 768 to 810 ml, and remeasured 24 hours later. Twenty-four–hour aldosterone secretory rate (ASR) was measured by isotope dilution before, during, and after hemorrhage. ¶ Transcapillary refilling of the blood averaged 105% at 24 hours. Plasma renin and aldosterone generally rose as the volume of hemorrhage increased. In all but one instance, ASR increased significantly with hemorrhage, a response persisting for 36 hours, as shown by 12-hour urinary aldosterone excretion. Plasma cortisol levels peaked at maximal hemorrhage volume and fell significantly the day after hemorrhage. Arterial, venous, slow, and rapid hemorrhage all provoked different endocrine responses.

***In Vivo* Measurement of Bone Mineral.** DAVID M. SMITH, C. CONRAD JOHNSTON, JR., AND WILLIAM P. DEISS, JR.,* Indianapolis, Ind.

An accurate reproducible method for meauring bone mass *in vivo* would be of considerable clinical value. Previous methods have depended largely upon X ray. Cameron and Sorenson described an improved method of measuring bone mass that is dependent upon absorption of a monochromatic beam of gamma radiation from a ^{125}I source. This technique has been modified to improve accuracy and speed. Continuous scans across the radius are done with a motor-driven apparatus. A scan can be completed in approximately 30 seconds, and the scan can be repeated over different areas of bone (i.e., representative cortical bone at 8 cm and cancellous bone at 3 cm). The decrease in scan time has reduced the problem of patient movement and has allowed a more reproducible value for bone mass to be obtained (coefficient of variation previously 4%, now 2%). The data are accumulated on a multichannel analyzer and punched onto paper tape for later computer analysis. A mean width of the radius for the area scanned and a mean bone mass are calculated. ¶ A number of "normal" people are being surveyed by this technique, and the data suggest that *1*) there is a decreased bone mass with age, *2*) the width of the bone correlates well with bone mass ($r = 0.8$), *3*) Negroes tended to have greater bone mass than Caucasians with similar sized bones, and *4*) there is no sig-

nificant difference in the mass of bone in the right vs. the left arm. When the normal population is compared to patients with clinical osteoporosis, the patients tend to have lower bone mass, particularly at the 3-cm scan distance. The technique is being utilized to study any change produced by sodium fluoride therapy of osteoporosis. ¶ Another advantage of this technique is that values obtained by different investigators would be comparable when a common standard is used.

Comparison of the Opsonocytophagic Activity of Mercaptoethanol Sensitive and Resistant Anti-Proteus Globulins. JAMES W. SMITH, JACK A. BARNETT, ROBERT P. MAY, AND JAY P. SANFORD,* Dallas, Texas.

Stimulation with various antigens has been demonstrated to result in the sequential formation of physicochemically distinct immunoglobulins IgM (19 S or 2-mercaptoethanol sensitive) and IgG (7S or 2-ME resistant). Although opsonins in normal and immune sera have been extensively investigated, the opsonocytophagic capabilities of the specific classes of immunoglobulins have not been elucidated. ¶ A phagocytosis system using polymorphonuclear leukocytes from rabbit peritoneal exudate was utilized to compare 19 S and 7 S antibodies as opsonins. Rabbits immunized with varying strains of heat-killed *Proteus mirabilis* were bled at 5 days for 2-ME sensitive anti-proteus antibody (exclusively 19 S by ultracentrifugation) and at 4 weeks for serum that contained 2-ME resistant antibody (predominantly 7 S). Dilutions of heated antisera were added to mixtures containing leukocytes and bacteria at a 10:1 ratio and incubated in a roller drum apparatus. After lysis of leukocytes, quantitative bacterial counts were performed. Decrease in counts from controls represented phagocytosis with intracellular killing. ¶ Sera that had equivalent agglutination titers were compared at varying concentrations, and in every instance 7 S antisera enhanced phagocytosis to a greater extent than did 19 S antisera. In four experiments the average $\log^{10}$ decrease in organisms between experimental and control systems at equivalence (1.0) was 2.1 for 7 S and 0.9 for 19 S. At higher dilutions, e.g., 0.4 of equivalence, the decrease was 1.8 for 7 S and 0.8 for 19 S; at 0.1, 1.4 vs. 0.6; and at 0.04, 1.2 vs. 0.5. In no experiment did 19 S antiserum achieve $> 99\%$ phagocytosis (log decrease of ≥ 2.0), whereas 7 S antiserum accomplished this frequently and at relatively low antibody titers. Opsonization activity could be specifically absorbed. ¶ These experiments demonstrate that antibody (19 S) appearing early in response to infection or stimulation with *Proteus mirabilis* antigen is less effective as an opsonin than is antibody (7 S) that appears later.

Lysosomal Enzymes in Rat Epidermis. J. GRAHAM SMITH, JR., HAROLD J. YARDLEY, THEODORE ROSETT, AND MORDECAI J. MOORE, Durham, N. C. (introduced by William M. Nicholson †).

Rat epidermis obtained by stretch separation was suspended in 0.25 M sucrose and ground in a Rosett homogenizer. Rat liver was treated similarly. Residues from centrifugations of 1×10^4, 3.3×10^4, 2.5×10^5, and 3×10^6 *g*-minutes were obtained. A portion of each residue was resuspended in sucrose or labilizer, and the mixtures were recentrifuged. The following supernates were assayed for acid phosphatase: β-D-glucuronidase, α-D-glucosidase, α-D-galactosidase, α-D-mannosidase, and N-acetyl-β-D-glucosaminadase. The supernates from the original 3×10^6 *g*-minute centrifugations were assayed similarly. ¶ Triton X-100 (TX100), dimethyl sulfoxide (DMSO), and freeze-thawing were used to labilize liver and epidermal lysosomes. The most effective labilizer for liver and epidermis was 1% TX100, which produced marked increases of activity in the residues; 0.1% TX100 was less effective, whereas 0.01% TX100 did not produce any detectable increase in enzyme activity. Ten per cent DMSO increased liver enzyme activity approximately as well as 0.1% TX100 but had no effect on epidermis. Freeze-thawing ten times increased liver but not epidermal enzyme activity. Therefore, 1% TX100 was used in all subsequent experiments. ¶ Most of the enzyme activity in liver was found in the 3.3×10^4 and 2.5×10^5 *g*-minute residues. In epidermis, however, most of the enzyme activity remained in the 3×10^6 *g*-minute supernate, with the sedimentable activity nearly evenly distributed between the 3.3×10^4, 2.5×10^5, and 3×10^6 *g*-minute residues. Negligible amounts of activity were present in the 1×10^4 *g*-minute residues. The distribution of activity among the residues was the same for all the enzymes studied.

Interactions between Haptoglobin and Isolated α and β Subunits of Human Hemoglobin. MARTIN J. SMITH AND WILLIAM S. BECK,* Boston, Mass.

The nature of the interaction between haptoglobin and hemoglobin is unknown. Previous work has tentatively suggested that to be bound by haptoglobin hemoglobin must contain α-chains plus one other chain species. Such results, if confirmed, would indicate that a specific conformation of the hemoglobin molecule arising from an interaction between its unlike chains is essential for the binding of hemoglobin by haptoglobin. The recent description by Bucci and Fronticelli of a new method for isolating α and β subunits of human hemoglobin in high yield and in native state presented the opportunity of performing definitive experiments to determine whether pure α and β subunits, consisting of heme groups linked to polypeptide chains, can be independently bound by haptoglobin. ¶ In experiments in which increased peroxidase activity was employed as the index of binding, addition of serum containing haptoglobin to isolated α and β subunits caused peroxidase activity to increase to approximately ten times that observed in the absence of serum. These indications of interaction between haptoglobin and each type of isolated subunit were confirmed by gel filtration studies. Isolated α and β subunits were retained by Sephadex G-100 when applied alone or in a mixture

with serum containing no haptoglobin. However, when mixed with serum containing haptoglobin, the subunits were rejected. We concluded that α and β subunits are bound by haptoglobin. ¶ The foregoing experiments considered the interaction of haptoglobin and single subunit species. When haptoglobin-containing serum was added to a mixture of α and β subunits, peroxidase activity rose to approximately 30 times the activity of the subunit mixture in the absence of serum, a rise three times that observed when subunits interacted singly with haptoglobin. In addition, the peroxidase activity of a mixture of α and β subunits (in the absence of haptoglobin) exceeded the activity that would have been predicted from summation of the activities of isolated subunits. These results indicate the occurrence of an interaction between α- and β-chains and an interaction between an α-β aggregate and haptoglobin. ¶ The peroxidase activity of hemoglobin is generally attributed solely to its heme moiety. However, the present results suggest that interactions between unlike polypeptide chains of globin enhance the peroxidase activity of the heme. The increase is presumably the result of conformational changes associated with aggregation.

The Effect of Diet on the Plasma Aminogram. SELMA E. SNYDERMAN, PATRICIA M. NORTON, ELLEN ROITMAN, AND L. EMMETT HOLT, JR.,† New York, N. Y.

The free amino acids of the plasma (plasma aminogram) studied under fasting conditions respond rapidly to alterations in the protein intake and the amino acid pattern of the diet. In all countries studied, patients with protein deficiency (kwashiorkor) showed a characteristic pattern with a decreased concentration of the essential amino acids most marked in the branched chain acids and least striking in phenylalanine and lysine, and a striking reduction of four unessential amino acids (tyrosine, arginine, citrulline, and butyrine), the other unessential amino acids being resistant to a decline. These changes cannot be interpreted as resulting from a limited intake of some essential amino acid. Experimental studies in which single essential amino acids have been deficient give characteristic but quite different patterns, which are determined by amino acid imbalances. The limiting factor in kwashiorkor diets appears to be nitrogen rather than an essential amino acid. It can be closely simulated by experimental diets low in good quality protein. ¶ When high protein diets are fed experimentally, the fasting aminogram also differs from what has been regarded as normal. Certain amino acids are elevated, notably methionine and proline. These effects are more striking when whole proteins rather than amino acid mixtures are fed. Excesses of single amino acids also produce their characteristic aminograms, occasioned apparently by phenomena of imbalance. The implications of these findings on the supplementation of human dietaries will be discussed.

Suppression of the Shunt Pathway in Primary Gout by Azathioprine. LEIF B. SORENSEN, Chicago, Ill. (introduced by Attallah Kappas *).

An increasing volume of studies lends support for a dual etiology of hyperuricemia in primary gout. In this disorder there may be either normal or excessive production of uric acid. Patients with primary gout related to overproduction of uric acid show a pattern of incorporation of glycine-^{14}C into urinary uric acid indicating the presence of a shunt pathway. ¶ When azathioprine was given to gout patients who displayed excessive excretion of uric acid, plasma and urinary uric acid fell to normal. To evaluate this finding more precisely, the incorporation of glycine-^{14}C into uric acid during treatment with azathioprine was studied in three patients who had previously been shown to possess the shunt pathway. Azathioprine was administered for 7 to 10 days in a daily dose of 3.4 to 4.6 mg per kg body weight. On the second or third day of treatment, glycine-^{14}C was injected intravenously. A striking conversion of the incorporation pattern to normal was observed in all three patients. The cumulative recovery of glycine-^{14}C in urinary uric acid during the 7-day control study was 0.41, 0.52, and 0.51% of the dose. During the azathioprine study these values fell to 0.09, 0.28, and 0.10% of the dose, respectively—values comparable to those obtained in normal subjects. No significant changes in urate metabolism were observed when azathioprine was given to two normal individuals and to a gouty subject whose hyperuricemia was related to a dysfunction in renal tubular transport of urate. ¶ Thus, for the first time, abolishment of the shunt pathway in primary gout has been demonstrated. The mechanism of action of azathioprine upon purine biosynthesis is not clear, but is currently under investigation.

Plasma Production Rates of Testosterone in Normal Men and Women; a Physiological Study Using Both a Single Injection and Constant Infusion Technique. A. LOUIS SOUTHREN, GARY G. GORDON, AND SATORU TOCHIMOTO, New York, N. Y. (introduced by Louis J. Soffer †).

Urinary testosterone glucuronoside was shown not to be a unique metabolite of plasma testosterone. As a consequence, testosterone production rates measured by dilution of labeled testosterone in urinary testosterone glucuronoside probably overestimated testosterone production rates to some extent. In the present investigation, we calculated the production rate (PR) of testosterone from the product of the metabolic clearance rate (MCR) of testosterone in plasma by using both single injection and constant infusion of the labeled steroid and the mean nonisotopic plasma concentration of the hormone. This method has the advantage of not requiring the presence of a hypothetical unique metabolite. A distinct diurnal variation of plasma testosterone was found in the male subjects, with maximal concentration in the morning. There were no significant differences

found between the estimates of the MCR of testosterone obtained by single injection or constant infusion. There was, however, a distinct sex difference in the plasma clearance of testosterone; the male values were approximately twice the female levels. Among the factors affecting the MCR of testosterone were body position and plasma concentration of testosterone. The calculated plasma production rates of testosterone were 8.1 mg per day, SD ± 1.8 (single injection) and 7.2 mg per day, SD ± 2.7 (constant infusion) in normal men, and 0.45 mg per day, SD ± 0.06 (single injection) and 0.41 mg per day, SD ± 0.11 (constant infusion) in normal women. The male values compared reasonably well with those reported for the urinary metabolite method, whereas the female values were considerably less than those obtained by this procedure. These data indicate that women, in contrast to men, synthesize a significantly greater proportion of their total testosterone from precursors in a separate anatomical compartment and conjugate it before it enters the general circulation. The plasma method for measuring testosterone production rates would thus appear to offer a more accurate estimate of testosterone production than that obtained by the urinary technique.

