ABSTRACTS

Comparison of Effects of Alpha Adrenergic Blockade on Resistance and Capacitance Vessels. FRANÇOIS M. ABBOUD* AND JOHN W. ECKSTEIN,* Iowa City, Iowa.

A foreleg of each of 20 dogs was perfused with blood at a constant rate through the brachial artery. The nerves of the brachial plexus were transected, and an electrode was applied to their distal ends. Pressures were recorded simultaneously from the brachial artery and cephalic vein and from a small artery and small vein in the paw. Pressor responses to injections of norepinephrine (1, 2, and 4 μg) into the brachial artery and to nerve stimulation (3, 6, and 12 cps) were measured before and after intra-arterial administration of 0.25 mg of phenoxybenzamine; measurements were repeated after a second dose of 0.5 mg. Increases in total foreleg vascular resistance that occurred in response to both norepinephrine and nerve stimulation were reduced moderately by phenoxybenzamine, but increases in venous resistance were blocked completely. ¶ Forearm blood flow (FBF) and forearm venous volume at a transmural pressure of 30 mm Hg (VV_{30}) were measured in 10 normal human subjects with a mercury-in-rubber plethysmograph. Observations on the responses of these parameters to infusions of norepinephrine (0.075 and 0.15 μg per minute) into the brachial artery were made before and after intra-arterial injection of 0.5 mg of phentolamine; observations were repeated after a second dose of 1.0 mg. Since blood pressure was unaltered, flow changes may be interpreted as changes in tone of resistance vessels. Changes in VV_{30} represent changes in compliance of veins or capacitance vessels brought about by changes in tone of venous smooth muscle. Decreases in FBF with norepinephrine were blocked only partially by phentolamine, whereas decreases in VV_{30} were blocked almost completely. ¶ In these experiments the alpha adrenergic blocking agents phenoxybenzamine and phentolamine were more effective in blocking venous resistance and capacitance responses to norepinephrine and nerve stimulation than they were in blocking prevenous responses to the same stimuli.

Human Platelet ATPase Activities: Relationship of Localization to Function. LOUIS M. ALEDORT, STANLEY B. TROUP, AND ROBERT I. WEED,* Rochester, N. Y.

This investigation was undertaken to relate the Na^+-K^+—activated ATPase activity of human platelets to the platelet ATPase activity known to be essential for clot retraction. The specific consumption of ATP by platelet ATPases was measured by the use of firefly luminescence. Inhibition of ATPase activities by ouabain, parachloromercuribenzoate (PCMB), and parachloromercuribenzenesulfonate (PCMBS) was studied. ¶ Membrane ATPase of osmotically prepared platelet "ghosts" is composed of Na^+-K^+-Mg^{++}—dependent ATPase (15 to 30%) and Mg^{++}-activated ATPase (60 to 85%). Ouabain (10^{-4} M), which inhibits the Na^+-K^+—dependent ATPase activity and produces a resultant loss of cellular K^+, gain of Na^+, and cell swelling, does not inhibit either ADP aggregation or clot retraction. ¶ PCMB, an organic mercurial compound that diffuses into cells, totally inactivates both ATPase activities in the ghost preparations and in the intact platelet. PCMBS, a highly polar, poorly diffusible agent, also inhibits platelet membrane ATPase activities as evidenced by a 35% cell swelling and the loss of up to 50% of the K^+ content, but does not inhibit clot retraction by the intact platelet. Both mercurials inhibited ADP aggregation. ¶ These observations indicate the existence of at least two distinct ATPase activities in the human platelet. The Na^+-K^+—dependent activity necessary for maintaining cation content and cell volume resides within the platelet membrane, can be inactivated by the PCMBS, but is not essential for clot retraction. ATPase activity essential for clot retraction, by contrast, can be localized beneath the outer membrane of the intact platelet and is unaffected by ouabain or PCMBS. The demonstration that PCMBS inhibits ADP aggregation but not clot retraction suggests that ADP aggregation may not be a necessary step in the process of clot retraction.

An Extrarenal Mechanism of Tolerance to Acute Potassium Loads. EDWARD ALEXANDER AND NORMAN G. LEVINSKY,* Boston, Mass.

Rats fed high potassium diets for several days usually survive acute potassium loads that are lethal to rats on regular diets. It is generally assumed that this tolerance is due to enhanced urinary potassium excretion. To study tolerance, we fed 150-g rats regular rat diet (unadapted) or diet containing 10% KCl (adapted) for 5 days. Food was withheld for 24 hours before experiment. Without an acute load, plasma K was equal: adapted, 4.2; unadapted, 4.4 mEq per L. Test rats were given KCl, 5 mEq per kg intraperitoneally; more than 90% of this was absorbed within 2 hours. In unadapted rats, plasma K was 5.8 ± 1.4 mEq per L 1 hour later and 5.0 ± 0.5 mEq per L 2 hours later. In adapted rats, plasma K was significantly lower: 5.0 ± 0.9 at 1 hour ($p < 0.05$) and 4.3 ± 0.5 at 2 hours ($p < 0.001$). Urinary K excretion was less in adapted than in unadapted rats: adapted, 274 ± 138; unadapted, 404 ± 131 at 2 hours ($p < 0.01$). To confirm the nonrenal mechanism suggested by these data, we performed studies on rats nephrectomized im-

* ASCI, active member.
† ASCI, emeritus member.
¶ New paragraph.

mediately before KCl loading. Plasma K was again lower in adapted rats 1 and 2 hours after loading: adapted, 4.4 ± 0.7 and 5.4 ± 0.8 mEq per L; unadapted, 5.6 ± 0.8 and 7.4 ± 2.1. The differences at both times were highly significant ($p < 0.01$). The plasma bicarbonate decreased 3 mmoles per L 2 hours after loading in both groups. Isolated diaphragms from adapted and unadapted rats maintained identical K contents at equal external K concentrations in Ringer's medium, suggesting that there is no intrinsic mechanism for adaptation. These experiments demonstrate a very potent nonrenal mechanism that regulates plasma K in adapted rats. Because of rapid lowering of plasma K by this mechanism, renal excretion is actually decreased in adapted rats during the first 2 hours after acute loading.

Effects of Left Ventricular Failure on the Pulmonary Circulation. J. K. ALEXANDER,* H. L. FRED, M. A. MODELSKI, D. A. GONZALEZ, AND J. A. BURDINE, JR., Houston, Texas.

In 14 patients with clinical and hemodynamic findings of left ventricular failure, structural changes in the pulmonary vasculature were identified by means of selective angiography, and pulmonary capillary blood flow was assessed by lung scanning after injection of ^{131}I macroaggregated albumin. Thirteen patients with a high calculated pulmonary vascular resistance and low cardiac output demonstrated the following angiographic alterations at the lung bases bilaterally or unilaterally: *1*) closer approximation of segmental vessels consistent with partial atelectasis, *2*) narrowing and retarded filling of the segmental arteries and veins, and *3*) abrupt attenuation of segmental branch vessels. Pulmonary capillary blood flow, as indicated by the scanning technique, was relatively reduced in the lower lobes of these patients, whereas normally flow to these regions is relatively increased. No change in the hemodynamic or angiographic findings took place during inhalation of 100% O_2 for 20 minutes. It has been concluded that with a high level of pulmonary vascular resistance and low cardiac output in left ventricular failure, reduction in perfusion as well as in vascular caliber of the lower lobes develops. These changes can be related to the regional distribution of interstitial edema, but not to hypoxia.

γA-Antibody with Anti-Gm Specificity in Rheumatoid Factor. JAMES C. ALLEN, Baltimore, Md. (introduced by Leighton E. Cluff *).

Human antibodies against γ-globulin occur as rheumatoid factor (RF) and as nonrheumatoid agglutinators specific for genetic (Gm and Inv) factors of γ-globulin. These antibodies have been found among γM- and γG-globulins. Anti-Gm hemagglutinating activity in RF may be distinguished by certain characteristics, especially its inhibition by Gm-negative heat-aggregated γG-globulin. Serum from a Gm(a-) patient with Sjögren's syndrome was found to be positive for RF in the latex-Fraction II fixation and Waaler-Rose tests. Agglutinating activity in Rh antibody/red cell systems was without prozone, was specific for the Gm(a) factor even when tested with an anti-Rh coat of broad specificity for rheumatoid factors, and was not inhibited by heat-aggregated Gm(a-) γG-globulin. Chromatography on Sephadex at low pH did not reveal hemagglutinating activity of other specificities. Waaler-Rose activity was apparently independent of anti-Gm specificity by inhibition studies. Anti-Gm(a) activity was removed by treatment with 0.1 M 2-mercaptoethanol and could not be inhibited by heat-aggregated Gm(a-) γG-globulin. This agglutinator was eluted in late fractions from DEAE cellulose and was found to sediment between the 19 S and 7 S regions in density gradient ultracentrifugation. Gm(a) specific agglutinating activity was absorbed by a specific anti-γA-antiserum used in the region of antibody excess. Studies of this unique serum offer a further confirmation of the antibody concept of human anti-γ-globulin activity by its demonstration in a third immunoglobulin class (γA). They provide a bridge between rheumatoid and nonrheumatoid anti-γ-globulin antibodies and present an example of defined isospecificity of hemagglutinating activity associated with classical rheumatoid factors.

Elevation Gradient of Lung Density. J. ALTOBELLI, R. ACKERLEY, S. E. KETNER, M. A. TATTERSALL, AND J. L. PATTERSON, JR.,† Richmond, Va.