Studies of Plasma Lipoproteins as Cholesterol and Phospholipid Are Lowered by Substitution of Unsaturated for Saturated Dietary Fat. NORTON SPRITZ, New York, N. Y. (introduced by George W. Frimpter *).

To define further the mechanism of the lipid-lowering effect of unsaturated fat, the lipid and protein contents and fatty acid composition of fasting lipoproteins were determined in humans during feeding with a formula containing saturated fat (40% coconut oil) and while cholesterol and phospholipid were lowered by isocaloric unsaturated fat (corn oil). This lowering occurred in both high (HDL) and low density lipoproteins (LDL) isolated ultracentrifugally or by precipitation. Lipid fall in LDL averaged 18.2% and in HDL 17.4% ($p < 0.05$), with insignificant change in protein resulting in lipid to protein ratios during unsaturated intake averaging 0.8 that during saturated intake. This suggests that the hypolipemic effect results, at least in part, from decreased lipid per lipoprotein macromolecule rather than decrease in their number. After substitution of unsaturated for saturated diet, linoleate content of each lipid class rose to equivalent degree in all lipoproteins (triglyceride from 9.1 to 33.2%, 6.4 to 52.8, and 8.3 to 29.8% in three subjects; cholesterol ester from 43.6 to 56.8, 41.7 to 71.7, and 44.2 to 69.8%; and phospholipid from 15.9 to 23.2, 18.9 to 29.9, and 18.4 to 32.1%). These maximal composition changes required 8 to 10 days, the time required for maximal decrease of cholesterol and phospholipid content. ¶ In summary, lowering of plasma lipid by unsaturated fat feeding is a phenomenon of all circulating lipoproteins, is associated with falling lipid to protein ratio, and occurs simultaneously with the change in esterified fatty acid content induced by diet. These findings permit the hypothesis that the lipid lowering reflects diminished cholesterol- and phospholipid-carrying capacity of lipoproteins as their lipids incorporate unsaturated acids. This could be due to irregular molecular configuration of unsaturates with less efficient "packing," a concept consistent with the hypolipidemic effect, shown by others, of more irregularly shaped cis compared to isomeric trans dietary fatty acids.

Responsiveness of Lymphoid Cells to Phytohemagglutinin; Unresponsiveness of Thymus Cells. PETER STASTNY AND MORRIS ZIFF,† Dallas, Texas.

In studying the *in vitro* proliferative response of rabbit lymphocytes, as measured by tritiated thymidine incorporation into DNA, we observed that cells from the rabbit thymus did not respond to stimuli that induced proliferation of other types of lymphoid cells. ¶ The addition of phytohemagglutinin or streptolysin S to cultures of rabbit spleen, lymph node, or peripheral leukocytes resulted in a 3- to 60-fold increase in thymidine-^{3}H incorporation. In rabbits immunized with hemocyanin, bovine serum albumin, or sheep erythrocytes, a similar response was obtained after addition of specific antigen. *In vitro* stimulation with phytohemagglutinin of spleen cells from spleen, lymph node, or peripheral leukocytes resulted in the development of specific hemolytic antibody for sheep RBC, not reacting with human RBC. ¶ When cells from the thymus were tested under similar circumstances, either no increase or small increases in incorporation were detected after addition of phytohemagglutinin, streptolysin S, or specific antigens, and hemolytic plaque-forming cells did not develop. Such observations were made with thymus cells from rabbits ranging from 2 weeks to 6 months of age. The suspensions of thymus cells appeared viable on the basis of *1*) their morphologic appearance, *2*) their ability to exclude trypan blue, and *3*) a baseline value of incorporation of thymidine-^{3}H that was similar to that of the other types of lymphoid cells and was reduced to zero by freezing and thawing or heating of the thymus cell suspensions. ¶ Although a lack of response to antigen in thymus cells may be a consequence of an absence of immunological memory because of exclusion of antigen from the thymus, the failure to respond significantly to phytohemagglutinin and streptolysin S suggests a difference in cellular mechanism between thymus and other lymphoid cells, since prior exposure is presumably not required for the development of a response to phytohemagglutinin or streptolysin S.

On the Metabolic Error in Refsum's Disease. DANIEL STEINBERG,* CHARLES MIZE, JOEL AVIGAN, HENRY M. FALES, LORENTZ ELDJARN, KENNETH TRY, ODDVAR STOKKE, AND SIGVALD REFSUM, Bethesda, Md., and Oslo, Norway.

Patients with Refsum's disease, an inherited autosomal recessive disease that affects primarily the nervous system, accumulate large amounts of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) in serum and tissues. The present studies show in a patient with Refsum's disease

that after iv injection of mevalonate-2-^{14}C, plasma phytanate contained only traces of ^{14}C. Incorporation into plasma cholesterol was normal. Attempts to demonstrate mevalonate incorporation into phytanate in rats were negative. The patient was given D_2O for 10 weeks, maintaining body water at 0.5 atoms % excess. Incorporation of deuterium, measured at 2-week intervals, was near the limits detectable by mass spectroscopy and showed no tendency to increase with time. The marginal incorporation found indicated at most replacement of 2 of 40 hydrogen atoms and virtually rules out *de novo* biosynthesis. Oral tracer doses of phytol-U-^{14}C were well absorbed by six control subjects (66 to 98%) and two subjects with Refsum's disease (74 and 80%). In both groups, phytanate-^{14}C was demonstrated in the plasma, reaching a peak at 3 to 6 hours. In control subjects, however, plasma phytanate-^{14}C virtually disappeared by 24 hours, whereas in the patients there was a secondary rise, and significant plasma phytanate-^{14}C levels persisted for over 90 days. Five of the six controls converted 17 to 29% of the absorbed dose to $^{14}CO_2$ in 12 hours, whereas the two patients converted only 3.5 and 5.8%, respectively. The sixth control showed the poorest absorption and converted only 3.2% to $^{14}CO_2$; her plasma radioactivity curve, however, was like that of the other controls. The present results indicate that the phytanate accumulating in Refsum's disease is primarily of exogenous origin, and there is a relative block in the degradation of phytanic acid.

Characteristics of Hydrogen Ion Transport in Urinary Bladder of the Turtle. PHILIP R. STEINMETZ AND HOWARD S. FRAZIER,* New York, N. Y., and Boston, Mass.

Urine acidification in the fresh water turtle is a function of both kidney and bladder. Hydrogen ion transport by the bladder was examined in an *in vitro* system permitting control of the electrical and concentration-driving forces for H^+ movement. By means of a pH stat technique, H^+ secretion rate was measured on the mucosal side (M) of the bladder suspended in a Lucite chamber between identical bicarbonate-free Ringer solutions buffered with 0.3 mM Na_2HPO_4. In 11 experiments H^+ secretion rate averaged 1.14 ± 0.09 (SEM) μmoles per hour per 8 cm^2 in the presence of the spontaneous electrical PD (M negative with respect to serosal side, S) and 0.96 ± 0.07 when the PD was nullified by an external current. The bladder was able to generate a H^+ gradient of 3.0 pH U in the "short-circuited" state. Acidification of M was associated with alkalinization of S without significant change in the buffer capacity of the solutions. H^+ secretion did not require exogenous HCO_3^- or CO_2 but was markedly reduced in 0.2 to 0.5 mM acetazolamide and was abolished when the solutions were deoxygenated with N_2, indicating dependence on endogenous CO_2 production. Since H^+ transport occurred in the absence of electrical and concentration-driving forces, the possibility of coupling to active transport of other ion species was examined. H^+ secretion was not abolished when Na^+ or Cl^- was replaced by Cs^+ or SO_4^- in M and S, or when K^+ was removed from M. ¶ These data indicate that H^+ transport in the turtle bladder is an "active" process coupled to cellular metabolism and dependent on endogenous CO_2 as a source of H^+. Electrical driving forces generated by the active transport of other ion species play a quantitatively small role in H^+ transport.

Intestinal Lymphangiectasia: A Protein-losing Enteropathy with Hypogammaglobulinemia Lymphocytopenia and Impaired Homograft Rejection. WARREN STROBER, R. DEAN WOCHNER, PAUL P. CARBONE, AND THOMAS A. WALDMANN,* Bethesda, Md.

Intestinal lymphangiectasia (IL) is a disease characterized by dilated intestinal lymphatics, protein-losing enteropathy, hypoalbuminemia, and edema. The immunological status of 19 patients with IL was studied. Concentrations of IgG, IgA, and IgM were measured by immune precipitation, and metabolism of these three immunoglobulins was studied using purified radioiodinated proteins. The serum concentration and total body pool of each immunoglobulin were greatly reduced. The fraction of the intravenous protein pool catabolized per day was increased to 38% for IgG, 58% for IgA, and 66% for IgM; this contrasts to values of 7%, 25%, and 18%, respectively, in controls. Synthetic rates of immunoglobulins were normal or slightly increased, indicating that a reduced serum concentration is not a potent stimulus to immunoglobulin production. Circulating antibody to Vi and tularemia antigen was produced in 5 patients; however, these antibody titers were lower than that of controls. Lymphocytopenia was almost invariably noted in patients with IL. The mean circulating lymphocyte counts were 800 ± 300 per mm^3 in contrast to $2{,}150 \pm 220$ per mm^3 in controls. The patients showed marked skin anergy. All of 12 patients showed no response to tuberculin; all of 5 patients showed no response to mumps antigen; 8 of 9 patients showed no response to tricophyton antigen; and 6 of 9 patients showed no response to candida antigen. Three patients received skin grafts from normal donors; these grafts are surviving for at least 10 months. The immunological disorders in patients with IL appear to result from loss of immunoglobulins and lymphocytes into the gastrointestinal tract secondary to disorders of lymphatic channels. Lymphocyte depletion then leads to anergy and impaired homograft rejection. Immunologically, these patients are similar to animals with chronic lymph drainage and may have great importance in the study of transplantation immunity.

The Acid-Base Behavior of Separated Renal Tubules. ALBERT STRUYVENBERG, R. B. I. MORRISON, AND ARNOLD S. RELMAN,* Boston, Mass.

Separated dog renal tubules were prepared by a modification of the Burg and Orloff technique, and intracellular pH was calculated from the distribution of

DMO-^{14}C, with inulin used to measure noncellular water. The cells remained in good condition, as shown by normal potassium content, excellent capacity for potassium transport, and preservation of normal histochemistry and ultrastructure. ¶ When incubated at normal P_{CO_2} and extracellular [HCO_3^-], renal cells were considerably more alkaline (mean pH, 7.32) than other mammalian cells previously studied by the DMO method. Unlike skeletal muscle, renal cell [H^+] varied as a continuous direct linear function of extracellular [H^+] when the latter was changed over a wide range by varying either P_{CO_2} or [HCO_3^-], but P_{CO_2} had a much greater effect than did [HCO_3^-]. Such linear relationships would be particularly appropriate if tubular cell [H^+] controlled the renal response to extracellular acid-base disturbances. ¶ Inhibition of carbonic anhydrase by 10^{-5} M acetazolamide increased cell pH. Exposure to 5° C also increased pH, but the alkalinity of the cells actually decreased relative to the temperature-corrected neutrality point. (Cold increased the cellular concentration of DMO, thus arguing against active accumulation of this compound.) Neither acute potassium depletion nor rapid repletion significantly affected cell pH. ¶ These data suggest that renal tubular cells are relatively alkaline because they actively secrete acid. This acid secretory process appears to be dependent on metabolism but is not tightly coupled to the carbonic anhydrase reaction and hence probably does not directly utilize H^+ ions derived from H_2CO_3. These results also suggest that changes in tubular acid secretion associated with disturbed potassium balance are not mediated by changes in tubular cell pH.

Induction of Megaloblastic Erythropoiesis by Ethanol in an Individual with Normal Folate Stores. LOUIS W. SULLIVAN AND Y. K. LIU, Jersey City, N. J. (introduced by Harry Robinson †).

Previous studies have demonstrated that ethanol may suppress the hematopoietic response to folic acid in patients with megaloblastic anemia due to folate deficiency. The present investigation was undertaken to determine whether ethanol affected erythropoiesis in an individual with normal folate stores. ¶ A 27-year-old male "heavy drinker" with chronic hemolytic anemia (hereditary elliptocytosis: hematocrit 34%, persistent reticulocytosis of 3.4 to 7.7%) was studied while consuming a normal diet. Serum and erythrocyte *Lactobacillus casei* folate activities and serum vitamin B_{12} were normal (6.6 ng per ml, 829 ng per ml, and 664 pg per ml, respectively). ¶ During the daily ingestion of 460 ml of 43% ethanol for 11 days, the reticulocytosis persisted, marrow erythroid cells were normoblastic, plasma ^{59}Fe clearance remained accelerated ($t_{\frac{1}{2}} = 13$ minutes), and erythrocyte utilization of ^{59}Fe continued to be rapid (73% after 96 hours). However, during ingestion of 690 ml of ethanol for 14 days, the erythrocyte ^{59}Fe utilization was only 55% after 96 hours and the bone marrow became megaloblastic. With ingestion of 920 ml of ethanol for 10 days, the megaloblastic abnormalities in the marrow increased and erythrocyte ^{59}Fe utilization remained depressed. ¶ Before ethanol administration, leukocyte and platelet counts averaged 14,010 per mm^3 and 341,000 per mm^3, respectively; during ethanol ingestion, these respective values decreased to 8,530 per mm^3, and 255,000 per mm^3. Excretion of formiminoglutamate in the urine was not increased before or during ethanol ingestion. ¶ These observations suggest that ethanol may suppress hematopoiesis in individuals with normal folate and vitamin B_{12} stores. The development of megaloblastic erythropoiesis during the ingestion of ethanol provides further evidence that ethanol affects folate metabolism. The lack of increased formiminoglutamate excretion during the induction of a megaloblastic marrow suggests that ethanol may not affect equally the various folate-dependent enzyme systems in man.

A Study of Hemoglobin and Nonhemoglobin Heme Metabolism by Means of Double Labeling with δ-Aminolevulinic Acid-4-^{14}C and Glycine-^{15}N. WILLIAM R. SWAIM, ROBERT RYDELL, JAMES LOWMAN, VINCENT FROMKE, SAMUEL SCHWARTZ,† AND C. J. WATSON,† Minneapolis, Minn.

Administered δ-aminolevulinic acid (ALA)-^{14}C is converted largely to nonhemoglobin (Hb) hemes, which are rapidly degraded and excreted as "early-labeled" bile pigment, mainly within 1 to 2 days. In contrast, glycine-^{15}N is converted appreciably to both Hb and non-Hb hemes. A relatively large amount of the former is excreted as bile pigment after 3 to 6 days. Because of this differential behavior, the simultaneous use of ALA-^{14}C and glycine-^{15}N permits superior definition of the magnitude of these two sources of early-labeled bile pigment. Such studies have been carried out in two patients. The first (F.S.) has a peculiar chronic hemolytic anemia characterized by red cell destruction limited mainly to young circulating erythrocytes, persistent reticulocytosis (5 to 19%), marked normoblastic hyperplasia with increased storage iron, normal serum iron, increased osmotic fragility, and absent haptoglobin. Urobilinogen excretion was greatly increased. Turnover of ^{59}iron was increased to 215 mg per 24 hours ($t_{\frac{1}{2}}$, 10 minutes). ^{51}Chromium studies revealed two erythrocyte populations, one with a $t_{\frac{1}{2}}$ of less than 6 days. Maximal Hb proto-^{14}C and ^{15}N levels were reached in 2 days and fell about 30% in the first week and more slowly thereafter. Stercobilin-^{15}N was markedly increased and maximal on day 6. After an initial stercobilin-^{14}C peak at 2 days there was a secondary "shoulder" from days 4 to 7, coinciding with the ^{15}N peak and pointing to a major contribution of Hb heme at this time in contrast to the earlier ^{14}C peak of the non-Hb heme contribution. ¶ The second patient, W.J., had hepatic porphyria cutanea tarda. Here there was relatively less labeling of circulating Hb proto- but peak labeling of stercobilin with both ^{14}C and ^{15}N in 24 hours. This, with relatively little labeling from days 4 to 7, pointed to a major contribution by nonerythropoietic sources. The quantitative evaluation of these contrasting data will be discussed.

Macroglobulinemia in Sjögren's Syndrome. N. TALAL AND W. BARTH, Bethesda, Md. (introduced by J. Seegmiller *).

Of 70 patients with Sjögren's syndrome, 7, symptomatic for 1 to 15 years, have developed a lymphoma-like illness characterized by lymphadenopathy, splenomegaly, purpura, cryoglobulinemia, macroglobulinemia, and PAS-positive intranuclear inclusions in plasma cell infiltrates. Quantitative determinations of serum IgG, IgM, IgA, and IgD immune globulins were performed in these 7 patients and in 31 others with Sjögren's syndrome, 5 of whom also had reticulum cell sarcoma. Serum IgM levels in the 7 patients were 3, 6, 7, 9, 13, 32, and 94 mg per ml (normal 1.5 ± 0.6 mg per ml). Levels above 10 mg per ml fall in the range seen in primary macroglobulinemia and, in fact, the patient with the highest IgM level manifested the hyperviscosity syndrome. By contrast, IgM in the other 31 patients was normal or only slightly increased (not over 3.3 mg per ml). IgG was elevated in 50% of all patients. Three reticulum cell sarcoma patients had a markedly low IgG. ¶ IgM was isolated by gel filtration on Sephadex G-200 and studied immunochemically. Two patients had a homogeneous monoclonal IgM, which was κ type in one and λ in the other. In another patient, IgM increased from 4 to 7 mg per ml as she developed lymphoplasmacytic pulmonary infiltrates. At 4 mg per ml the IgM was heterogeneous. At 7 mg per ml an electrophoretic "spike" appeared, and immunoelectrophoresis suggested the appearance of a homogeneous IgM component. After prednisone treatment for 5 weeks, IgM decreased to 3 mg per ml, the "spike" disappeared, the titer of rheumatoid factor fell from over 1:4,096 to 1:256, the chest X ray cleared, and the patients improved. These changes in IgM, possibly analogous to the development of myeloma proteins in Aleutian mink disease, demonstrate that there is no sharp distinction between primary and secondary macroglobulinemia. The present experience tends to support an earlier hypothesis that Sjögren's syndrome predisposes to the development of lymphomas.

Prevention of Death from Endotoxin with Homologous or Heterologous O Antisera. WILLIAM TATE III, HERNDON DOUGLAS, AND ABRAHAM I. BRAUDE,† Pittsburgh, Pa.

Failure of antibiotics in treating shock from infection by gram-negative bacteria led us to re-examine antiserum against endotoxin. Heretofore, the multiplicity of O antigens made antiserum appear impractical. Discovery of uniform polysaccharide cores in different endotoxins suggested that antiserum against core antigens might protect against endotoxins of diverse gram negatives. Studies were undertaken, therefore, to produce antisera with wide protective activity. ¶ Rabbit antisera against boiled *Escherichia coli* O:113 prevented death in mice given endotoxin 6 hours later, but nonimmune rabbit serum gave no protection (LD_{50} and 95% confidence limits determined by probit analysis). Protection was statistically significant, reproducible, a function of serum dosage, and obtained by different routes of administration: subcutaneous antiserum prevented death from intraperitoneal endotoxin, and intraperitoneal antiserum protected against intravenous endotoxin. Protection was also independent of O antibody. Antiserum lacking O antibody (prepared against endotoxin in galactose-deficient rough mutant *E. coli* O:113), O antiserum (against smooth *E. coli* O:113), and heterologous antiserum (against *Salmonella abortus equi* endotoxin) each protected against O:113 endotoxin. Conversely, O:113 antiserum prevented death from *S. abortus equi* endotoxin. ¶ Protection by antiserum was accompanied by accelerated clearance of circulating radioactivity in mice given lethal doses of ^{51}Cr-labeled endotoxin. Density gradient fractionation of plasma from unprotected mice demonstrated a gradual shift in radioactivity from heavier to lighter fractions *in vivo,* indicating degradation of polydisperse circulating endotoxin macromolecules into smaller particles. In protected animals the heaviest radioactive particles disappeared more rapidly from the circulation. ¶ The results demonstrate that antiserum prevents death from intravenous or intraperitoneal endotoxin, and that protection is independent of O antibody. They support the possibility that antibody against core polysaccharide protects against endotoxins of different bacterial species and suggest that antiserum potentiates the normal detoxification process of degrading large circulating toxic macromolecules.

Extra-adrenal Effect of ACTH on Cortisol Metabolism in Normal and Obese Subjects. RONALD W. TATUM AND CHRISTINE WATERHOUSE,† Rochester, N. Y.

Obese persons frequently have increased urinary excretion of 17-hydroxycorticosteroids and hyper-responsiveness to ACTH. Plasma cortisol is usually normal; tracer studies demonstrate no differences in fractional turnover rates. However, when complete disappearance curves of isotopically labeled cortisol are obtained using 2-, 4-, 6-, 8-, 10-, 16-, 30-, 45-, 60-, and 70-minute samples and plotted as disintegrations per minute per milliliter, the initial volume of distribution (IVD), as calculated from the intercept at 0 time, is greatly magnified in obese subjects given ACTH. This study was designed to further define the factors in the IVD of cortisol and to determine whether ACTH had an influence apart from increasing adrenal secretion. ¶ Conventional compartmental analysis of disappearance curves of cortisol-^{14}C in normal individuals has been compatible with a two pool system, the first or injected compartment comparable in volume to the plasma volume and the second or "extravascular" of unknown volume, but about equal in milligrams to the plasma pool. The injected compartment doubled in volume with ACTH infusion, and only a small portion of the increase could be accounted for by cortisol on red blood cells. In obese subjects, the basal IVD was larger than in control subjects and larger than the plasma volume of the obese person (determined by RISA). Under

ACTH, doubling of the IVD occurred (up to 17 L) while measured plasma volume remained unchanged. ¶ These findings reveal an additional third pool, which rapidly equilibrates with plasma and is measured as part of the injected compartment. Because equilibration occurs in less than 2 minutes, the traditional two compartment (injected and extravascular) rate analysis may still be used to calculate secretion rates. ¶ When exogenous cortisol was infused into lean and obese surgically adrenalectomized patients at high and low infusion rates and this was repeated with ACTH added, the additional pool increased with high F infusion and further expanded with ACTH. Thus, ACTH has an extra-adrenal effect of increasing the IVD, due to expansion of a third rapidly equilibrating pool. The difference between lean and obese subjects is one of degree. This appears to explain hyper-responsiveness observed in obese subjects given ACTH. The nature of this third pool remains uncertain, although adipose tissue, liver tissue, or both, seem likely sites.

Non-Cytochrome Dependent Na^+ Transport in a Nucleated O_2 Dependent Erythrocyte. J. Theodore, H. V. Murdaugh, Jr.,* and E. D. Robin,† Pittsburgh, Pa., and Salisbury Cove, Me.

In most vertebrate tissues, energy for Na^+ transport is provided by O_2 dependent metabolism, specifically that fraction involving the cytochrome chain. The nonnucleated mammalian erythrocyte, however, uses anaerobic glycolysis as the energy source for Na^+ transport. We investigated the relationship between Na^+ transport and oxygen metabolism in nucleated erythrocytes with a known brisk O_2 consumption. ¶ Simultaneous measurements of O_2 consumption and ^{22}Na efflux were performed in the erythrocyte of the shark *Squalus acanthias* under a variety of experimental conditions with the following results. At 30° C, O_2 consumption values in microliters per gram per hour for control, CN^- (10^{-3} mole per L), antimycin (12.5 μg per ml), DNP (10^{-5} mole per L), and ouabin (10^{-4} mole per L) were 49.9 ± 12.0 (22 studies), 17.1 ± 3.9 (8 studies), 17.8 ± 3.6 (11 studies), 68.9 ± 23.5 (16 studies), and 44.3 ± 8.5 (11 studies), respectively. Values for Na^+ efflux in milliequivalents per kilogram per hour were 23.3 ± 6.9 (7 studies), 26.3 ± 6.2 (6 studies), 21.9 ± 8.7 (4 studies), 22.9 ± 2.7 (8 studies), and 9.4 ± 3.1 (8 studies), respectively. At 13° C, O_2 consumption values for 11 control and 10 ouabain studies were 15.4 ± 5.5 and 15.0 ± 6.1 μl per g per hour. Values for Na^+ efflux in 8 control and 8 ouabain studies were 9.9 ± 3 and 1.8 ± 0.7 mEq per kg per hour. ¶ It is apparent that inhibition of cytochrome-mediated O_2 consumption either by CN^- (cytochrome A_3 inhibition) or by antimycin A (cytochrome b inhibition) reduces Q_{O_2} by 70% but produces no significant decrease in total Na^+ efflux or in "active" Na^+ transport (total efflux − ouabain-inhibited efflux). This finding is consistent with two possibilities, *1*) anaerobic glycolysis is the energy source for cation transport in this oxygen-consumption unit; and *2*) non-cytochrome dependent O_2 utilization provides energy for cation transport. ¶ The following three lines of evidence suggest, albeit indirectly, the former possibility: *1*) uncoupling with DNP leads to an increased Q_{O_2} without affecting transport, *2*) temperature dependence for Q_{O_2} and Na efflux differ significantly, and *3*) ouabain inhibition leads to a depression of Na efflux without significant change in Q_{O_2}. These findings raise the intriguing possibility that anaerobic glycolysis in mammalian erythrocyte cation transport reflects a general evolutionary pattern.