In an earlier study, intrapleural pressure in the anesthetized dog, held vertical, was found to increase 0.21 cm H_2O for each cm of vertical distance. Mean lung density in the end-tidal position was 0.22 g per ml lung. The mechanism for the intrapleural pressure (Ppl) gradient is uncertain, but among the possibilities are *1*) the lung behaves as a homogenous fluid, or *2*) a vertical gradient of lung density exists that affects the Ppl. The present investigation explored this second possibility. Ten anesthetized (pentobarbital) mongrel dogs were studied after 45 minutes in the vertical head-up position, one in the horizontal, and one in the vertical head-down position. Circulation was stopped by electrical fibrillation and the trachea clamped and thorax entered; pulmonary vessels were clamped and lungs removed en bloc, wrapped in polyethylene sheet, and immersed in liquid nitrogen (−197° C). The trachea was unclamped to admit air as lung gas shrank. Later, the completely frozen lung was cut from apex to base into cubes 1 to 2 cm on each edge and the position of each cube measured. The density (weight/volume) of each cube was determined. A composite curve, fitted by least squares to the density vs. vertical distance data, had the formula, density $= 0.094\ e^{0.078H}$, where H = vertical distance from the apex in centimeters. This indicates a density at the apex of 0.094 g per ml and at the base 0.41 g per ml, for an apex-to-base distance of 20 cm. The apex-to-base density gradient was absent in the horizontal position and was anatomically reversed in the head-down position. This density gradient indicates major differences in ratios of lung gas to tissue plus blood at different elevations. It cannot be solely responsible for the linear gradient of Ppl vs. elevation.

The Chemical Plasticity of the Brain: The Effect of Environmental Changes on Brain Polysomes and Protein Synthesis. S. H. APPEL AND W. DAVIS, Philadelphia, Pa., and Durham, N. C. (introduced by J. B. Wyngaarden*).

Several experiments have associated brain RNA with information processing and storage. The capacity for brain protein synthesis *in vitro* is dependent upon ribosomal, messenger, and transfer RNA; yet it is unclear which of these species of RNA is associated with increased neural activity. ¶ Weanling rats were placed in the dark for 3 days, after which some were exposed to light for 10 minutes. Rates of protein synthesis *in vivo* in occipital cortex indicated a consistent 30 to 50% increase in the light-exposed animals compared to controls; but because of possible vascular and permeability changes, the availability of an RNA moiety may not have been the rate-limiting step in isotope incorporation into protein. ¶ Although there was no net increase in RNA, ribosomes isolated from the occipital cortex of light-exposed animals were found to contain 30% more polysomes and fewer single ribosomes than their dark-exposed controls as noted in patterns of sucrose density gradients and confirmed by electron microscopy. This effect was seen in the remainder of the brain as well as the occipital cortex, although it was not seen in ribosomes isolated from experimental and control livers. These brain ribosomes incorporated 60 to 90% more amino acid into protein per mg of ribosomal protein *in vitro* when compared with dark-exposed controls. Neither the levels of ribosomal RNA, t-RNA, or activating enzymes seem to have changed significantly in the two groups. At present, evidence is not available to determine whether synthesis of new messenger RNA has occurred. However, the aggregation of ribosomes into polysomes and the increased capacity for protein synthesis demonstrates more functioning messenger RNA and indicates at least one of the ways by which the brain may react to environmental changes and process information.

The Measurement of Thyrocalcitonin in Human and Pig Plasma by Radioimmunologic Means. CLAUDE ARNAUD AND TRAVIS LITTLEDYKE, Philadelphia, Pa., and Ames, Iowa (introduced by Howard Rasmussen*).

Homogenous thyrocalcitonin (TCT) isolated from porcine thyroid glands was conjugated to rabbit albumen by carbodiimide condensation and was used to produce antibodies in a rabbit to this new peptide hormone. Immunoelectrophoretic and gel diffusion studies showed that the antibody was specific for TCT. The antiserum in a dilution of 1:20,000 with homogenous, high specific activity ^{131}I-labeled TCT was employed to develop a radioimmunoassay for the measurement of TCT in plasma. A modification of the Berson-Yalow technique was used. Antibody was incubated at 4° C with unlabeled TCT or an unknown plasma sample for 4 days and then with the ^{131}I-labeled hormone for 18 hours. After separation, the ratio of the values of the antibody bound to free ^{131}I TCT decreased progressively with increasing concentrations of added unlabeled TCT. Reproducible standard curves were obtained, and a lower limit of sensitivity of 0.5 nanogram per ml was achieved. The curves produced by immunoassay of purified TCT and by dilutions of porcine thyroid vein plasma and human peripheral vein plasma were virtually the same. This suggests reactions of complete immunologic identity between the isolated porcine hormone and endogenous hormone in porcine and in human plasma. Precise recovery studies have not been possible because all of the plasmas examined to date have contained immunoassayable thyrocalcitonin. The concentration of TCT in the plasma of 25 human subjects with normal levels of plasma calcium varied from 30 to 85 nanograms per ml with a mean of 54. Plasma TCT levels appeared to be higher in older than in young pigs. In the latter, TCT increased from 170 to 350 nanograms per ml during induced hypercalcemia and to 800 nanograms per ml during vigorous direct thyroid gland massage. Thyroidectomy was followed by a decrease to levels significantly lower than control.

Abnormal Metabolism of Vitamin D_3 in Vitamin D-resistant Rickets and Familial Hypophosphatemia. LOUIS V. AVIOLI, JOSEPH E. MCDONALD, AND T. FRANKLIN WILLIAMS, Jersey City, N. J., and Chapel Hill, N. C. (introduced by Walter Hollander, Jr.*).

One hypothesis advanced to explain the pathogenesis of hypophosphatemia and skeletal disease in vitamin D-resistant rickets (VDRR), and persistent hypophosphatemia in genetically related adults (FamH), is a disorder of vitamin D metabolism. Five to 10 μc of tritiated vitamin D_3 (D_3-^{3}H) was therefore administered intravenously to each of ten normal subjects, four patients with VDRR, and three adult relatives with persistent hypophosphatemia, and its metabolic fate studied for the subsequent 48 to 72 hours. D_3-^{3}H and metabolites thereof were extracted from plasma with chloroform, separated by thin layer and methanol-ether gradient elution column chromatography, and identified by gas chromatographic techniques. Twenty-four hours after D_3-^{3}H injection, D_3-^{3}H accounted for 67.4 (±3.9), 82.8 (±7.3), and 94.1 (±3.9) per cent of the total lipid soluble plasma radioactivity in normal subjects, FamH, and VDRR, respectively. The relative decreased rate of vitamin D metabolism in VDRR and FamH was also reflected by a corresponding decrease of 48 (±3.7) and 8 (±2.4) per cent in D_3-^{3}H fractional turnover times. In normal subjects two D_3-^{3}H chloroform soluble metabolites (peak II and peak III), each of greater polarity than vitamin D_3, accounted for 7.4 (±3.6) and 25.2 (±4.8) per cent of plasma lipid soluble radioactivity, respectively. One metabolite (peak III) retained vitamin D activity by biological "line testing" techniques. In patients with VDRR peak II and peak III were both significantly decreased in plasma, accounting, respectively, for 3.7 (±1.2) and 2.2 (±0.7) per cent of total lipid soluble radioactivity. Peak II and III changes in FamH were similar but less severe than those observed in VDRR. These observations provide evidence of abnormal vitamin D metabolism in VDRR and FamH and suggest

that "resistance" to vitamin D in VDRR and persistent hypophosphatemia in genetically related FamH may both result from a defect in the conversion of vitamin D to biologically active metabolites.

Effects of Small Amounts of Carboxyhemoglobin on Oxygen Transport. STEPHEN M. AYRES AND STANLEY GIANNELLI, JR., New York, N. Y. (introduced by Donald B. Louria *).

The effects of small amounts of carboxyhemoglobin (COHb) on oxygen transport were studied by administering measured volumes of carbon monoxide (CO) to six normal subjects and five dogs. Arterial and mixed venous blood was sampled and analyzed for P_{O_2}, P_{CO_2}, and pH by electrochemical techniques. Oxygen and CO contents were measured by a gas chromatographic method. In contrast to accepted theory, arterial P_{O_2} decreased linearly as COHb was raised from 3.9 to 80.5% saturation (change in $P_{O_2} = 0.87$ COHb + 0.42, $r = 0.99$, $p < 0.001$). Mixed venous P_{O_2} decreased proportionately more than arterial. Alveolar ventilation was unchanged and venous admixture kept constant by frequent maximal inflations. The mean alveolar-arterial and alveolar-end capillary oxygen gradients, measured while breathing 14% O_2, averaged 27 and 4 mm Hg in three dogs. After elevation of COHb to 28%, the gradients increased to 56 and 30 mm Hg. Tissue P_{O_2}, estimated by reverse Bohr integration, correlated closely with mixed venous P_{O_2}. Over-all equilibrium constants, calculated from *in vivo* hemoglobin dissociation curves, increased with increasing P_{O_2} during control periods but decreased with increasing P_{O_2} in the presence of COHb. We suggest that COHb decreases the reaction rate of the residual reduced hemoglobin with oxygen in the pulmonary capillary, producing an alveolar-end capillary "diffusion" gradient. A similar decrease in the dissociation rate explains the low tissue and mixed venous P_{O_2}. Since as little as 5% COHb significantly decreases arterial and mixed venous oxygen tensions, smokers and nonsmoking city dwellers, who may have as much as 15% COHb, probably have chronic tissue hypoxia.

The Effect of Dietary Purines on the Purine Excretion of a Xanthinuric Subject. JOHN H. AYVAZIAN AND SOLOMON SKUPP, New York, N. Y. (introduced by Robert H. Green †).