A Comparison of the Antihypertensive Potency of Kidneys from One Strain of Rats Susceptible to Salt Hypertension and Kidneys from Another Strain Resistant to It. Louis Tobian,* Karen Coffee, Polly McCrea, and Lewis Dahl, Minneapolis, Minn., and Upton, N. Y.

Starting from one "parent" rat strain, Dahl bred a "sensitive" strain, which does and a "resistant" strain, which does not get hypertension after eating 8% salt. This contrast prompted comparison of the antihypertensive action of kidneys from both groups. Both strains were fed 8% salt for 8 weeks, after which the sensitives had a pressure of 172 and the resistants, 122. Then a kidney from either a sensitive or a resistant rat was transplanted into the neck of a hypertensive rat from the original parent strain. Rats of the parent strain were made hypertensive by narrowing one renal artery and excising the contralateral kidney. When a kidney was transplanted to the neck of a hypertensive rat, the ischemic abdominal kidney was removed from this rat. Thus, the only kidney in the hypertensive rat was the transplanted kidney being tested for antihypertensive potency. Imuran was given to prevent transplant rejection. Intra-arterial pressures were obtained on the hypertensive rats before and 5 days after transplant. Kidneys from resistant rats were transplanted into 12 hypertensive rats and after 5 days lowered their pressures from 196 to an average of 106. Kidneys from sensitive rats were transplanted into 17 hypertensive rats and after 5 days lowered their pressures from 198 to an average of 143. Thus, the antihypertensive action of kidneys from resistant rats was considerably greater than that from sensitive rats ($p < 0.0001$). The serum urea and creatinine were virtually identical in the sensitive and resistant donor rats before transplant. The urea and creatinine rose no higher in the recipient rats receiving sensitive kidneys than in those receiving resistant kidneys. In summary, the kidneys of the sensitive hypertensive rats had significantly less antihypertensive potency than did the kidneys from the resistant normotensive rats. This difference may explain why the sensitive strain is susceptible to hypertension.

Serum Hyperviscosity and Metabolic Acidosis Due to Circulating Hyaluronic Acid. T. B. Tomasi, Jr.,* W. Van B. Robertson, R. Naeye, and M. Reichlin, Buffalo, N. Y.

A routine leukocyte smear on a 2-year-old patient with metastatic Wilms's tumor revealed irregular thread-

like precipitates, which stained dark blue with Wright's stain. We felt initially that these represented proteinaceous precipitates in a patient with a usual plasma cell dyscrasia. However, the total serum protein and serum electrophoresis were normal. Serum viscosity was four times normal. Ultracentrifugation of the serum revealed a striking abnormality with a sharp peak on the light side of the albumin boundary. The abnormal component was isolated by sequential application of precipitation at pH 5.0 with acetic acid, Sephadex G-200 chromatography, and pevikon block electrophoresis. Chemical analyses revealed less than 4% protein and equimolar concentrations of hexosamine and hexuronic acid. Microseparation of glycosaminoglycans based on a modification of the method of Antonopoulos and associates demonstrated the abnormal component to be hyaluronic acid. The sedimentation coefficient was $S^{o}_{20} = 3.0$ S, [N] = 1,300 g per ml, and calculated molecular weight was 6.9×10^5. Examination of the tumor tissue revealed mucinous material between the tumor cells that stained positively for acid mucopolysaccharides. This staining was removed by prior treatment with testicular hyaluronidase. ¶ The patient's serum contained approximately 2.5 mg per ml of hyaluronic acid, which was thought to be responsible for the hyperviscosity (treatable with hyaluronidase) and compensated metabolic acidosis. Treatment of the patient with actinomycin D resulted in remission of tumor and disappearance of the hyaluronic acid from the serum. The serum abnormality recurred after exacerbation of the tumor. A screening procedure for detecting small amounts of circulating hyaluronic acid based on a mucin clot test and its prevention by hyaluronidase was devised. Apparently similar cases in two patients, one with neuroblastoma and the other with reticulum cell sarcoma, have been reported by Deutsch.

The Effect of β-Receptor Blockade upon the Plasma Free Fatty Acid and Growth Hormone Response to Exercise. W. G. TROYER, JR., S. J. FRIEDBERG,* E. S. HORTON, AND M. D. BOGDONOFF,* Durham, N. C.

The purpose of these studies has been to investigate the neuroendocrine mechanisms that mediate the process of lipid mobilization during exercise. Eleven young student volunteers exercised on a bicycle for 45 minutes on two separate days. On one day, saline was administered intravenously during exercise; on the other day, an infusion of propanolol, an autonomic beta-receptor-blocking compound was given. Serial plasma FFA and growth hormone (GH) levels were measured. In the saline-exercise studies, FFA and GH levels increased sharply, as did the heart rate; in the propanolol-exercise studies, the magnitude of FFA response was significantly decreased, as was the heart rate response ($p < 0.005$), whereas GH level increases were unmodified. Palmitate-^{14}C turnover times were the same in both types of exercise periods. These results have been interpreted to suggest that the autonomic nervous system contribution to lipid mobilization during exercise represents the dominant response mechanism, the elaboration by the pituitary of GH being unchanged by beta-receptor blockade.

Gel Filtration of Human Gastric Intrinsic Factor. MICHAEL D. TURNER, GERARDO C. GARRIDO-PINSON, LEON L. MILLER, AND HARRY L. SEGAL, Rochester, N. Y. (introduced by Ralph F. Jacox†).

In experiments on the isolation of human gastric intrinsic factor, human gastric juice and the soluble components of human gastric mucosal homogenates were subjected to Sephadex gel filtration. The intrinsic factor was detected by its ability to bind radioactive vitamin B_{12}, by abolition of this binding activity by human intrinsic factor-blocking antibody, and by the formation of a radioactive precipitin line on immunodiffusion with radioactive B_{12}, human intrinsic factor antibody, and rabbit anti-human γG-globulin. ¶ We found that the intrinsic factor of both mucosa and gastric juice was excluded from Sephadex G-75 and appeared in the void volume. With Sephadex G-100, however, gastric mucosa was found to give three major protein peaks and one large B_{12}-binding peak, giving the reactions of intrinsic factor and appearing in the included volume of the column. This B_{12}-binding material did not correspond in position to any of the major protein peaks. Comparison of the gel filtration behavior of intrinsic factor with that of some reference proteins of known molecular weights showed that intrinsic factor had an effective molecular volume close to that of hemoglobin. Filtration of neutralized human gastric juice through Sephadex G-100 revealed a B_{12}-binding substance with the same elution characteristics as the B_{12}-binding material from human gastric mucosa. There was no evidence for the presence of any B_{12}-binding material with an approximate molecular weight larger than 100,000 in either gastric juice or gastric mucosa. ¶ Fractions of the Sephadex column eluate that contained the major peak of B_{12}-binding material were isolated; these fractions had enhanced B_{12}-binding activity. In a typical experiment, a gastric juice concentrate binding 0.27 μg of B_{12} per mg protein gave, after gel filtration, a purified fraction binding 2.69 μg B_{12} per mg protein.

Potassium Depletion in Hypertensive Patients. IAN TYSON, S. GENNA, R. L. JONES, V. BIKERMAN, AND B. A. BURROWS,† Boston, Mass.

Total body potassium in 24 healthy control subjects (20 males and 4 females 18 to 50 years of age), using a body counter for determination of naturally occurring ^{40}K and related to body weight, showed a significant sex difference, 51.2 ± 4.1 mEq per kg in males and 38.2 ± 4.7 mEq per kg in females. Serum potassium concentration was 4.2 ± 0.5 mEq per L in males and 4.2 ± 0.3 mEq per L in females. We found body potassium in 13 hypertensive patients with normal serum potassium values to be 42.8 ± 4.7 mEq per kg in 6 males and 38.0 ± 4.6 mEq per kg in 7 females. This represented a significant reduction in males ($p = 0.05$). ¶ Body potassium related to the difference between apparent volumes of distribution of H_2O-^3H (V_{3H}) and

inulin-^{14}C (V_{In}) did not show sex difference in the controls, 153 ± 11.9 mEq per L ($V_{s_H} - V_{In}$) in males and 149 in females. Eleven of the 13 patients had low body potassium values, 99 to 129 mEq per L ($V_{s_H} - V_{In}$). Ten of these patients had received antihypertensive medication. One patient with a body potassium of 113 mEq per L ($V_{s_H} - V_{In}$) had no history of prior treatment and showed no adrenal or serum electrolyte abnormality. ¶ We concluded that body potassium measurements related to the difference between ^{3}H and inulin-^{14}C apparent volumes of distribution showed a reduction more often than when related to body weight. Body potassium was frequently reduced in hypertensive patients with normal serum potassium values. However, in 11 such patients only 1 was found in whom potassium depletion could not be explained.

Renal Concentrating Ability in Cirrhosis of the Liver. II. Changes Associated with the Clinical Status of the Disease. CARLOS A. VAAMONDE, LILIANA S. VAAMONDE, HUGO J. MOROSI, EUGENE L. KLINGER, JR., AND SOLOMON PAPPER,* Albuquerque, N. M.

Patients wth cirrhosis of the liver have some impairment of renal concentrating ability. The detailed nature of this impairment and its possible relationship to the clinical status and course of the disease were studied. ¶ Six decompensated cirrhotics (marked ascites and edema) were compared to five compensated cirrhotics (no ascites or edema) and to nine chronically ill patients without liver disease ("controls"). All received the same diet (Na, 10 mEq; K, 80 mEq; protein, 1.5 g per kg daily). In each subject we determined: *1)* U_{osm} max after 16 hours of dehydration, *2)* U_{osm} max after a 3-hour infusion of vasopressin during a sustained water load, and *3)* $T^{c}_{H_2O}$ during a mannitol diuresis. ¶ Decompensated cirrhotics had significantly lower U_{osm} max ($p < 0.01$) and $T^{c}_{H_2O}$ ($p < 0.001$) than controls. Mean $T^{c}_{H_2O}$ was 4.5 ± 0.7 ml per minute, and U_{osm} max was 774 ± 116 mOsm per kg H_2O in the control patients. Decompensated cirrhotics had significantly lower mean $T^{c}_{H_2O}$ (1.5 ± 0.8 ml per minute) than compensated cirrhotics (3.6 ± 1.7 ml per minute) ($p < 0.02$). When factored by GFR the values were also different ($p < 0.05$). No significant differences in U_{osm} max after dehydration (decompensated cirrhotics, 606 ± 85, and compensated cirrhotics 705 ± 177 mOsm per kg H_2O) and after vasopressin administration (546 ± 115 and 561 ± 105 mOsm per kg H_2O, respectively) were observed. Decompensated cirrhotics excreted less sodium ($p < 0.01$) and urea ($p < 0.05$) than compensated cirrhotics. No significant differences were observed in C_{In}, C_{PAH}, and serum sodium and potassium concentrations among the three groups of patients. In three decompensated cirrhotics the studies were repeated after compensation. Improvement of concentrating ability was observed in all: Δ U_{osm} max + 104 mOsm per kg H_2O and Δ $T^{c}_{H_2O}$ + 1.6 ml per minute per 100 C_{In}. ¶ These data suggest that the inability of decompensated cirrhotics to concentrate the urine normally, whatever the mechanism, may be reversible.

Effect of Dehydration on Urinary Concentration in the Absence of Vasopressin. HEINZ VALTIN, Hanover, N. H. (introduced by Gilbert H. Mudge†).

In earlier studies showing that urine could be rendered hypertonic to plasma in the absence of vasopressin, the urine was only slightly concentrated to about 400 mOsm per kg. The present study sought to determine whether much more concentrated urine could be formed in rats that lack vasopressin. Eight adult rats with hereditary hypothalamic diabetes insipidus (DI) that appeared to have an absolute defect for synthesizing vasopressin were kept in metabolism cages without drinking water for 48 hours. Urine was collected at 6- or 12-hour intervals. During the first 12 hours of dehydration, DI rats increased U_{osm} from a mean of 153 to 390 mOsm per kg. During the subsequent three 12-hour periods the urine became progressively more concentrated to 805, 1,013 and 1,155 mOsm per kg. By the end of 48 hours of water withdrawal, the animals had lost an average of *22%* of body weight, but they remained in good condition. After only 6 hours of rehydration ad libitum, mean U_{osm} had decreased to 309, and after 12 hours of rehydration it was 193 mOsm per kg. By this time water intake and urine flow had returned to control values even though mean body weight had been restored to only 91% of the initial value. Urinary sodium excretion decreased from 31 μEq per 100 g BW per hour during the control period to 0.38 after 48 hours of water withdrawal. There was much less decrease in urinary potassium excretion. Although the mechanisms by which such striking concentration of urine can be achieved in the absence of vasopressin remain unknown, the present results and published studies suggest major roles for reductions in glomerular filtration rate and medullary blood flow.