The purine excretion of a xanthinuric man was studied while he was maintained on a purine-free diet supplemented for intervals of 7 days each by yeast RNA, 3′-guanylic acid (GMP), and 3′-adenylic acid (AMP). The purines of each daily urine were separated, identified, and quantitated by chromatographic, enzymatic, and spectrophotometric methods. During the 7 days of the purine-free diet his daily oxypurine output averaged 2.27 mmoles; 1.91 was xanthine and 0.36 hypoxanthine. For 7 days supplementary RNA added 1.2 mmoles of purine to his daily diet; his oxypurine output averaged 2.88 mmoles per day; 2.48 was xanthine and 0.40 hypoxanthine. While he received 0.6 mmole of 3′-GMP daily, an equivalent increase in urinary purines was observed, and his daily oxypurine excretion averaged 2.96 mmoles; 2.59 was xanthine and 0.37 hypoxanthine. No change in his purine excretion was noted while he ingested 0.6 mmole of 3′-AMP daily; his oxypurine excretion averaged 2.26 mmoles, of which 1.92 was xanthine and 0.34 hypoxanthine. There were no significant changes in the urinary excretion of the other purines, including guanine and adenine, throughout the study. Statistical analysis of the data confirms the significance of the increments noted in the total oxypurine and xanthine fractions of the RNA and 3′-GMP periods as compared to the purine-free and 3′-AMP periods. During the 3′-AMP study the subject was given 10 μc of 3′-AMP-8-^{14}C orally. The ^{14}C excretion was similar in rate and purine distribution to a previous study when adenine-8-^{14}C was given intravenously, indicating the 3′-AMP was readily absorbed. These studies demonstrate that this subject retains the adenine component of ingested RNA or 3′-AMP and excretes, as xanthine, the guanine component of RNA and 3′-GMP. The failure to observe any change in urinary purines while he was ingesting 3′-AMP implies an equivalent inhibition of the *de novo* synthesis of purines.

Suppression of Vulnerability to Induced Ventricular Fibrillation after Coronary Occlusion by Chemical Sympathectomy. MARVIN BACANER, Minneapolis, Minn. (introduced by Paul Aggeler †).

In the course of other experiments it was observed that taking a single biopsy from the ventricle of open chest but otherwise intact dogs immediately produced ventricular fibrillation. In contrast, fibrillation never occurred despite repeated biopsies taken over many hours from the totally isolated blood-supported heart removed from the chest but beating spontaneously. Believing that the invulnerability of the isolated heart to fibrillation might be related to its sympathetic denervation, we explored the efficacy of bretylium tosylate (whose action is roughly that of a chemical sympathectomy) in suppressing induced fibrillation in intact hearts both with and without coronary ligation. Control episodes of ventricular fibrillation were induced in open chest dogs by applying an electrode pair (1 cm apart) to the ventricle. The fibrillatory stimulus consisted of a continuous train of unidirectional 2.5 msec pulses at a repetition rate of 100 pulses per second; each pulse had a peak current of 20 to 40 ma. Before treatment (with or without coronary occlusion) fibrillation was invariably induced by touching the ventricle with the electrode for 0.25 to 0.35 second. Fibrillation could be terminated by counter shock from an a-c difibrillator. After administration of bretylium (20 mg per kg intravenously) the ventricles tolerated hundreds of applications of the fibrillatory stimulus for periods ranging from 3 (minimum) to 300 times the control duration without fibrillating. If fibrillation was induced by prolonged stimulation of the protected ventricle, it would usually revert to sinus rhythm as soon as the electrode was removed or continue fibrillating for a few seconds (up to 20) before spontaneously reverting to sinus rhythm.

Since no discernible toxic effects were observed, this drug may have potential clinical value in prevention or treatment of ventricular fibrillation or both, as well as providing some insight into its mechanism.

Studies on Distribution and Excretion of Human Urokinase in Man. FEDOR BACHMANN, St. Louis, Mo. (introduced by Gerald T. Perkoff *).

Urokinase is a potent plasminogen activator extracted from human urine. Although it is of considerable interest physiologically, studies of its fate in man are incomplete. In the present study highly purified urokinase (Abbott Laboratories, specific activity > 35,000 CTA U per mg) was administered to three patients after cholecystectomy and T-tube drainage; one also had thoracic duct cannulation. An additional patient with complete biliary obstruction also was studied. After 420,000 to 500,000 CTA U was injected intravenously over a 10-minute period, clearance from plasma ($t_{\frac{1}{2}}$) and distribution into urine, bile, and, in one, into thoracic duct lymph were determined. In the three cholecystectomized patients $t_{\frac{1}{2}}$ was 5 to 9 minutes (^{125}I clot lysis test), 6 to 11 minutes (fibrin plate test), and 7.5 to 13 minutes (euglobulin lysis). Minimal activity (<25% that of plasma) was found in lymph for 10 minutes after infusion of urokinase. Total recovery of urokinase in urine was less than 2% after 6 hours. Activity was first detected in bile 5 minutes after injection; peak activity in bile appeared after 35 to 60 minutes, was two to three times higher than the highest activity in plasma, and persisted for 140 to 190 minutes. Recovery in the bile was only 5 to 8%, but the T-tube did not allow complete collection. Guinea pig antiurokinase antiserum inhibited bile activity completely in the ^{125}I clot test. In the patient with complete biliary obstruction, $t_{\frac{1}{2}}$ was significantly prolonged (15, 16, and 47 minutes, respectively). These findings provide the first direct evidence that urokinase is cleared by the liver and excreted into the bile. The high urokinase bile/serum activities suggest that the liver has an active transport system for urokinase and perhaps for other physiological activators of the thrombolytic system.

Structural Aspects of Human Antierythrocyte Antibodies: Light and Heavy Chain Determinants. RICHARD F. BAKEMEIER AND JOHN P. LEDDY, Rochester, N. Y. (introduced by John H. Vaughan *).

In extension of our studies of human γG erythrocyte autoantibodies, only κ light chains have been detected in ten cases, only λ light chains in five, and both types in eight. Some of the populations "monotypic" for light chains were quite homogeneous electrophoretically. Twenty-two populations of γG isoantibodies have exhibited both light chain types with two exceptions. One anti-Rh_0 (D) and one anti-rh' (C) population each revealed only κ light chains; the anti-Rh_0 population also showed electrophoretic homogeneity. This suggests that apparent structural homogeneity of antibodies is not necessarily pathologic. ¶ By use of myeloma proteins typed by W. Terry, specific monkey or rabbit antisera have been prepared to the four known heavy chain subclasses of γG-globulin: γ_{2a} (Ne), γ_{2b} (We), γ_{2c} (Vi), and γ_{2d} (Ge). Concentrated preparations (50 to 430 μg N per ml of γG) of six autoantibodies and six isoantibodies have been studied by micro-Ouchterlony technique. All twelve samples contained γ_{2b}-globulins. $Gamma_{2a}$ molecules were also detected in one autoantibody and one isoantibody population. $Gamma_{2c}$ molecules were detected in another autoantibody and another isoantibody population, respectively. Several weak reactions with anti-γ_{2d} serum cannot yet be interpreted with confidence. One autoantibody population in which only κ light chains were detected contained at least two heavy chain subclasses. Because the γ_{2b} subclass markedly predominates in normal γG-globulin, the apparent absence of γ_{2a} and γ_{2c} molecules from a given antibody population may reflect limited sensitivity of the method. More sensitive methods are currently being applied to these and to other erythrocyte antibodies. Of significance may be the failure to find autoantibody populations either lacking γ_{2b} or with clear predominance of γ_{2a} or γ_{2c}. ¶ Thus, from the present data, erythrocyte autoantibodies and isoantibodies may not differ in representation of heavy chain subtypes. From evidence of others that the content of light chain type or types in an antibody population may be influenced by the immunizing antigen, it is possible that the much greater tendency of autoantibodies than isoantibodies to show restricted representation of light chain types may reflect differences in the stimulating antigens.

Erythrocyte Catalase Activity in Thalassemia. STANLEY P. BALCERZAK AND WALLACE N. JENSEN,* Pittsburgh, Pa.

Increased erythrocyte catalase activity per gram hemoglobin or per milliliter red cells was found in 32 of 32 patients with thalassemia minor, and in 2 of 7 patients with thalassemia major. Thalassemia trait was associated with increased catalase activity even when combined with a qualitatively abnormal hemoglobin, sickle hemoglobin. Increased enzyme activity was not found in patients with sickle cell disease, iron deficiency, or a wide variety of other hematologic conditions except in one patient with sideroblastic anemia and in his apparently healthy daughter. Four other patients with sideroblastic anemia had normal activity. ¶ The increased catalase activity could not be explained as a reflection of a younger red cell population. Neither was the increased enzyme activity simply relative to diminished hemoglobin production. ¶ This evidence suggests that increased erythrocyte catalase activity may be used to identify the thalassemia trait. The results add a new point of similarity between thalassemia and certain types of sideroblastic anemia. Finding of normal to increased activity of this heme-containing enzyme in thalassemia major and minor provides indirect evidence that reduced heme synthesis is not the limiting defect causing the reduction in hemoglobin synthesis.

S-Adenosylmethionine in the Liver after Portacaval Venous Anastomosis. Ross J. Baldessarini and Josef E. Fischer, Bethesda, Md., and Boston, Mass. (introduced by Seymour S. Kety †).

Pathologic changes in the hepatic parenchyma may follow portal-systemic venous anastomoses. In man the problem is complicated, since liver disease is usually present before such operations, and may continue. In animal studies, metabolic alterations have been noted, but the mechanisms involved are not fully clarified. ¶ S-Adenosylmethionine (SAMe, "active methionine") is a major donor of methyl groups for mammalian transmethylation reactions, which alter the biological activity of many endogenous materials, and administered substances. The liver is the major site of SAMe utilization and its enzymatic formation from methionine and ATP. ¶ After portacaval anastomoses in rats, tissue levels of SAMe and methionine-activating enzyme were assayed postoperatively for 3 months. SAMe was measured by a sensitive and specific enzymatic, isotope dilution technique. The activating enzyme was measured *in vitro* by the formation and isolation of SAMe-^{14}C from methionine-^{14}C and ATP. After end-to-side, but not side-to-side, portacaval shunts, rats lost then regained body weight and had smaller livers, with SAMe decreased in concentration and total amount. Brain levels were normal. When methionine supplements were administered, marked elevation of hepatic SAMe occurred equal to normal. Direct measurements of methionine-activating enzyme's activity confirmed that it functions normally. ¶ A major factor producing the metabolic defect in question appears to be the lack of an essential substrate. Depriving the liver of nutrient-rich portal blood appears to be capable of producing the observed defect. In patients, portal-systemic anastomoses, which retain some portal flow, might be expected to induce less hepatic metabolic damage. Furthermore, modest dietary methionine supplements might benefit patients with portacaval anastomoses.

Role of Excess α-Chain Synthesis in the Pathogenesis of β Thalassemia. Arthur Bank and Paul A. Marks,* New York, N. Y.

This study defines the kinetics of hemoglobin synthesis in β thalassemia by measuring the rates and extent of formation of specific globin chains (^{14}C amino acid incorporation) in erythroid cells of thalassemic and nonthalassemic subjects. Complete separation and quantitation of α, β, and γ chains were achieved with a carboxymethylcellulose chromatography system. In cells of thalassemic subjects, β-chain synthesis is markedly decreased (<20% of nonthalassemic values); γ-chain synthesis is variable, being increased in amount per reticulocyte in several patients. Most strikingly, α-chain synthesis is markedly greater than β-chain synthesis. In contrast, in nonthalassemic cells, α-chain synthesis equals β-chain synthesis. ¶ Thalassemic reticulocytes have four- to tenfold as much radioactivity incorporated into α chains as into β chains during 1 hour of incubation. Further, radioactivity incorporated into α chains exceeds that into β chains plus γ chains by 30 to 80%. Over 95% of the total radioactivity incorporated into α chains is recovered from the supernatant fraction of the cells, indicating that these chains have been released from the ribosomes. ¶ The excess of α chains may exist as Hbα4, an unstable component reported by Fessas in cells of patients with β thalassemia. Alternatively, excess α chains may be bound to unlabeled β chains. This seems unlikely since the pool of β chains is too small to combine with the excess of α chains. The excess α chains might predispose cells that contain them to rapid preferential removal from the circulation. This would account for the failure to detect accumulations of unlabeled α chains in peripheral red cells, whereas newly synthesized α chains are demonstrable in these cells since they have not yet been destroyed. Such a mechanism could explain the increased rate of hemolysis of erythroid cells in β thalassemia.

Direct Measurement of Regional Ventilation/Perfusion Ratios after Pulmonary Embolism. Harry Bass, Nicholas R. Anthonisen, and Thomas Heckscher, Montreal, Canada (introduced by David V. Bates *).

Measurements of regional ventilation/perfusion ratio, regional perfusion, and regional ventilation have been made on eight supine patients with ^{133}Xe. Studies were performed 2 to 24 days after the last documented episode of pulmonary embolism. Eight to ten collimated scintillation counters were positioned over different lung regions. All patients breathed ^{133}Xe in air from either an open or closed circuit. ^{133}Xe in saline was then injected as a continuous constant volume infusion during tidal breathing and as separate slug injections during breath holding. Regional ventilation/perfusion ratios, regional flow per unit volume, and regional ventilation per unit volume were calculated for each separate counter zone. The relative regional ventilation per unit lung volume was calculated from these data and also computed from regional washout curves. Embolized areas of lung were found to be characterized by high ventilation/perfusion ratios and a low perfusion per unit lung volume. The data did not show a systematic decrease of ventilation per unit volume in embolized regions unless they were also infarcted. Serial studies in one patient showed a constant level of ventilation in embolized regions both during hypoperfusion and after recovery. Concomitant airway disease did not interfere with the localization of emboli. Macroaggregated iodinated albumin lung scans performed at similar times in six of the patients showed the same areas of decreased perfusion as had been mapped with ^{133}Xe.

Left Ventricular Ejection Fraction and Efficiency in Heart Disease. William A. Baxley, Harold T. Dodge,* and Richard R. Hawley, Seattle, Wash.

Previous studies indicate a large total left ventricular (LV) O_2 consumption relative to LV work (low efficiency) in patients with known myocardial disease. A

small stroke volume (SV) relative to end-diastolic volume (EDV), or low ejection fraction (SV/EDV), has also been demonstrated in patients with myocardial disease. In this study the SV/EDV and LV efficiency were compared in 32 patients with heart disease of various etiologies. Coronary blood flow and LV oxygen consumption per 100 g per minute were determined by the nitrous oxide method after coronary sinus catheterization. LVEDV (116 to 490 ml), SV (42 to 328 ml), and mass (106 to 609 g) were determined from biplane angiocardiograms by previously described methods. Total LV O_2 consumption (12.8 to 55 ml per minute) was calculated as LV mass/100 × LV O_2 consumption/100 g. LV work (4.23 to 31.1 kg-m per minute) was calculated as mean systolic ejection pressure × SV × heart rate per minute. LV efficiency is LV work per energy equivalent of LV O_2 consumption. For all patients SV/EDV (0.10 to 0.77) and LV efficiency (4.1 to 40.0%) were significantly correlated ($r = 0.51$, $p < 0.1$). In 5 patients with nonobstructive cardiomyopathy, SV/EDV (0.10 to 0.30) and LV efficiency (4.1 to 19.4%) were low. In 25 patients with valvular heart disease, SV/EDV (0.39 to 0.74) and LV efficiency (8.6 to 40.0%) were compared. Of 9 patients with valvular heart disease and low SV/EDV (< 0.5), 8 had LV efficiency < 18%, and of 16 with SV/EDV > 0.5, 14 had LV efficiency > 20%. These data demonstrate that patients with primary myocardial disease have excessive LV hypertrophy and O_2 consumption relative to LV work as well as a low ejection fraction. Furthermore, these data suggest that determination of LV ejection fraction and efficiency provides a method for evaluating LV myocardial function in patients with hemodynamic abnormalities due to valvular heart disease.

Protein Synthesis by Reticulocytes: Presence of a Subribosomal Pool of Stimulatory Nucleoprotein. N. Shelley Beard, Steven Armentrout, and Austin S. Weisberger,† Cleveland, Ohio.

A subribosomal fraction capable of stimulating the incorporation of amino acids into protein in mammalian cell-free systems has been described by Hoagland. This fraction (X fraction) has been obtained from rat liver homogenates by prolonged high speed centrifugation of postmicrosomal supernatant fractions and contains stimulatory activity unrelated to amino acid-activating or transfer enzymes. The present report concerns a similar fraction obtained from reticulocytes and presents evidence that this fraction promotes the formation of ribosomal aggregates that are highly efficient in protein synthesis. ¶ Reticulocytes were obtained from phenylhydrazine-treated rabbits, and the X fraction was sedimented from postribosomal supernatants by centrifugation at 105,000 × g for 12 hours. These crude fractions stimulated cell-free protein synthesis by reticulocyte ribosomes approximately threefold when assayed with optimal concentrations of activating enzymes and had no activity in the absence of ribosomes. Sedimentation of this fraction through 5% to 20% sucrose gradients in 0.0001 M Mg^{++} and 0.010 M Tris pH 7.8 revealed the presence of two subribosomal particles and a separate peak of stimulatory activity with a sedimentation value of less than 10. Removal of interfering substances by this technique increased the stimulatory effect of X fraction to more than 15-fold. Sephadex gel filtration also isolated a single peak of stimulatory activity that had approximately the same stimulatory capacity as that obtained from sucrose gradients. The 260 to 280 absorbance ratio of these active peaks was approximately 1.5. Electron micrographs demonstrate that the X fractions promote the formation of ribosomal aggregates in preparations that contain predominantly single ribosomes. ¶ The precise role of this fraction in reticulocyte protein synthesis requires further study. The evidence presented here suggests that it may function in initiating or stabilizing ribosomal aggregates essential for protein synthesis. The possibility also remains that X fractions may contain a pool of unattached messenger RNA.

Methionine Biosynthesis in Leukemic Leukocytes. J. R. Bertino * and A. R. Cashmore, New Haven, Conn.

5-Methyltetrahydrofolate (5-MeFH_4) and a coenzyme form of vitamin B_{12} are known to be required for methionine biosynthesis. Since the formation of 5-MeFH_4 from N^5-N^{10}-methylene FH_4 is physiologically irreversible, it has been postulated that methionine biosynthesis may serve to regulate cellular FH_4 levels, inasmuch as this is the only known mechanism whereby 5-MeFH_4 may be converted to FH_4. It was of interest, therefore, to determine whether methionine biosynthesis could occur in mammalian tissues other than liver, and in particular, in human leukocytes. With the L1210 murine leukemia as a model system, an enzyme(s) that forms methionine and FH_4 from 5-MeFH_4 and homocysteine has been purified twofold by ammonium sulfate fractionation (30 to 55% saturation) and DEAE cellulose chromatography. Methionine formation has been measured by a radioassay in which the incorporation of the methyl group of methyl-^{14}C labeled 5-MeFH_4 into methionine is measured and also by a microbiological assay of methionine with *Leuconostoc mesenteroides*. A catalytic requirement for S-adenosylmethionine, similar to that for the pig liver system, has been demonstrated. A reducing agent, such as 2-mercaptoethanol, is also required. Cyanocobalamin, under certain conditions, stimulates the rate of methionine formation. Attempts to demonstrate a direct involvement of methylcobalamin in the reaction are in progress. Enzyme activity has been found in leukocyte extracts from patients with acute and chronic granulocytic leukemia as well as from normal leukocytes. These studies demonstrate that 5-MeFH_4 may be converted by leukemia cells to FH_4 and provide support for the concept that the methionine synthetizing system plays a regulatory role in folate metabolism.

17-Hydroxylation Deficiency in Man. EDWARD G. BIGLIERI,* San Francisco, Calif.