Relationship between Bone Blood Flow and Distribution of Erythropoietic Marrow. DONALD VAN DYKE,* Berkeley, Calif.

Use of the short-lived positron-emitting isotope of fluorine ^{18}F and the positron camera provides a unique method for demonstrating the distribution of fluoride in the living animal or human subject. Fluoride ion taken into the body is promptly deposited in the skeleton or excreted via the kidneys. The initial uptake of fluoride by bone occurs primarily by exchange. The distribution of ^{18}F administered intravenously as fluoride ion is uneven in the normal skeleton and markedly altered by age and in pathological conditions. ^{18}F accumulates in the heated extremity, in fracture and tumor sites, and in the lesions of Paget's disease. The initial uptake of fluoride in bone is dependent on the rate of delivery of the isotope and the extraction efficiency. Evidence has been obtained indicating that fluoride distribution in the skeleton is determined by differences in blood flow to the various bones rather than differences in extraction efficiency. ¶ In the same subject in whom relative bone blood flow has been determined using ^{18}F, the distribution of erythropoietic marrow can be recorded with the positron camera, using the positron-emitting isotope of iron, ^{52}Fe. ¶ In both

normal animals and humans there is a remarkable similarity in the distribution of ^{18}F and ^{59}Fe that indicates a relationship between bone blood flow and activity of the contained hemopoietic marrow. The finding of increased erythropoietic activity adjacent to areas of increased bone blood flow after implantation of the tip of the rat's tail into the abdomen, after fracture, and in the lesions of Paget's disease indicates a more than casual relationship between bone blood flow and marrow function and suggests that the distribution of hemopoietic marrow within the skeleton is determined by adequacy of the blood supply.

The Regulation of Heme Synthesis: Studies on the Inhibition of Aminolevulinic Acid Synthetase. JOHN D. VAVRA AND SALLY A. POFF, St. Louis, Mo. (introduced by Elmer B. Brown *).

Aminolevulinic acid (ALA) synthetase, the initial enzyme in the synthesis of heme, has been implicated as the site of feedback inhibition in control of the over-all rate of heme synthesis. The kinetics of a preparation of this enzyme, obtained from the reticulocyte of anemic chickens, has been studied to elucidate the mechanism of ALA synthesis and to assess the importance of different potential metabolic and product inhibitors. The rate of enzymatic synthesis of ALA in the presence of optimal concentrations of cofactors is described by Michaelis-Menton kinetics for a two substrate reaction. The following Michaelis constants were obtained by $1/V_{max}$ plots: glycine, 12 mmoles per L; α-ketoglutarate (αKG), 0.22 mmole per L (reaction synthesizing succinyl CoA); and succinate, 1.7 mmoles per L (succinate and CoA as substrates). Enzymatic activity was maximal in the presence of ferrous iron concentration of 0.01 mg per ml (0.2 mmole per L), and was only 70% as active at iron concentrations tenfold higher or lower. Mixed competitive inhibition of ALA synthetase was produced by chicken and human oxyhemoglobin, human methemoglobin, and human globin solubilized by partial alkali digestion. The inhibitor constant K_I (concentration of inhibitor producing 50% inhibition) for each was 13 to 16 g per 100 ml. No inhibition was observed with bovine serum albumin in the same concentration. Human α- and β-globin chains produced mixed competitive inhibition with K_I of 0.7 to 1.0 mg per ml and 1.0 to 1.7 mg per ml, respectively. Heme probably does not inhibit ALA synthesis from glycine and αKG in the mitochondrial system. In rabbit reticulocytes heme (0.4 mmole per L) inhibited the over-all synthesis of heme 88%, as determined by glycine-^{14}C incorporation, compared with 40%, as determined by ALA-^{14}C incorporation into heme. The results of these studies indicate that the various components of hemoglobin influence the rates of ALA synthesis. The primary site of heme inhibition remains to be determined.

The Direct Effect of Insulin on Hepatic Glucokinase: Kinetics. JOHN W. VESTER, Pittsburgh, Pa. (introduced by I. Arthur Mirsky †).

It has been previously reported from this laboratory that insulin is capable of directly augmenting rat hepatic glucokinase activity. The procedure consists of the incubation of a particle-free supernatant of rat liver with a solution of amorphous insulin that is low in zinc content and soluble in distilled water. The 4-minute incubation period is followed by a spectrophotometric assay of glucokinase activity. This effect has a linear-log dose response. ¶ Kinetic studies have demonstrated that insulin could be interpreted as removing a noncompetitive inhibitor to the binding of glucose in that the hormone doubled V_{max} without altering Km. This effect was mimicked by L-cysteine in 10^{-4} M final concentration. EDTA (10^{-4} M) likewise augmented V_{max} but decreased Km by a factor of 3. Essentially, all three substances augmented glucokinase in a fashion compatible with removal of a noncompetitive inhibitor to the binding of glucose. Recent studies indicate that these three substances also appeared to affect kinetics in a similar fashion when ATP concentrations were varied. Here, the substances used appeared to both augment V_{max} and diminish Km for ATP. The net interpretation, again, is that of blocking a noncompetitive inhibitor to the binding of ATP. ¶ When magnesium concentrations were varied, the control V_{max} was 3.68 μmoles TPNH per g liver per minute, and the insulin-treated V_{max} was 4.08. Km was correspondingly decreased. A composite graph of the effects of cysteine and EDTA indicated that these substances appeared to be acting in a similar, if not identical, fashion. ¶ These observations indicate that insulin appears to produce its direct augmentation of glucokinase activity in a fashion kinetically interpretable as that of removing a competitive inhibitor to the action of magnesium. The essential role of magnesium in other insulin dependent systems suggests that facilitation of magnesium interaction as a universal mode of insulin action may be a worthy hypothesis.

Metaphyseal and Epiphyseal Accretion of ^{87m}Strontium as an Indicator of Growth. H. N. WAGNER, JR.,* D. E. TOW, R. A. MILCH, AND W. A. NORTH, Baltimore, Md.

Skeletal activity is usually evaluated by examination of stages of ossification in radiographs. Such studies fail to convey quantitative kinetic data. We have measured the accretion of ^{87m}strontium by the metaphyses and epiphyses of the knees to provide a quantitative index of skeletal growth. This nuclide was chosen because none of the isotopes of calcium is suitable. Advantages of ^{87m}Sr as an analog of calcium are that the physical half-life is 2.9 hours, and decay is by isomeric transition emitting a photon of 388 kev energy. Thus, the radiation dose is well within permissible limits even in children, and the nuclide can be readily localized by external radiation detectors, including scanners. One mc of ^{87m}Sr as nitrate or chloride (less than 1 mg of stable strontium) was injected intravenously. Serial uptakes of the Sr were measured over the epiphyseal and metaphyseal areas of the knees. Serial quantitative scans were also performed. The radioactivity was measured with a flat field collimator and gamma spectrometer. The uptake was expressed as a percentage of the administered dose

by comparison with a standard encased in a Lucite phantom. In six adult subjects aged 42 to 74 without known skeletal disease, the mean was 1.2% (range, 0.6 to 1.7) at 2 hours and 1.4% (range, 0.7 to 1.9) at 4 hours after injection. The peak of accretion was between 10 to 12 hours. In five children aged 6 to 14, the means of uptakes were 3.4% (range, 3.0 to 3.8) and 4.1% (range, 3.5 to 4.6) at 2 and 4 hours, respectively. In two children aged 7 and 14 with congenital hypothyroidism treated with thyroid hormone, the uptakes were 2.6 and 2.2% at 2 hours and 2.7 and 2.6% at 4 hours. The data indicate a correlation of rate of accretion with chronological age and are consistent with the hypothesis that this method may be a simple, safe, objective, and effective parameter of growth.

The Nature of Thrombin Stimulation of Glucose Oxidation by Platelets. ANDREW L. WARSHAW, LEONARD LASTER, AND N. RAPHAEL SHULMAN,* Bethesda, Md.

Interaction of thrombin with platelets is necessary for clot retraction. We found that agglutination of human platelets by purified thrombin (1 U per 10^9 platelets) *in vitro* is accompanied by a sustained (over 3 hours) increase in glucose oxidation. This report presents investigations of biochemical mechanisms underlying this stimulation. When platelets and thrombin were incubated in Krebs-Ringer bicarbonate buffer, oxidation of glucose-6-^{14}C to $^{14}CO_2$ increased 100%, but oxidation of glucose-1-^{14}C increased only 35%, and lactate accumulation increased only 15%. Thus, thrombin appears to stimulate glucose oxidation primarily via the Embden-Meyerhof pathway and citric acid cycle rather than the hexose monophosphate shunt. Production of $^{14}CO_2$ from other sugars phosphorylated by hexokinase, mannose-1-^{14}C, and fructose-U-^{14}C, increased 50% with thrombin, but $^{14}CO_2$ from galactose-1-^{14}C and pyruvate-3-^{14}C decreased 50%. With glucose omitted from the incubation medium, thrombin inhibition of $^{14}CO_2$ production from galactose or pyruvate fell to 15%. The findings suggest that thrombin stimulates a reaction, possibly the hexokinase reaction, earlier in the metabolic pathway than those at which galactose or pyruvate enter, and that this stimulation increases concentrations of metabolites that either inhibit utilization of exogenous galactose and pyruvate or dilute metabolic pools, thereby decreasing radiochemical yields. Stimulation by thrombin apparently does not require new enzyme formation, since pyromycin did not affect the stimulation. Aggregation per se cannot account for the stimulation since ADP, which also agglutinates platelets, did not affect glucose metabolism. That thrombin does not act by nonspecifically increasing transport of organic substrates into platelets is suggested by its failure to increase decarboxylation of orotate-$^{14}COOH$ or oxidation of galactose-1-^{14}C. However, thrombin has been reported to increase platelet permeability to K^+, and perhaps by thus changing intracellular ionic environment it may alter enzyme activity and thereby stimulate glucose oxidation.

A Common Mechanism for the Fungicidal and Nephrotoxic Effects of Amphotericin B. GERALD WEISSMANN,* MORDECAI PRAS, AND ROCHELLE HIRSCHHORN, New York, N. Y.

Amphotericin B and other polyene antibodies are considered to be fungicidal because of their interaction with sterols present in fungal cell walls. Intravenous amphotericin regularly induces pyrexia in man resembling the pyrogenicity of other lysosome disruptive agents such as etiocholanolone. The antibiotic also causes renal tubular lesions recently associated with damage to lysosomes. Therefore, several polyene antibiotics were tested for their effects upon lysosome-rich fractions of rabbit liver, kidney, and granulocytes. At concentrations above 10^{-5} mole per L, filipin, etruscomycin, nystatin, and pimaricin, in that order, released up to 75% of total lysosomal enzymes (beta-glucuronidase, acid phosphatase, and aryl sulfatase) from liver, kidney, and granulocyte lysosomes at neutral and acid (3.0 to 7.5) pH. These antibiotics are too toxic for systemic use. In direct contrast, amphotericin B was lytic only for kidney lysosomes and only at acid pH (3.0 to 5.6). Under conditions at which the polyenes induced maximal release of enzymes from lysosomes, no damage to mitochondria could be detected judged by mitochondrial swelling, release of mitochondrial enzymes, or both. Since lysosomal membranes have a higher cholesterol to phospholipid ratio than do mitochondria, the effect of polyenes upon artificial phospholipid spherules prepared with and without cholesterol was studied. Although filipin and etruscomycin disrupted artificial membranes prepared with and without cholesterol, amphotericin and nystatin caused leakage of marker ions or molecules only from cholesterol-containing spherules. These studies indicate that *1*) polyene antibiotics disrupt the membranes of lysosomes, but not of mitochondria, from mammalian tissues; *2*) amphotericin B interacts exclusively with lysosomes from kidney; and *3*) amphotericin B only affects natural or artificial membranes possessing a relatively high cholesterol to phospholipid ratio. Therefore, the polyene antibiotics appear to indiscriminately disrupt fungi and mammalian organelles by a common mechanism, in direct contrast to more selective antimicrobials such as penicillin.

Metabolism of Circulating Disaccharides in Man and the Rat. ELLIOT WESER AND MARVIN H. SLEISENGER,* New York, N. Y.