A deficiency of 17-hydroxylation was deduced from the study of an adult genotypic female whose attendant alterations in endocrine function consisted of hypercorticosteronism and lack of estrogen production. Salient clinical features were frequent respiratory infections, 20 years of diastolic hypertension, primary amenorrhea, and absent secondary sex characteristics. Serum electrolyte concentrations in mEq per L were as follows: sodium, 148 to 152; potassium, 2.2 to 2.8; chloride, 95 to 102; CO_2, 29 to 35. Urinary Porter-Silber chromogens, 17-ketosteroids, and tetrahydro 11-desoxycortisol were not detectable; urinary androsterone (0.150 mg per 24 hours), etiocholanolone (0.200 mg per 24 hours), and dehydroepiandrosterone (0.150 mg per 24 hours) were markedly diminished. ¶ By isotope dilution and derivative techniques, plasma cortisol (F) was not detected; plasma corticosterone (B) was 26 μg per 100 ml (N, 1 μg per 100 ml); progesterone was 0.21 μg per 100 ml. The secretory rates of F were 0 (N, 10 to 30 mg per 24 hours); of aldosterone, 10 μg per 24 hours (N, 50 to 170 μg per 24 hours); and of B, 112 mg per 24 hours (N, 0.9 to 4.0 mg per 24 hours); urinary aldosterone was less than 1 μg per 24 hours; tetrahydro 18-OH compound A was 675 μg per 24 hours (N, less than 50 μg per 24 hours). Plasma ACTH was slightly elevated, plasma renin activity was absent, and gonadotropins were elevated. Vaginal cytology showed no estrogen effect. Treatment with 1 and 2 mg per day of dexamethasone effected a natriuresis, potassium retention, correction of the hypokalemic alkalosis and hypertension, reduction of the secretory rate of B to 3.8 mg per 24 hours, and reduction of plasma B to 5 μg per 100 ml. Aldosterone excretion and secretion still remain virtually absent on this treatment. ¶ From the study of this new syndrome the following important observations are made: *1*) hypercorticosteronism, *2*) correctable ACTH-dependent hypertension, *3*) *in vivo* evidence of the key role of 17-hydroxylation in both ovarian and adrenal hormone synthesis, *4*) an additional variant of the hydroxylation defects observed in congenital adrenal hyperplasia.

Inhibition of Intestinal Absorption by Agents That Affect the Kidney. HENRY J. BINDER, HOWARD M. SPIRO,* AND RICHARD P. SPENCER, New Haven, Conn.

The amino acid transport defects in cystinuria and Hartnup disease suggest a functional similarity between the renal tubules and the small intestine. Further evidence of the similarity in the transport system in these morphologically distinct organs was sought by studying the ability of nonelectrolyte transport systems in the gut to function in the presence of agents that inhibit or modify renal tubular transport. Transport of a neutral amino acid, a basic amino acid, glucose, and a pyrimidine was evaluated by the everted gut sac technique (hamster) in the presence of probenecid, chlorothiazide, and ethacrynic acid. ¶ The transport of L-lysine (10^{-4} M), L-methionine (10^{-6} M), glucose (2×10^{-3} M), and uracil (5×10^{-6} M) was inhibited more than 80% by 5 mM ethacrynic acid. The inhibition of L-lysine absorption by ethacrynic acid was dose related and noncompetitive, but could not be prevented by ergothioneine (a sulfhydryl compound). The inhibition of nonelectrolyte transport by ethacrynic acid is possibly secondary to inhibition of cation transport. ¶ The presence of probenecid at a concentration of 5 mmoles per L, but not at 0.5 mmole per L, caused inhibition of nonelectrolyte transport by 38 to 53%. This was also dose related and noncompetitive. Chlorothiazide (2 mM) did not result in any significant inhibition of L-lysine, L-methionine, glucose, or uracil transport. ¶ This study suggests further functional similarities between the transport systems of the small intestine and the renal tubules.

Triglyceride Levels in Familial Hypercholesterolemia. DAVID H. BLANKENHORN,* JULIUS JENSEN, AND VALDEMAR KORNERUP, Los Angeles, Calif., and Varde, Denmark.

Triglyceride is one of the major determinants of serum lipoprotein mobility. Triglyceride levels are therefore important in classifying hypercholesterolemic phenotypes for genetic study. An important recent hypothesis is that elevated triglyceride levels appear in all or none of the affected individuals of hypercholesterolemic kindreds. At present an insufficient number of family studies of triglyceride level in familial hypercholesterolemia is available to confirm or deny this hypothesis. ¶ We have completed a 20-year follow-up of 12 large Danish hypercholesterolemic families first reported by Kornerup. These families comprise approximately one-eighth of all hypercholesterolemic families reported since 1939. Concurrent triglyceride and cholesterol levels have been determined in 185 family members, 98 with the hypercholesterolemic trait. ¶ Triglyceride levels were grossly elevated in all hypercholesterolemic members of one family. Triglyceride levels were in the range seen in normal individuals in all members of the remaining 11 families. In the 11 normotriglyceridemic families, triglyceride levels of all hypercholesterolemic individuals, averaged by family, were the same. However, in 5 of these 11 families, hypercholesterolemic individuals had higher average triglyceride levels than normocholesterolemic members of the same family. These findings clearly confirm that part of the hypothesis of Fredrickson and co-workers which postulates that if elevated triglyceride level does not occur in all affected members of a hypercholesterolemic family, none of the kindred will have them. These findings also suggest two additional hypotheses: *1*) It may be possible to subdivide hypercholesterolemic families with normal triglyceride levels into two new classes by comparison of triglyceride levels of hypercholesterolemic and normocholesterolemic individuals within each family, and *2*) triglyceride levels in hypercholesterolemia may represent a continuum with present typing schemes classifying outstanding members as separate types.

Potassium Dependence of Growth Hormone-activated Lipolysis *In Vitro*. SHELDON J. BLEICHER, Brooklyn, N. Y. (introduced by David M. Kydd †).

Regulation of lipolysis is generally viewed in terms of direct hormonal interactions on fat tissue. That cation flux might mediate, or modify, hormonally induced lipolysis *in vitro* was inferred from previous studies in which spontaneous lipolysis was found to be two to three times greater in all-potassium than in all-sodium media. One hundred μg per ml bovine growth hormone (BGH) failed to induce lipolysis in rat epididymal fat tissue incubated in glucose-free all-sodium media. However, less than half this concentration (40 μg per ml) of BGH or ovine growth hormone (OGH) effected a two- to fourfold increase in lipolysis above control in mixed sodium-potassium media. Equimolar substitution of lithium or choline for potassium canceled this lipolytic response to either BGH or OGH. Ouabain (10^{-3} M) wholly blocked BGH-activated lipolysis in mixed sodium-potassium media, whereas EDTA (1 mg per ml) did not impair it. Penetration of ^{42}K into adipose tissue fragments was increased 15% above control by 40 μg per ml BGH. Insulin (0.2 U per ml), although without effect on lipolysis in tissue incubated in all-sodium media, reduced lipolysis in, and ^{42}K penetration into, adipose tissue incubated in glucose-free all-potassium media. Thus, lipolysis induced by low concentrations of growth hormone *in vitro* specifically requires the presence of potassium in the incubation medium, proceeds by a mechanism inhibitable by ouabain but unaffected by EDTA, and is associated with increased penetration of potassium into adipose tissue. Insulin, in contrast, inhibits both potassium-activated lipolysis and potassium penetration into fat tissue. These experiments suggest that the reciprocal roles of growth hormone and insulin in regulating lipolysis may relate, at least in part, to their effects on potassium entry into adipose tissue.

An Inherited Serum Isoantigen in Leukemia and Down's Syndrome. BARUCH S. BLUMBERG,* Philadelphia, Pa.

A "new" serum isoprecipitin system has been described that is readily distinguishable from the first isoprecipitin system (beta-lipoprotein) described in our laboratory. The protein cannot be identified with any of the characterized serum proteins. It appears to contain lipid and has the mobility of α-globulin on agar immunoelectrophoresis. It was detected with specific antibody developed in the serum of a multiply transfused patient. Specific antibody has also been produced by rabbit immunization. ¶ Since the antigen was first studied in the serum of an Australian aborigine, it has been tentatively given the geographic name "Australia antigen" (Au) until it can be better characterized. Au is rare in U. S. populations (1 per 1,000) but is relatively common (approximately 6 per 100) in Southeast Asia populations and in some South American Indian and Mediterranean populations. Recent family studies in Southeast Asia are consistent with the hypothesis that Au is inherited as a simple autosomal recessive trait. ¶ Although very rare in normal and most diseased U. S. populations Au is present in about 15% of acute myelogenous leukemia patients and less commonly in other leukemia patients. It is also present in about 25% of patients with Down's syndrome, a disease characterized by a high risk of developing leukemia and leukemia-like syndromes. The protein has also been found in patients with other diseases in which the reticuloendothelial system or leukocytes or both are involved (i.e., Hodgkin's disease and Fanconi's anemia). ¶ The present findings are consistent with the hypothesis that Au(1) is an inherited protein associated with increased susceptibility to some forms of leukemia, Down's syndrome, and, possibly, other diseases.

Human Leukocyte Pyrogen after Activation of Blood Cells *In Vitro*. PHYLLIS T. BODEL AND MORRIS DILLARD, New Haven, Conn. (introduced by P. K. Bondy †).

The role of leukocytes in mediating clinical or experimental fever in man is not known. In rabbits, various stimuli release pyrogen from polymorphonuclear leukocytes *in vitro*, and a pyrogenic substance can be detected in the blood of febrile rabbits. However, in man, circulating pyrogen has not been found during fever, and most attempts to demonstrate leukocyte pyrogen have been unsuccessful. Injection of certain naturally occurring steroids produces fever in man, but their mode of action is unknown. In a search for possible mechanisms of microbial and steroid-induced fevers in man, human blood leukocytes were incubated *in vitro* with gram-negative bacterial endotoxin, heat-killed staphylococci, and several steroids, including etiocholanolone, androsterone, lithocholic acid, and sodium taurolithocholate. A leukocyte-rich fraction was obtained from heparinized blood by dextran sedimentation, and most erythrocytes were removed by hypotonic lysis. After incubation in a 15% Krebs-Ringer phosphate buffer for 18 hours, the supernatant fluids were injected into rabbits. Supernatants from control leukocytes produced little or no fever. However, a pyrogen was released from cells that had been incubated with endotoxin, staphylococci, or serum-buffer solutions of etiocholanolone, lithocholic acid, and sodium taurolithocholate. Like rabbit leukocyte pyrogen, the pyrogenic material obtained from human leukocytes produced fevers of early onset and brief duration in rabbits and was inactivated by trypsin. These data provide evidence that human leukocytes can be activated by various stimuli to release pyrogen and hence may mediate certain fevers in man.

Humoral Regulation of Neutrophil Release from Bone Marrow. DANE R. BOGGS, JOHN C. MARSH, AND GEORGE E. CARTWRIGHT,† Salt Lake City, Utah.

Vinblastine sulfate transiently interrupts neutrophil production, and in dogs blood neutrophil concentration falls sharply at 4 days, is rising again by 6 days, and is

above normal by 10 days. Factors in plasma that influence neutrophils were searched for by harvesting plasma from vinblastine-treated dogs and determining the effect of infusing 15 ml per kg upon the neutrophil concentration of normal recipients. ¶Normal plasma did not influence the recipient's neutrophil concentration significantly (21 infusions). Plasma obtained 2, 4, 10, and 20 days postvinblastine was similarly ineffective (17 infusions). However, plasma obtained 6 and 8 days postvinblastine produced prompt neutrophilia (2-, 4-, and 6-hour postinfusion neutrophil concentration after 31 infusions equaled 156% of control). Similar neutrophilia-inducing activity (NIA) was demonstrable in 6-day post-HN2 plasma (4 infusions) but not in 10-day post-HN2 plasma (4 infusions). ¶ Postvinblastine plasma NIA could not be ascribed to cortisol, epinephrine, or endotoxin, for it differed qualitatively and quantitatively in its effect upon neutrophils and other leukocytes. Furthermore, response to plasma NIA was undiminished by induction of endotoxin tolerance in recipients. ¶ Endotoxin, given intravenously, produces prompt neutropenia, but at approximately 1.5 hours postendotoxin, neutrophils begin to increase and by 4 hours marked neutrophilia is present. Plasma harvested during the first hour or 4 to 5 hours after endotoxin did not produce neutrophilia in recipients (13 infusions), but 1.5- to 4-hour postendotoxin plasma did (16 infusions = 181% of control). In donors rendered tolerant to endotoxin by daily injections, transferable plasma NIA disappeared as donors stopped developing neutrophilia. ¶ Accelerated release from marrow was the mechanism whereby plasma NIA produced neutrophilia, as judged by morphologic studies and infusion of ^{32}DFP-labeled neutrophils. The close correspondence of the presence of demonstrable NIA in plasma with developing neutrophilia in the donor suggests that plasma NIA may represent a physiologic, humoral regulator of neutrophil release.

Serum Coagulation Factors in Renal Disease. JAIME BORRERO, MARGARET E. TODD, AND E. LOVELL BECKER, New York, N. Y. (introduced by David D. Thompson *).

Fourteen patients with the nephrotic syndrome secondary to glomerulonephritis and eight patients with uremia were studied. There were significant differences between a control group and patients with the nephrotic syndrome. Increased fibrinogen concentration, accelerated thromboplastin generation test (TGT), increased antihemophilic globulin (AHG Factor VIII) assays, and altered thromboelastograms were observed. The silicone clotting times and the heparin tolerance tests were prolonged. The platelet adhesive index was not significant in nine patients, and prothrombin times in undiluted plasma were not significant in the 14 patients with the nephrotic syndrome. ¶ The patients with uremia had prolonged silicone clotting times, accelerated TGT, increased fibrinogen values, and shorter prothrombin times. The changes observed were all significant. The thromboelastograms, the heparin tolerance test, and the platelet adhesiveness were not significant, and the AHG concentrations in eight patients were too variable to be significant. ¶ The results of the studies made on these two groups of patients suggest a hypercoagulable state, but certain alterations are interpreted as indicative of activation of antithrombin mechanisms.

Beat-to-Beat Changes in Hemodynamics and Ventricular Work during Reactive Hyperemia in Subjects with Ganglionic Blockade. JAMES T. BOTTICELLI AND RAMON L. LANGE,* Milwaukee, Wis.

Previously, indicator dilution methods indicated that, in human subjects with ganglionic blockade (TEAC, 5 mg per kg), rapid release of arterial tourniquets applied to the upper thighs for 15 to 20 minutes is followed in 1 minute by increased stroke work (SW) and stroke volume (SV) that persists for up to 15 minutes although vascular resistance (R) returned toward control. These changes are associated with initial mild increased heart rate (HR) followed by pronounced cardiac slowing. These effects are shortened by tourniquet reapplication 1 to 2 minutes after release, suggesting humoral stimulation. ¶ Information on the immediate effect of release was desirable. The aortic pressure contour method was employed to estimate beat-to-beat changes in cardiac performance during the first minute after release in six subjects. The average of 3 beats immediately after release provided reference values. Change (%) in HR, SV, SW, R, cardiac output (CO), stroke power (SP), the ratio of systolic duration to cycle length (TS/RR), and the slope of the ascending aortic pressure curve (S) was determined ± standard error of the mean for 5 successive beats within 10 seconds of release and after aortic pressure had returned toward prerelease levels (15 to 25 seconds). R decreased slightly (-11.8 ± 1.4), although aortic pressure increased. Other variables increased: HR, 7.0 ± 2.4; SV, 23.0 ± 4.0; CO, 33.0 ± 2.7; SW, 40.0 ± 4.0; SP, 37.0 ± 3.0; S, 22.0 ± 4.0; TS, $+2.4 \pm 1.9$; and TS/RR, $+8.6 \pm 0.4$. Changes begin earlier (10 to 15 seconds) and persist longer than post-tourniquet hyperpnea. The rapid application of automatic tourniquets precludes volume trapping as a cause of altered ventricular work. Alterations in ventricular filling pressure do not correlate with CO, SV, and SW (indicator dilution method). We suggest that humoral substances produced in ischemic tissue are responsible for the biphasic chronotropic and positive inotropic effects and further imply these may contribute to the cardiac response to exercise.

The Biphasic Effect of ACTH on Repressed RNA Synthesis in Adrenal Cortex. E. D. BRANSOME, JR., La Jolla, Calif. (introduced by W. P. VanderLaan †).

Changes in nucleic acid metabolism may not be required for many of the immediate effects of ACTH on aldrenal cortex, but the differentiated information allowing response to this small molecule might be presumed to be transcribed in RNA. We have investigated RNA synthesis in guinea pig adrenal cortex with short *in vivo*

exposure to uridine-^{32}P or 5-uridine-^{3}H. After nuclei and cytoplasm were separated, nucleic acids were extracted with detergent-phenol treatment and fractionated by MAK column chromatography. We have regarded RNA specific radioactivity as indicative of synthetic rate and have found considerable heterogeneity in the synthesis of different RNA. ¶ A stimulation of the synthesis of several cytoplasmic and many nuclear RNA fractions is evident 15 minutes after ACTH administration, is maximal in less than 1 hour, and persists for 12 hours. In animals with endogenous ACTH suppressed by dexamethasone treatment, RNA specific radioactivity also increases but falls below control levels in several hours, remaining significantly depressed for several days. This "repressive" phase of ACTH action, as well as the return to base line in normal animals, is antagonized by additional exposure to ACTH. If a small amount of actinomycin D is injected, the rate of adrenocortical RNA synthesis (and steroid biosynthesis) is first depressed but then increases for several days, suggesting that some RNA moieties are involved in repression and that RNA synthesis is normally in a state of partial repression. Comparison of the relative amounts of cytoplasmic RNA fractions from adrenals "derepressed" with actinomycin or stimulated with ACTH, with those of controls and of steroid-treated animals exhibiting exaggerated repression after ACTH, suggests that information specifying and modifying the response to ACTH may be regulated through the biphasic effect of the hormone upon heterogeneous repression of RNA synthesis.

Genetic Aspects of Autoimmune Disease in NZB/NZW Mice. IRWIN M. BRAVERMAN AND JOAN MARINO, New Haven, Conn. (introduced by Allan V. N. Goodyer †).

NZB/NZW hybrid mice provide an experimental model for lupus erythematosus: LE cell phenomena, renal disease, antinuclear factor (ANF), positive Coombs' test, and hemolytic anemia. Three hundred hybrids of F_1, F_2, and F_3 generations 1 year of age and older were tested for ANF, Coombs' positivity, and LE cell phenomena. Data indicate that all F_1 mice have one or more serologic abnormalities and histologic evidence of glomerulonephritis. Seventy-eight per cent and 72% of F_2 and F_3 mice, respectively, exhibit identical findings. Serologically normal F_2 and F_3 mice (22% and 28%, respectively) have renal disease demonstrated by conventional histologic techniques. NZW mice are not completely normal as has been thought: the majority exhibit histologic evidence of glomerulonephritis including deposition of γ-globulin in glomeruli demonstrated by immunofluorescent techniques. The renal lesions in the F_2 and F_3 mice appear histologically to be less severe than those occurring in the F_1 generation and the NZB parent. Ten animals in the F_1 generation showed spontaneous disappearance of serologic abnormalities after 12 to 15 months of age. Classic LE cells in the absence of ANF were found in ten animals. The following genetic hypothesis is offered: the NZB genotype is AAKK, and the NZW, aaKK, where A represents the gene for autoimmune serologic markers, and K the gene for renal disease. Both are autosomal dominants. This formulation will account for the observed incidences of serologic and renal abnormalities. However, the possibility of an infectious agent inducing autoimmune disease in genetically susceptible mice has not been excluded.