Increased amounts of unhydrolyzed disaccharides are absorbed into blood and excreted in urine by patients with celiac disease. To compare the metabolism of different circulating disaccharides, three human subjects were given separate iv infusions of 10 g of lactose, sucrose, and maltose. Urine was collected for 24 hours and assayed enzymatically for the respective disaccharides. After iv lactose and sucrose, 45 to 95% of the disaccharide was excreted unchanged in the urine, whereas after iv maltose, less than 2% was excreted in the urine. There was no significant rise in blood glucose after any of the di-

saccharide infusions. These results suggested that maltose, but not lactose or sucrose, was metabolized. ¶ The metabolic fate of circulating disaccharides was studied in nonfasting rats. After iv injection of 5 mg (0.5 μc) of ^{14}C-labeled disaccharides, we measured the radioactivity of expired $^{14}CO_2$ and urine for 24 hours. Less than 8% of the iv dose of lactose or sucrose was oxidized to CO_2 and more than 62% of these disaccharides was excreted unchanged in the urine. After maltose injection, 54% was oxidized to CO_2 and less than 5% was excreted in the urine. We noted a rise in blood glucose only after the maltose injection. Maltase activity was assayed in several rat organs. Relative activities (U per g prot.) were the following: mucosa, 485; kidney, 17; brain, 14; liver, 2; spleen, 1; muscle, 0.1; and serum, 0.4 U per ml. Small bowel resection, hepatectomy ($\frac{2}{3}$), or bilateral nephrectomy did not significantly reduce oxidation of circulating maltose. ¶ We concluded that circulating maltose, but not lactose or sucrose, is metabolized by both man and rat. In man, extraintestinal tissue maltases may be important factors in metabolism of circulating maltose, whereas in the rat circulating maltose is probably hydrolyzed to glucose by serum maltase.

Thyroxine (T_4) Secretory Rates in Steady and Nonsteady States Calculated from Serum T_4 Specific Activity Decay Curves. C. D. West,* L. F. Kumagai, V. J. Chavré, and A. H. Bigler, Salt Lake City, Utah.

T_4 secretory rates (SR) in steady and nonsteady states have been calculated from the dilution in T_4 specific activity (T_4SA) in serum after the intravenous administration of T_4-^{131}I. Thin layer chromatography was used to determine serum T_4SA (cpm per μg), T_4-^{131}I (cpm per 100 ml), and T_4I (μg per 100 ml) levels. Semilog plots of T_4SA and T_4-^{131}I against time were essentially linear in both steady and nonsteady states. In steady states the slopes were equal, and serum T_4I levels were constant. In nonsteady states, slopes were unequal and T_4I levels varied. From these observations the following equation for calculating SR (μg per day) was derived: $SR = (A_oKs/S_o)e^{(K_s-K_a)t}$, where A_o = dose of iv T_4-^{131}I (cpm), S_o = T_4SA at 0 time, t = days, and K_s and K_a = fractional turnover per day for T_4SA and T_4-^{131}I. Calculated SR values were 75 to 96 μg per day in four normals, 0 to 6 μg per day in three hypothyroids, and 523 to 737 μg per day in two hyperthyroids. Treatment with Lugol's solution or propylthiouracil (PTU) for 2 weeks markedly reduced SR in the two hyperthyroids but not in normals. One hyperthyroid had a normal SR after 3 months on PTU. Oral T_3 or T_4 for 3 weeks suppressed SR by 50% in euthyroid patients. One hypothyroid patient was given T_4 intravenously every other day for 2 weeks. His average SR was calculated to be 88 μg per day, and he actually received 76 μg per day. The rate of removal (RR) of T_4 from serum = $(A_oKa/Ks)e^{(K_s-K_a)t}$. If the difference between SR and RR is known, the theoretical serum T_4I level can be calculated for any time (t) from the amount of T_4 in the body (A_o/S_o) and its volume of distribution (A_o/T_4-$^{131}I_o$). Actual serum T_4I levels and calculated values were in excellent agreement in 12 patients in nonsteady states. These observations support the validity of this SR method.

Inhibition of Virus-induced Leukemia in Mice by a Nontumor Virus. E. Frederick Wheelock, Cleveland, Ohio (introduced by John H. Dingle †).

The virus etiology of certain murine leukemias and possible virus etiology of human leukemia suggest the treatment and prevention of these diseases in terms of viral inhibition, perhaps through viral interference. Sendai virus (SV), a parainfluenza nontumor virus, inoculated intraperitoneally into mice before Friend leukemia virus (FV) is administered intraperitoneally, markedly inhibits the subsequent splenomegalic, lymphocytotic, and erythroblastotic responses that normally occur 3 weeks after FV inoculation. Sendai virus protects mice against Friend virus leukemia when the interval between virus administrations is as long as 6 weeks, and the protection may be permanent—it lasts for at least 3 months after FV inoculation, and longer intervals are being studied. Sendai virus inoculated intraperitoneally induces interferon production. Friend virus, however, induces no interferon. We hypothesized *1*) that SV-induced interferon might be involved in the inhibition of FV disease when SV is inoculated before FV, and *2*) that the reason SV does not inhibit splenomegaly when inoculated after FV might be the inability of FV-infected mice to produce interferon in response to Sendai virus. We found that within 1 day after FV inoculation the ability of mice to produce peritoneal interferon in response to an intraperitoneal SV challenge is markedly diminished, and the interferon-producing capabilities return to normal levels 12 days later. ¶ The peritoneal macrophage may be the key cell in the reciprocal interference between Sendai and Friend viruses. Macrophages from FV-infected mice produce FV *in vitro*, and macrophages from SV-infected mice produce interferon *in vitro*. Therefore, since both viruses use the macrophage as a host cell, the possibility exists that the reciprocal interference between viruses may be on an intracellular competitive basis. These experiments suggest that leukemia viruses may be inhibited in animals and perhaps in man by nontumor viruses.

Hepatic Production of Bilirubin and Carbon Monoxide *In Vitro*. Peter White, Abigail A. Silvers, Mary L. Rother, Brenda C. Shafer, and William J. Williams,* Philadelphia, Pa.

Carbon monoxide has been shown to be an end-product of the degradation of heme *in vivo*, and the breakdown of circulating hemoglobin normally accounts for approximately 80% of the CO produced endogenously. Recently, it has been shown that after the administration of glycine-2-^{14}C to man ^{14}CO is produced before circulating heme is significantly labeled. This "early-appearing" CO is thought to originate from sources other than circulating heme that presumably also give rise to the concomitant

early-appearing stercobilin. Others have suggested a hepatic origin for part of the early-appearing stercobilin. To determine whether early-appearing CO may also originate in the liver, we incubated rat liver homogenates with glycine-2-^{14}C and delta-aminolevulinic acid-4-^{14}C (ALA-^{14}C). Bilirubin was isolated from the homogenates with the aid of carrier and assayed for radioactivity after repeated recrystallization. To collect CO, we added ferricyanide to the reaction mixtures after incubation, and flushed the gas through a train consisting of CO_2 absorbers for quantitative removal of CO_2 followed by a Hopcalite column to convert CO to CO_2. This CO_2 was then trapped and its radioactivity determined with a liquid scintillation counter. Rat liver homogenates generate labeled bilirubin from both glycine-2-^{14}C and ALA-^{14}C. These reactions require a mixture of mitochondria and soluble fraction. Suspensions of mitochondria in soluble fraction convert glycine-2-^{14}C to bilirubin-^{14}C and ^{14}CO. Yields of bilirubin-^{14}C and ^{14}CO are higher when room air is used as gas phase than with either 100% O_2 or 100% nitrogen. The concomitant appearance of ^{14}CO and bilirubin-^{14}C in this *in vitro* system indicates a common origin and supports the concept that "early-labeled" CO arises in part from pathways involving tetrapyrrole synthesis in the liver.

Prophylaxis of Myohemoglobinuric Acute Renal Failure in the Rat: A Micropuncture Study. DOUGLAS R. WILSON, GILBERT THIEL, MANUAL L. ARCE, AND DONALD E. OKEN, Boston, Mass. (introduced by Lewis Dexter †).

Rats given 50% glycerol (10 ml per kg im) after 24 hours of water deprivation develop oliguric acute renal failure, the BUN of 35 animals rising to 226 ± 11 (SE) mg per 100 ml in 48 hours. Intravenous 25% mannitol (3 ml per kg) or intraperitoneal isotonic saline (80 ml per kg) prevented oliguria and decreased the severity of azotemia in 28 rats (BUN, 98 ± 57 and 122 ± 13 mg per 100 ml, respectively, at 48 hours, $p < 0.001$). Intraperitoneal saline and iv mannitol given together to 14 rats totally prevented azotemia (48-hour BUN, 17 ± 1 mg per 100 ml). In an attempt to delineate the mode of action of these agents, tubular fluid flow rate (TFR), intratubular pressure (ITP), water absorption, and GFR were measured in individual proximal tubules of untreated glycerol-injected rats (group 1), and similarly prepared rats given mannitol (group 2), saline (group 3), or both (group 4), as outlined above. ¶ GFR and flow rate were minimal in all nephrons examined 1 to 4 hours after glycerol, but ITP was considerably higher in 203 tubules of 31 rats in groups 2, 3, and 4 (11.6 ± 0.7, 11.3 ± 0.9, and 12.2 ± 0.3 cm H_2O) than in 58 tubules of 10 untreated rats (4.8 ± 0.3 cm H_2O) ($p < 0.001$). The groups with higher intratubular pressure initially were not oliguric in the succeeding 20 hours, although the BUN of groups 2 and 3 rose considerably. GFR and TFR values 24 hours after glycerol in 85 nephrons with sufficient flow to permit measurement in 27 rats were the same in all groups (maximal difference $p < 0.2$), but the proportion of such nephrons in a single kidney varied according to treatment and correlated well with the 48-hour BUN value of the group. We concluded that the abolition of oliguria and the degree of protection from azotemia afforded by these agents primarily reflects a modification of the number of nephrons with significant filtration rather than a diffuse salutary effect on all nephrons.

The Biosynthetic Origin of Serum Cholesterol in the Monkey. JEAN D. WILSON * AND JOHN M. DIETSCHY, Dallas, Texas.

Although it has previously been demonstrated in man that 40 to 60% of serum cholesterol arises from endogenous nonhepatic sources, the biosynthetic site for this component of serum cholesterol is unknown. To elucidate the tissue of origin of this cholesterol in a primate, we performed three different types of experiments in squirrel monkeys fed diets containing either low or high quantities of cholesterol. First, animals were tube-fed each day diets containing 300 mg of cholesterol-4-^{14}C in the form of egg lipoprotein, and serum cholesterol specific activity was assayed periodically for 3 to 6 weeks. When an isotopic steady state was attained, 20 to 40% of the serum cholesterol was derived from endogenous sources, demonstrating quantitative and qualitative similarities between monkey and human. Second, cholesterol synthesis was studied *in vitro* in tissues obtained from animals fed either low (10 mg per day) or high (250 mg per day) cholesterol diets. In animals fed the high cholesterol diet, cholesterol synthesis in liver was markedly suppressed, whereas synthesis was unaltered in the other seventeen tissues examined, including the intestinal wall. Thus, liver was not the endogenous source of serum cholesterol in the cholesterol-fed animal. Third, cholesterol specific activity-time curves were determined in intestinal lymph and serum for periods up to 48 hours after single injections of acetate-2-^{14}C. On low cholesterol diets lymph cholesterol specific activity was lower than that of serum, whereas on high cholesterol diets lymph cholesterol specific activity was invariably higher than that of serum, which was virtually unlabeled when intestinal lymph was completely diverted from the circulation. These results strongly suggest that the intestinal wall may be a major site for the biosynthesis of serum cholesterol.

The Syndrome of Milk-induced Occult Gastrointestinal Bleeding. JOHN F. WILSON AND M. E. LAHEY, Salt Lake City, Utah (introduced by John W. Athens *).

In the pathogenesis of iron deficiency anemia (IDA) of infancy, occult fecal blood loss (FBL) has received scant attention. Our preliminary studies showed that the ingestion of whole cow's milk (WCM) precipitated occult enteric bleeding in three of five anemia subjects. ¶ Further observations have now been carried out on a total of 34 infants with IDA. FBL was quantitated by the RBC-^{51}Cr labeling technique and measurement of fecal radioactivity. To identify WCM as the factor precipitating

FBL several criteria were met: *1*) FBL ceased or improved significantly when soya formulas (SMS) or heated milk formulas (HMF) were substituted for WCM in the diet; *2*) abnormal FBL recurred when WCM was returned to the diet; and *3*) FBL again ceased during a second period of WCM elimination. ¶ The results indicate an over-all 47% incidence of WCM-induced FBL, which increased to 68% in those with accompanying hypoproteinemia (TSP < 6.0 g per 100 ml); these data further incriminate FBL as the major cause of the anemia. In eight subjects continued on WCM and treated with iron (five orally, three parenterally) bleeding continued; this rules out iron lack as contributing to the enteropathy. In two subjects a quantitative relation was demonstrated between the amount of WCM ingested and the FBL. In another subject given bovine serum and SMS, FBL increased two and one-half-fold over control periods, suggesting that heat-labile protein or proteins in WCM may be of etiologic importance. Finally, since four subjects studied 9 to 17 months later failed to show WCM-induced bleeding, a factor of maturation may be significant in developing tolerance to milk. ¶ This study emphasizes the need to give greater consideration to WCM-induced occult FBL as a cause of IDA in infancy.