The Role of Carnitine and Carnitine Acyltransferase in Biological Acetylations. R. BRESSLER * AND K. BRENDEL, Durham, N. C.

The use of acetyl CoA (AcCoA) for fatty acid synthesis (FAS) and the acetylation of sulfanilamide has been shown to be an extramitochondrial process, whereas the production of AcCoA from pyruvate occurs within the mitochondria. The AcCoA formed must be transferred across the mitochondrial barrier, and it has been proposed that the process is not a direct one, but one in which AcCoA condenses with oxaloacetate (OAA) within the mitochondria to form citrate that is either oxidized to CO_2 via the tricarboxylic acid (TCA) cycle or diffuses out of the mitochondria where the citrate cleavage enzyme (CCE) reconverts it to AcCoA and OAA. It has also been shown that AcCoA formed within the mitochondria may be converted to acetylcarnitine (AcCarn) in the presence of acetyl CoA-carnitine acyltransferase (CAT) and carnitine, and that AcCarn serves as a direct transport form of activated acyl groups that diffuse out of the mitochondria and are reconverted to AcCoA by CAT in the presence of coenzyme A. ¶ Pigeon liver homogenates (PLH) converted citrate-1-^{14}C and pyruvate-2-^{14}C to $^{14}CO_2$ and either fatty acid (FA)-^{14}C or acetylsulfanilamide (AcS)-^{14}C. The production of $^{14}CO_2$ from pyruvate-2-^{14}C occurs by the entry of pyruvate to the TCA cycle via citrate, where it becomes part of a pool from which AcCoA is generated by the CCE. On the basis of $^{14}CO_2$ produced it was shown that over 80% of FAS from pyruvate occurred via citrate and the CCE. ¶ The addition of (−)-carnitine to PLH augmented the conversion of pyruvate-2-^{14}C to FA-^{14}C and AcS-^{14}C by a noncitrate pathway. The addition of (+)-carnitine, a competitive inhibitor of CAT, caused a marked decrease in AcS-^{14}C production, whereas it stimulated the conversion of pyruvate-2-^{14}C to $^{14}CO_2$. This suggested that (−)-carnitine and CAT play a role in AcCoA transfer out of the mitochondria and that CAT inhibition results in a shunting of AcCoA to oxidation. ¶ In homogenates from fasted pigeons FAS and CCE activity were markedly decreased, whereas AcS formation and CAT activity were increased. These data suggest that carnitine and CAT play an important role in the translocation of AcCoA for biological acetylations, rather than for FAS.

Mechanism of the Hemodynamic Changes Associated with Angiocardiography. J. DAVID BRISTOW,* FRANK E. KLOSTER, GEORGE A. PORTER, AND HERBERT E. GRISWOLD, Portland, Ore.

Intracardiac injection of radiocontrast agents produces a prompt rise in cardiac output (CO), a fall in systemic

arterial blood pressure (SAP), and hence a decrease in sytemic vascular resistance. These changes were evaluated in 14 intact dogs anesthetized with pentobarbital. The contrast agent, 80% sodium iothalamate, was injected through a retrograde catheter into the left ventricle in doses of 0.5 ml per kg. With 17 control injections, the mean SAP fell 19 mm Hg. During the hypotension the CO rose an average of 1.07 L per minute, or 49.9%. Arterial serum osmolality increased 59.4 mOsm per kg a few seconds after injection and then fall rapidly. Sixteen injections of contrast agent were given during ganglionic blockade with trimethaphan. CO increased 70% but mean SAP did not fall; rather systolic SAP rose 12 mm Hg ($p < 0.01$). Similar results were obtained with sodium iothalamate injections after SAP was raised to normal levels by constant infusion of norepinephrine during ganglionic blockade. The alterations of SAP with sodium iothalamate before and during ganglionic blockade were reproduced to a lesser degree by sudden injections of 50 ml of isotonic saline into the inferior vena cava. These observations demonstrate several mechanisms in the hemodynamic response to this radiocontrast agent. There is an abrupt expansion of intravascular volume in response to the marked increase in serum osmolality. Venous return to the heart is augmented and the CO rises. The increased intravascular volume is buffered, however, by an autonomic vasodepressor mechanism resulting in a decrease in SAP despite the elevated CO. A final component of the decreased systemic vascular resistance might be direct loss of water content of arteriolar walls due to the marked, although transient, osmotic gradient.

Biochemical Mechanism of Muscle Relaxation: Relaxing Factor in Right and Left Heart and in Tonic and Phasic Muscles. IRWIN A. BRODY, Durham, N. C. (introduced by Albert Heyman †).

The binding of calcium ions to sarcoplasmic reticulum constitutes a muscle-relaxing factor, since it deprives the myofibrils of calcium necessary for contraction. After contraction, phasic muscles, such as gastrocnemius, undergo a greater magnitude of relaxation than tonic muscles, such as soleus. Similarly, myocardial relaxation is normally of greater magnitude in the left ventricle than in the right. In the present study, relaxing factors of right-ventricular and left-ventricular myocardium and of phasic and tonic skeletal muscles were compared in order to examine the relation between physiologic performance and the biochemical mechanism of muscle relaxation. Left and right ventricles, gastrocnemius, and soleus from freshly sacrificed adult guinea pigs were each homogenized and ultracentrifuged. The microsomal fractions, containing sarcoplasmic reticulum, were incubated in a solution labeled with ^{45}Ca. The reaction mixtures were then passed through Millipore filters, and the amount of calcium bound to microsomes was calculated from the radioactivity of the filters measured in a liquid scintillation counter. Significantly greater calcium binding was found in left ventricle than in right and in gastrocnemius than in soleus. The mean values were as follows: left ventricle, 0.5 μmole Ca per mg microsomal protein; right ventricle, 0.3; gastrocnemius, 1.5; soleus, 0.7. This is the first report of a difference between relaxing factor of left and right ventricles. The greater calcium binding found in the left ventricle parallels its greater magnitude of relaxation. In contrast to a previous report of a twentyfold difference between the calcium binding of rabbit gastrocnemius and soleus, the twofold difference reported here in guinea pigs corresponds to the magnitude of known electrophysiologic and biochemical differences between these muscles. This study suggests that the ability of a muscle to undergo relaxation is related to the capacity of the sarcoplasmic reticulum to bind calcium.

Autoimmune Hemolytic Anemia: Failure of Normal and Neoplastic Lymphocytes to Produce Red Cell Iso- and Autoagglutinins. JEROME I. BRODY * AND LAWRENCE H. BEIZER, Philadelphia, Pa.

The purpose of this study was to determine whether malignant lymphocytes, postulated as being the source of erythrocyte-coating proteins in certain categories of autoimmune hemolytic anemia, actually are capable of synthesizing red cell antibody in the form of type-specific isohemagglutinins or Coombs-positive globulins. Peripheral blood lymphocytes were collected from normal volunteers, individuals who had recently been immunized with type-specific blood group substances, and patients with chronic lymphocytic leukemia and lymphocytic lymphoma with positive and negative Coombs' tests. The cells were grown in suspension cultures in media fortified with autologous and homologous AB plasma from a compatible single donor. Each triplicate set of vials contained no stimulant, lyophilized parallel blood group substance, and phytohemagglutinin (PHA), the latter serving as a prototype blastogenic agent. On the fifth day, colchicine was added to arrest mitosis several hours before termination of the cultures. After centrifugation, all supernatants were dialyzed for concentration purposes and were tested for direct type-specific hemagglutination and reactivity against the Coombs antiglobulin reagent with appropriate erythrocytes. The stained cellular sediments were examined quantitatively for lymphocyte blast-cell transformation and mitotic figures as indicators of antigenic response. In all instances, the postculture media were singularly free of all forms of detectable antibody, and blastogenesis and mitotic figures were seen only when PHA was used as a stimulant. The appearance of the normal lymphocytes, theoretically sensitized *in vivo* during immunization with blood group substance, remained unchanged from the unstimulated control cells. These observations suggest that normal lymphocytes probably are not a source of isoagglutinins, that neoplastic lymphocytes produce neither specific nor Coombs-reactive red cell-sensitizing antibody, and that the present clonal hypothesis of autoimmune hemolytic anemia in lymphoproliferative disorders should be re-examined on this basis.

Hormonal Control of Enzymes Mediating Lipid Synthesis from Carbohydrates. JOSIAH BROWN, PAT MCLEAN, AND A. L. GREENBAUM, Los Angeles, Calif., and London, England (introduced by David H. Solomon *).

To determine the influence of pituitary and thyroid hormones on the synthesis of fat from carbohydrate, we determined the activity of enzymes mediating important metabolic pathways. Hexokinase, malic enzyme, isocitric dehydrogenase, and citrate cleavage enzyme were measured in the soluble fraction of the cell. The effect of hypophysectomy or thyroidectomy and subsequent administration of physiological doses of thyroxin, ACTH, or luteinizing hormone (LH) was determined. The tissues studied were rat adipose and liver, which synthesize triglycerides, and testis and adrenal, which synthesize steroid hormones. Dietary control of the animals was obtained by limit feeding and addition of glucose to the drinking water. Results: *1*) In adipose tissue, hypophysectomy was followed by a fall in activity of the four enzymes to approximately 20% of control. Thyroxin, 5 μg per day, returned these to normal or above. *2*) In liver, hypophysectomy or thyroidectomy lowered citrate cleavage to 40% of control. Thyroxin returned this to normal in thyroidectomized but not hypophysectomized rats. Comment: The activity of these enzymes in adipose tissue and liver is closely related to the availability of thyroxin. Hypophysectomy results in additional influences on liver not reversed by thyroxin. *3*) In adrenal and testis, hypophysectomy resulted in a fall in activity of the four enzymes in proportion to the decrease in weight of the organ. Thyroxin had no effect on enzyme activity in either organ. ACTH injected with thyroxin stimulated adrenal citrate cleavage and malic enzymes fourfold and hexokinase twofold without significant change in adrenal weight. LH in addition to thyroxin stimulated testis citrate cleavage significantly. In summary, thyroxin appears to strongly control the activity of enzymes mediating important pathways of lipid synthesis from carbohydrate in adipose tissue and liver. In contrast, thyroxin alone does not affect the activity of these enzymes in adrenal and testis, but addition of the trophic hormone results in dramatic stimulation. These studies emphasize the important role of thyroid hormone in the control of fat synthesis.