Effects of Epidural Sympathetic Block on the Hemodynamic Responses to Epinephrine in Triiodothyronine-induced Hypermetabolism. WILLIAM R. WILSON,* ERNEST O. THEILEN, VINCENT S. AOKI, WILLIAM K. HAMILTON, AND PAUL E. LEAVERTON, Iowa City, Iowa.

The importance of the sympathetic nervous system in the hemodynamic changes of hypermetabolism was evaluated in seven volunteers given triiodothyronine (400 to 500 μg per day) for 2 weeks. The average sleeping pulse increased from 64 to 103 beats per minute and oxygen consumption from 139 to 199 ml per minute per m^2 ($p < 0.01$); body weight fell slightly, and serum cholesterol decreased (229 to 126 mg per 100 ml ($p < 0.01$). Hemodynamic measurements were made during the hypermetabolic session (A), and during the euthyroid session (B) before and during the fifth minute of infusion of three graded doses of epinephrine (0.037 to 0.15 μg per kg per minute) and before and during continuous epidural sympathetic blockade (lidocaine, 0.5% — 105 to 120 ml). Abolition or significant attenuation of the Valsalva overshoot, anhydrosis, miosis, and increased skin temperature were criteria for sympathetic blockade. Triiodothyronine significantly increased heart rate (74 to 105 beats per minute), systolic arterial pressure (114 to 132 mm Hg), and cardiac index (3.3 to 5.3 L per minute per m^2); systemic resistance fell (1,007 to 694 dynes per second per cm^{-5}). Epidural block reduced systolic pressure 36 mm Hg and mean arterial pressure 18 mm Hg in B ($p < 0.01$) and 10 mm Hg and 7 mm Hg, respectively, in A ($p > 0.05$). Control heart rate, cardiac index, and systolic and mean arterial pressures after sympathetic block remained higher ($p < 0.05$) and resistance lower ($p < 0.05$) in A than in B. Responses to epinephrine were similar before and after block in A. Sympathetic block in B potentiated the rise in mean arterial pressure and attenuated the fall in resistance during epinephrine, but increments in heart rate, systolic pressure, and cardiac index during epinephrine were similar before and after block in both sessions. These findings suggest that the circulatory changes of drug-induced hypermetabolism are not mediated via the sympathetic nervous system, and that triiodothyronine does not augment the hemodynamic responses to epinephrine.

Studies of Natural and Synthetic Angiotensins. BERTRAM M. WINER AND WILHELM F. LUBBE, Boston, Mass. (introduced by Benjamin Alexander †).

The chemical structure of angiotensin II is known in several species, but not in man. In assays of plasma renin activity certain differences were observed in the responses of rabbit aortic strips to synthetic asparaginyl-1 valyl-5 angiotensin II and natural forms of angiotensin. Natural angiotensin was obtained by incubation of dialyzed plasma of patients or dogs with salt depletion, renal arterial stenosis, or pregnancy. The contraction after asparaginyl-1 valyl-5 angiotensin II was followed by a rapid smooth relaxation phase after washout. In contrast, the relaxation phase after the contractile response to natural human or dog angiotensin was triphasic, with an early rapid phase interrupted by a delay or distinct contraction, with subsequent complete relaxation. In addition, the contractile response after asparaginyl-1 octapeptide was of more rapid onset and rate of shortening than that after the natural angiotensins. These differences were also observed after the synthetic and natural angiotensins were extracted by Dowex column and butanol paper chromatography. To extend these observations, we made studies of aortic strip responses to the synthetic angiotensin II analogues, asparaginyl-1 isoleucyl-5, aspartyl-1 valyl-5, aspartyl-1 isoleucyl-5, β-aspartyl-1 valyl-5, and natural hog angiotensin II. The triphasic response after washout consistently followed contraction elicited by natural human, dog, and hog angiotensin and those analogues with aspartic acid in the 1-position, but not by the asparaginyl-1 or β-aspartyl-1 forms; it was independent of the amino acid in the 5 position, valine, or isoleucine. In pentolinium-blocked vagotomized rats, aspartyl-1 and asparaginyl-1 octapeptides had equal pressor activity, but in rabbit aortic strips the vasoconstrictor activity of the aspartyl-1 forms was approximately four times that of the asparaginyl analogues. These studies demonstrate biologic differences between natural angiotensins and synthetic asparaginyl-1 angiotensin II and suggest that the 1-group in human, as well as dog and hog, angiotensin II is in the aspartyl and not asparaginyl form.

Kinin Release from Human Skin. R. K. WINKELMANN, Rochester, Minn. (introduced by Aaron B. Lerner *).

Kinin release in human skin has been assessed by perfusion of the dermal-subcutaneous compartment of the

forearm with isotonic saline. The rat uterus bioassay of the perfusates standardized with bradykinin is sensitive to nanogram quantities of kinin. Normal human skin demonstrates temporary kinin release after the trauma of needle insertion. Kinin is released from normal human and atopic skin by the histamine-releasing agent 48:80. Large amounts of kinin were released by trauma, by 48:80 from the skin of nine patients with dermographia and nine with urticaria pigmentosa, and by cold and 48:80 from five patients with cold urticaria, and all kinin release was prevented by occlusion with the blood pressure cuff. Histamine was released by 48:80 but not by the cold or trauma in these patients, whereas kinin was released by both types of stimulus. Study of a boy with congenital sensory analgesia (no peripheral sensory nerves or nerve endings) demonstrated kinin release with 48:80. The appearance of kinin was correlated with the bluing of the cutaneous wheal by intravenously injected Evans blue dye, and the kinin release was blocked by blood pressure cuff occlusion. Kinin may occur in the absence of histamine release and does not appear to arise from cutaneous nerves or sweat glands. It seems that the appearance of kinin cutaneous perfusates is the result of vasopermeability and may be used to judge normal physiologic responses and pathologic responses that involve vasoactivity.

Lymphatic Transport of Bromsulfalein and Lymph Flow in Normal Subjects and Patients with Hepatobiliary Disease. MARLYS HEARST WITTE, ALLAN E. DUMONT, WILLIAM R. COLE, AND NORMAN LEVINE, New York, N. Y., and Saint Louis, Mo. (introduced by Sol Sherry †).

The rate of BSP removal from blood has been used extensively to evaluate hepatobiliary function. Although BSP does not enter ascites, chylous pleural effusions, or peripheral edemal fluid, unexplained high concentrations of BSP have been found in hepatic hilar lymph of experimental animals. Liver lymphatics normally contribute more than one-half of thoracic duct lymph (TDL), a fluid more accessible to sampling in man. Therefore, concentrations of total BSP (BSPT) and BSP "metabolite" (BSPM) were determined in TDL and plasma at frequent intervals up to 90 minutes after a single intravenous injection of BSP (5 mg per kg) in 31 patients with hepatic cirrhosis, 9 with extrahepatic biliary obstruction, and 3 normal subjects. In 5 patients, common duct bile and liver homogenates also were examined. BSPT attained peak levels in lymph rapidly (15 to 45 minutes). Lymph BSPT at 45 minutes did not correlate with simultaneous plasma BSPT and was highest ($\sim$plasma) in cirrhosis without portal hypertension or ascites (group I), lowest ($< \frac{1}{3}$ plasma) in cirrhosis with contracted liver and portal hypertension (group II), and intermediate in biliary obstruction and normal subjects. BSPM/BSPT in lymph generally was greater than in plasma in cirrhosis and less than in plasma in biliary obstruction. These observations indicate that BSP in TDL arises mainly by exchange of liver lymph with BSP from bile and liver cells, rather than by direct transfer of protein-bound dye filtered from plasma. BSPT in TDL was uniformly low in group II. In one such patient restudied after successful portacaval shunting, lymph flow fell and BSPT rose. Thus, liver lymph appears to contribute less to TDL as cirrhosis and portal hypertension progress. Finally, greatly elevated TDL flow clearly distinguished patients with cirrhosis from those with biliary obstruction, in whom flow was normal or decreased.

A Postulated Role of Long Chain Carnitine Acyl Transferase in the Red Cell Membrane. B. WITTELS, P. HOCKSTEIN, AND R. BRESSLER,* Durham, N. C. (introduced by E. A. Stead, Jr. †).

Carnitine esters of long chain fatty acids have been shown to be the transport form in which activated long chain fatty acyl (LCFA) groups are translocated across the mitochondrial barrier to the sites of fatty acid oxidation. The formation of LCFA carnitine is catalyzed by the long chain carnitine acyl transferase in the presence of LCFA CoA and carnitine. ¶ Membranes prepared from mature human red cells synthesized palmitylcarnitine-^{14}C when incubated with DL-carnitine, ATP, and palmitate-1-^{14}C, or with DL-carnitine and palmityl CoA-1-^{14}C. No conversion of the LCFA groups to $^{14}CO_2$ occurred in this system. The membranes also possessed the capacity to form lecithin-^{14}C or phosphatidylethanolamine-^{14}C from the respective lysophosphatide and either palmitate-1-^{14}C and ATP, palmityl CoA-1-^{14}C, or palmitylcarnitine-^{14}C. The addition of lysophosphatides to the incubation medium containing carnitine stimulated the incorporation of LCFA groups into phospholipids, whereas it depressed the incorporation into palmitylcarnitine. ¶ The identification of the long chain carnitine acyl transferase as an integral component of the red cell membrane is consonant with its role in the transfer of activated LCFA groups across membrane barriers. Thus, the enzyme and carnitine may play a role in regulating the transport of long chain fatty acids into the red cell. The synthesis of palmitylcarnitine could also be involved in the entry of LCFA groups into the membrane compartment where phospholipid synthesis occurs. In this function, the long chain carnitine acyl transferase may take part in the turnover of LCFA groups in the red cell membrane.

Urinary Normetanephrine and Metanephrine Excretion Separately Assayed in Normotensive, Hypertensive, and Pheochromocytoma Patients. ROBERT L. WOLF, MILTON MENDLOWITZ, † JULIA ROBOZ, ERIC NAFTCHI, AND STANLEY E. GITLOW, New York, N. Y.

We performed separate normetanephrine (NM) and metanephrine (M) determinations on urine specimens from (A) 30 normotensive, (B) 10 untreated primary benign hypertensive, and (C) 17 pheochromocytoma patients. NM and M assays were performed by high voltage paper electrophoresis on morning urine specimens after prior preparation of aliquots by hydrolysis at pH 1.0, passage through an Amberlite CG-50 resin column, elution with 4 N NH_4OH, and *in vacuo* evaporation of

the eluates followed by ethyl acetate extraction. The quantities of separate NM and M on the high voltage paper electrophoretograms were assayed with an automatic densitometer employing a regulated light source connected to an integrating linear log recorder. ¶ Mean urinary NM (micrograms per milligram creatinine) values for A, B, and C were 0.106, 0.095, and 1.338, respectively. Mean urinary M (micrograms per milligram creatinine) values for A, B, and C were 0.124, 0.099, and 1.518, respectively. The p test statistics for both NM and M were > 0.4 for A vs. B and < 0.01 for A vs. C and B vs. C. We concluded that at least as much M is generally excreted as NM. The quantity of either NM and M in each pheochromocytoma urine tested was greater than in normal urine. Three pheochromocytoma urine specimens contained no detectable M.

The Passive Transfer of Endotoxin Tolerance. SHELDON M. WOLFF, STANLEY B. WARD, AND PETER D. MOTT, Bethesda, Md. (introduced by James H. Baxter †).

The observation that induced resistance to endotoxin (endotoxin tolerance) can be passively transferred has focused attention on the role of humoral factors in this state. The duration of passively tranferred endotoxin tolerance is unknown. Rabbits (2 kg) were rendered tolerant by ten daily intravenous injections of 3 μg of *E. coli* endotoxin and exsanguinated on the eleventh day. The sera, sterile and pyrogen free, were pooled and injected into normal rabbits (20 ml per kg). Control animals received either normal or no sera. At frequent intervals until 35 days, separate groups of control and tolerant sera recipient rabbits were challenged with varying doses of endotoxin (1.0, 0.50, or 0.1 μg) and febrile responses measured. Pyrogenic tolerance to 1.0 μg of endotoxin disappeared by the fifth day. Significant tolerance ($p < 0.01$ *t* test) to both 0.5- and 0.1-μg doses persisted up to 25 days and disappeared between 30 and 35 days. These findings were obtained in four separate experiments. Antibody titers, as determined by bentonite flocculation, were measured on the donor sera before transfer and ranged from 1:1,024 to 1:2,048. Sucrose density gradient ultracentrifugation revealed that antibody in donor serum pools was mainly, but not solely, of the 19 S macroglobulin type. Before each challenge with endotoxin, recipients were bled and the titers of passively transferred antibody determined. Passively administered antibody persisted in the recipients' circulation up to 36 days and disappeared at an exponential rate (half-life of 4.1 days). In other experiments, no detectable antibody was found in sera obtained 24 hours after a single iv injection of 10 μg of endotoxin. However, these sera passively transferred some tolerance to normal recipient rabbits after challenge with 0.1 μg endotoxin. These studies suggest that the humoral factors responsible for the passive transfer of endotoxin tolerance are independent of serum antibody and persist for greater than 3 weeks.

Control of Epinephrine Biosynthesis by ACTH and Adrenal Glucocorticoids. RICHARD J. WURTMAN AND JULIUS AXELROD, Bethesda, Md. (introduced by Jan Wolff *).

The epinephrine content of the rat adrenal and of human urine declines after hypophysectomy. The activity of the adrenal enzyme that synthesizes epinephrine from norepinephrine (phenylethanolamine-N-methyl transferase, PNMT) also falls, reaching 10 to 15% of control levels within 1 week. The ability of the rat to make epinephrine can be restored by replacement doses of ACTH (i.e., doses of the order of magnitude needed to maintain adrenal weight), or by very large doses of glucocorticoids. ACTH elevates PNMT activity indirectly by stimulating the secretion of adrenal glucocorticoids. (PNMT activity is not inhibited in normal animals given doses of dexamethasone sufficient to cause adrenocortical atrophy.) These steroids then appear to induce the formation of the enzyme protein; their effect in the hypophysectomized animal can be blocked by actinomycin D or by puromycin. ¶ A dose of ACTH equipotent with a certain amount of hydrocortisone in depressing splenic weight is 100 times more potent in stimulating PNMT activity. This suggests that the enzyme is controlled by the level of glucocorticoid within the adrenal venous blood; hence, the location of the adrenal medulla within the adrenal cortex is an important factor in controlling the rate of epinephrine synthesis. (The medulla receives a major fraction of its blood supply from an intra-adrenal portal circulation; this blood has already drained the adrenal cortex.) ¶ It has been observed that the insulin supersensitivity of certain hypopituitary patients responds better to ACTH than to usual doses of glucocorticoids. It is possible that this is a consequence of the greater action of the polypeptide in restoring epinephrine synthesis.

Thyroxine as a Terminator of Chain Oxidations. JAMES WYNN,* Durham, N. C.

Investigation of Fe^{++}-induced oxidation of microsomes reveals that thyroxine prevents these oxidations. The rapid Fe^{++}-induced chain oxidatives are related solely to the oxidation of highly unsaturated lecithin. Such chain reactions are not initiated by peroxide or OH radical. They are presumably initiated by ferryl or perferryl radicals. The oxidative sequence is presumed to be of the following nature: $2\ RH + Fe(OH)_2^{++} \rightarrow 2\ R^{\cdot} + Fe^{+++} + 2\ H_2O$; $R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$; $ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$, etc. ¶ Thyroxine terminates these reactions by reacting with $R^{\cdot}$. In the presence of thyroxine there is no oxygen uptake, nor does thyroxine alter the rate or extent of air oxidation of Fe^{++}. It does not interact itself with Fe^{++}. Solutions containing molar ratios of Fe^{++} to lecithin to thyroxine of 500:500:1 are not peroxidized. These are small quantities of thyroxine and huge quantities of iron. The effect of thyroxine analogues on this process is directly related to their predicted ability to form stable free radicals. ¶ Since thyroxine degradation reactions are initiated in the presence of Fe^{++} and O_2, it is suggested that

the interaction with lecithin as a terminator of chain oxidation may be the initial reaction leading to degradation. Furthermore, a useful biologic function of thyroxine is described. It is a highly specific antoxidant in the preservation of unsaturated membrane lecithin.

Further Studies on the Hemolysis of Human Red Cells by Late-acting Complement Components. STANLEY YACHNIN,* Chicago, Ill.

Our previous studies have shown that red cells from patients with paroxysmal nocturnal hemoglobinuria (PNH-E) can be hemolyzed directly by late-acting complement (C′) components without the participation of C′1, C′4, or C′2. Since various enzyme-treated human red cells are also susceptible to acid hemolysis in human serum, we explored the effect of enzyme treatment upon the susceptibility of human red cells to lysis by partially purified late-acting C′ components. Trypsin, papain, ficin, and to a lesser extent, neuraminidase, were all capable of making red cells susceptible to direct lysis by late-acting C′ components and the reaction of enzyme-treated red cells with C′3a was in all respects analogous to that reported for PNHE. The intermediate complex PNH-EC′$_{3a}$ (terminology of Taylor and Leon) was originally made with a human C′3a reagent containing C′3, C′5, and C′6. Fractionation of human C′3a on hydroxylapatite columns revealed that C′3 (β_{1c}-globulin) was not involved in PNH-EC′$_{3a}$ formation, and that this intermediate complex could be designated PNH-EC′$_{5,6}$. Similar findings were made with enzyme-treated human red cells. Hemolysis of the various E-C′$_{5,6}$ complexes could be effected by dilute Na_3 HEDTA-serum or by partially purified C′3b (?C′8) + C′3c (?C′9). Of particular importance is the fact that in these lytic systems employing artificially altered red cells, the participation of heteroantibody or early complement components on the cell could be rigorously excluded. These findings suggest that the sensitivity of PNHE to late-acting C′ components is due to membrane damage. They also raise the possibility that early C′ components may act to impose changes upon the cell membrane, as well as participate in a cascade system of C′ component activation at the cell surface.

Hydroxyurea Inhibition of Histone Synthesis. J. W. YARBRO, Minneapolis, Minn. (introduced by I. D. Frantz, Jr.†).

Hydroxyurea is an effective agent for the treatment of chronic myelogenous leukemia and especially useful in patients whose disease has become refractory to busulfan. It has been shown to be a potent inhibitor of DNA synthesis in HeLa cell culture, mouse ascites tumor, and regenerating rat liver. It has little effect upon RNA or protein synthesis. When histone synthesis was studied in 6C3HED mouse ascites tumor by incorporation of lysine-^{14}C or ^{14}C-mixed amino acids, a reduced incorporation of isotope into acid soluble protein (histone) was observed in the absence of any effect upon saline soluble protein. Fractionation of the histones suggested that the lysine-rich fraction was the most markedly inhibited. Under similar conditions, orthophosphate-^{32}P incorporation into DNA was inhibited, whereas incorporation into RNA was not significantly depressed. These data are consistent with the hypothesis that hydroxyurea has a selective inhibition of the synthesis of one or more histone fractions in addition to its inhibition of DNA synthesis. That such an effect is not secondary to inhibition of RNA or general protein synthesis is indicated by the normal incorporation of ^{32}P into RNA and ^{14}C-amino acids into saline soluble protein in the presence of hydroxyurea. This suggests that either hydroxyurea has an independent effect upon histone synthesis in addition to its DNA effect, or that histone synthesis is in some way related directly to DNA synthesis. ¶ Whether the observed therapeutic effect of hydroxyurea against chronic myelogenous leukemia results from inhibition of DNA synthesis, histone synthesis, or both, is as yet undetermined.

Genetic Regulation of Apoferritin Synthesis in Rat Liver. YOSHIO YOSHINO, DAVID SCHACHTER,* AND JAMES MANIS, New York, N. Y.

The molecular mechanisms that activate or repress genes in mammalian cells are unknown. The following experiments demonstrate that the biosynthesis by rat liver of apoferritin, a well-characterized and crystallizable protein, is subject to genetic regulation and provides a tool for studying gene activation. ¶ We studied apoferritin synthesis by incubating slices of rat liver in a medium containing unlabeled amino acids plus L-leucine-^{14}C. Subsequently, the slices were homogenized, heated to 72° C for 10 minutes, centrifuged, and apoferritin in the supernatant precipitated with a rabbit antihorse spleen ferritin antiserum that had been shown to cross-react with crystalline rat liver ferritin. ¶ We estimated both the absolute quantity of immune precipitate and its radioactivity. ¶ Administration of iron (10 to 80 μg per g body weight iv, usually as iron-dextran) at least 2 to 4 hours before sacrifice increased incorporation of leucine-^{14}C into apoferritin *in vitro* five- to tenfold. Similarly great stimulation was observed with slices of spleen and lesser stimulation with duodenum and kidney. Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, was then given (1 to 4 μg per g body weight ip) 30 minutes before iron. At sacrifice, livers from actinomycin-treated animals contained 40 to 84% less apoferritin than controls treated with iron alone. On incubation, slices from actinomycin-treated rats incorporated 44 to 91% less leucine-^{14}C into apoferritin, with similar reduction in incorporation of uridine-^{14}C into RNA. Thus, gene-dependent synthesis of RNA is necessary for the enhanced synthesis of apoferritin in response to iron.

Massive Proteinuria Induced in Man by 2-Fluoroadenosine. C. W. YOUNG, S. HODAS, E. L. BECKER, AND D. A. KARNOFSKY,† New York, N. Y.

2-Fluoroadenosine (2-FAR), a cytotoxic antimetabolite selected as a potential anticancer drug, has produced toxic effects on the heart, blood vessels, liver, and kidney. Cardiovascular toxicity consisting of T-wave inversion, heart block, and hypotension was not apparent at drug infusion rates below 70 μg per minute. Three adults with advanced cancer who received 2-FAR by continuous iv infusion for a minimum of 5 days developed proteinuria (0.2 to 70 g per 24 hours). The first patient, who expired of pulmonary metastases 4 days after a course of 2-FAR (95 mg daily × 8), had massive proteinuria, urinary lipoid bodies, azotemia, hypercholesterolemia, and hypocalcemia. At postmortem examination there was fatty change in the liver and fatty degeneration of renal tubules with thickening and hypercellularity of the glomeruli. Two other patients developed reversible proteinuria after receiving 57 mg and 100 mg of 2-FAR daily × 5. The second of these showed azotemia, hypercholesterolemia, hypoalbuminemia, and edema. The clinical syndrome appears to be similar to that induced in rats and monkeys by the aminonucleoside of puromycin (AMN), another analogue of adenosine. The effects of 2-FAR and AMN upon synthesis of protein and nucleic acids were studied in HeLa cells with radioactive nucleosides and amino acids. 2-FAR (3.5 μmoles per L) inhibited protein synthesis within 2 hours; between 5 and 6 hours there was slight inhibition of RNA synthesis. AMN (mmoles per L) inhibited RNA synthesis immediately; inhibition of protein was detectable by 3 hours. DNA synthesis was not altered by either drug in 6 hours. We suggest that these drugs induce a disturbance of protein metabolism in glomerular and tubular cells *in vivo*. This may impair normal transport mechanisms, with resultant proteinuria.

Characterization of a Defect in Cortisol Metabolism in Cirrhosis and Its Reversible Reproduction by Norethandrolone (Nilevar). BARNETT ZUMOFF, H. L. BRADLOW, AND LEON HELLMAN,* New York, N. Y.

Previous studies from this laboratory had indicated a defect in peripheral cortisol metabolism in cirrhosis. This defect has now been specifically characterized and reversibly reproduced by administration of norethandrolone. Cortisol-^{14}C was given iv to 8 subjects with Laennec's cirrhosis and to 12 normal controls. The metabolites tetrahydrocortisol (THF), allotetrahydrocortisol (ATHF), tetrahydrocortisone (THE), cortols, and cortolones were measured in the neutral steroid extract after β-glucuronidase hydrolysis. Steroid glucuronide formation was subnormal (41 to 87% of normal) in all of the cirrhotics. THE was sharply decreased from normal (25% → 14%), and cortolones were increased comparably (19% → 35%). The sum of these two 11-ketometabolites remained normal. The 11-hydroxymetabolites, THF, ATHF, and cortols were normal. This specific distortion of the cortisol metabolite pattern was unrelated to the amount of glucuronide formation or to the presence or absence of a portacaval shunt. The fact that the pathway from cortisone to cortolones is augmented in the face of the decreased conversion of cortisone to THE may account for the previously reported finding that cortisone turnover is normal in cirrhosis, in contrast to an observed reduction in cortisol turnover. The administration of norethandrolone, 60 mg daily for 45 days, to a subject free of liver disease reproduced the characteristic cortisol metabolite pattern of cirrhosis without depression of glucuronide formation. The effect disappeared on drug withdrawal. Since both cirrhosis and norethandrolone produce similar ultrastructural changes in the bile secretory apparatus, this observation provides a model system for study of the mechanism of disturbed steroid hormone metabolism in cirrhosis.