The Positive Inotropic Effect of Acetylcholine on Ventricular Myocardium and Its Relation to Cardiac Norepinephrine Stores. ROBERT A. BUCCINO, EDMUND H. SONNENBLICK, THEODORE COOPER,* AND EUGENE BRAUNWALD,* Bethesda, Md., and St. Louis, Mo.

Although it is generally held that vagal stimulation depresses the contractility of the intact ventricle, acetylcholine has been reported to have variable effects on isolated ventricular myocardium. It has been proposed that it exerts both a direct negative inotropic effect and an indirect positive, adrenergic-like action. Moreover, Burn and Rand have suggested that acetylcholine is the actual mediator for norepinephrine release. To examine this hypothesis critically we compared the effects of acetylcholine (1×10^{-3} to 3×10^{-2} μg per ml) on normal myocardium and on cardiac tissue previously depleted of norepinephrine. Isometric force changes were determined in atrial myocardium and papillary muscles removed from nine normal cats and ten cats whose cardiac norepinephrine stores had been totally depleted by chronic cardiac denervation. Unexpectedly, acetylcholine exerted a dominant positive inotropic effect on ventricular myocardium, increasing isometric force an average of 0.80 g per mm^2 in normal and 0.79 g per mm^2 in denervated muscles. This effect was not blocked by treatment of the muscles with atropine or propranolol. In contrast, acetylcholine consistently reduced the force developed by isolated atrial tissue, whether beating spontaneously or at a constant rate; 1 μg per ml acetylcholine lowered force by 56% and 45% of control in normal and norepinephrine-depleted atria, respectively, whereas atropine shifted the acetylcholine dose-response curve to the right. In conclusion, acetylcholine exerted a predominantly positive inotropic effect on isolated ventricular myocardium, and since this effect was observed in the absence of norepinephrine, it cannot be attributed to norepinephrine release. Thus, these finding are not consonant with the Burn and Rand hypothesis that acetylcholine mediates the release of norepinephrine. In addition, these studies indicate the necessity for reassessing the role of the parasympathetic transmitter in the regulation of ventricular performance, and provide a unique demonstration of a substance having opposite inotropic effects on atrial and ventricular myocardium.

The Control of Renin and Aldosterone Secretion: Influence of Sodium and Potassium Balance and Changes in Blood Volume. MARCIA B. BULL, ROBERT S. HILLMAN, PAUL J. CANNON, AND JOHN H. LARAGH,* New York, N. Y.

Factors influencing renin activity and aldosterone secretion were investigated in six normal subjects under conditions of controlled metabolism. Sodium depletion, induced by dietary deprivation and diuretic administration, produced a mean negative balance of 164 mEq. Mean blood volume (RISA and ^{51}Cr) declined from 5,625 to 5,260 ml. This was associated with a four- to fivefold serial increase in aldosterone secretion (mean values rising from 186 to 841 μg per day) and a rise in plasma renin levels from 5.2 to 27.5 nanograms per ml per 4 hours. ¶ Potassium supplements further increased aldosterone secretion (mean change +147 μg per day). This occurred with variable changes in renin (mean −7.9 nanograms per ml per 4 hours), slight increases in blood volume (+135 ml), and no significant change in sodium balance. These results indicate that potassium stimulates aldosterone secretion in man by a separate, and perhaps direct, effect on the adrenal gland without involving renin and angiotensin. ¶ To effect a dissociation between changes in sodium balance and blood volume, we

combined serial plasmaphereses or phlebotomies with the concurrent administration of salt, producing a markedly positive sodium balance (mean +237 mEq) in the face of a sustained lowering of the blood volume by a mean of 640 ml. This procedure regularly produced 50% reductions in both aldosterone secretion and plasma renin activity. In all studies it was established that changes in renin activity were not the result of changes in either renin substrate concentration or angiotensinase activity. ¶ This demonstration that sodium loading suppresses renin and aldosterone secretion during maintained hypovolemia implies that changes in available sodium ion are of greater importance than are changes in the absolute intravascular volume in the regulation of renin and aldosterone secretion.

Dynamic Exchange of Intact Hemoglobin Hemes. H. FRANKLIN BUNN AND JAMES H. JANDL,* Boston, Mass.

A unique plasma pigment that accumulates during severe hemolytic processes in man is the ferriheme-albumin complex, methemalbumin. To investigate how intact heme is detached from globin and transferred to albumin under natural conditions and whether free heme is released normally during hemoglobin catabolism, we isolated labeled human and animal hemoglobins from reticulocyte suspensions that had been incubated with transferrin-^{59}Fe, glycine-2-^{14}C, and leucine-^{14}C. The variously labeled hemoglobins were then isolated, and mixtures of normal hemoglobins or of hemoglobin and albumin were separated by ion exchange chromatography or electrophoresis on cellulose acetate. Under physiologic conditions heme readily transferred to human albumin from methemoglobin but not from methemoglobin that was bound to haptoglobin, or from cyanmethemoglobin, oxyhemoglobin, or carboxyhemoglobin. Although the calculated affinity of human albumin for ferriheme was less than that of human globin, it considerably exceeded that of animal albumins, which formed little if any methemalbumin. The hemes of human methemoglobin and methemalbumin reached a complete exchange equilibrium within a few hours of admixture. When labeled human hemoglobins A and F were admixed under physiological conditions, their intact hemes exchanged continuously when in the oxidized (methemoglobin) form but were "fixed" to the globins by reduction to hemoglobin, by external ligands (cyanide) or by internal ligands (hemoglobin M_{Boston}). Intermolecular heme exchange followed first order kinetics, was markedly temperature dependent, and was accelerated by blocking globin thiols. Iron as such did not exchange, nor did globin subunits. These studies establish that hemes of intact normal human hemoglobin move rapidly from molecule to molecule when in the reversible ferriheme state of methemoglobin. This remarkable property permits the formation of methemalbumin when haptoglobin is absent and may be critical to heme metabolism in general and to the initial phase of hemoglobin catabolism.

Biologic Support of Hepatic Coma. J. M. BURNELL, E. D. THOMAS,* AND W. VOLWILER,† Seattle, Wash.

In 1963 a favorable response to human cross circulation by a patient in deep hepatic coma from massive liver necrosis encouraged further investigation. Three subsequent patients were managed by cross circulation through indwelling Silastic-Teflon arteriovenous cannulas (unidirectional flows approximately 150 ml per minute). Patient I had four 4-hour exchanges during 3 days (144 L). After the first 4 hours of cross circulation the patient awakened and did well for 15 days only to develop gastrointestinal hemorrhage and die. In patient II technical problems limited cross circulation to a total exchange of only 28 liters without improvement during the 4 days preceding death. In patient III an initial 6-hour cross circulation (54 liter exchange) alleviated coma. After 13 additional 1-hour cross circulations (15 liters) the patient recovered. All patients showed prompt biochemical improvement; between each cross circulation bilirubin rose and prothrombin time was prolonged. ¶ Donors included one cancerous patient and three normal volunteers. No donor exhibited encephalopathy. All donors developed fever, malaise, and thrombocytopenia. One developed dermatitis and severe transient bronchospasm after 5 days. Two had postural hypotension. No persistent sequelae were observed. ¶ An additional patient was supported for 5 days by daily exchange transfusions of 5 liters. Coma was not ameliorated, and the patient died of hemorrhage. ¶ Human cross circulation appears to provide life-saving support in acute hepatic coma. The initial support needed to reverse coma may require hours of cross circulation. The lack of serious sequelae observed in donors and the magnitude of problems associated with profound coma suggest that earlier use would yield greater survival. Despite this initial favorable experience, the decisions regarding selection of donors and their ultimate safety remain difficult and complex.

Digoxin-specific Antibodies. VINCENT P. BUTLER, JR., BERNARD F. ERLANGER, AND JAMES P. CHEN, New York, N. Y. (introduced by Charles Ragan†).

Digoxin (Dig) was conjugated covalently to bovine serum albumin (BSA) by the periodate oxidation method of Erlanger and Beiser. Rabbits immunized with synthetic BSA-Dig conjugates formed antibodies capable of binding Dig-^{3}H. ¶ When Dig-^{3}H (0.45 μc, 4 μc) was added to a 1:5 dilution of anti-BSA-Dig serum from rabbit DB-6 and dialyzed against an equal volume of Tris-buffered saline, pH 7.5, at 4° C for 5 days, less than 0.1% of the added radioactivity was found outside the dialysis bag. When Dig-^{3}H was added to a 1:5 dilution of preimmunization serum from this same rabbit and dialyzed under similar conditions, 49.5% of the added radioactivity was found outside the dialysis bag. Fourteen of 15 anti-BSA-Dig sera showed significant binding of Dig-^{3}H; less than 1% of the added Dig-^{3}H diffused outside the dialysis bag with 1:5 dilutions of 12 of these antisera. Preimmunization sera from these animals and

antisera to other antigens permitted 44 to 51% of the added radioactivity to diffuse outside the bag under comparable conditions. Purified γ-globulin from anti-BSA-Dig serum DB-6 was shown to possess significant digoxin-binding capacity. Nonradioactive digoxin was a very potent inhibitor of the binding of Dig-^{3}H by antiserum DB-6; digitoxin was a good inhibitor. Oubain was a poor inhibitor, and cortisol, corticosterone, and cholesterol possessed negligible inhibitory capacities. ¶ When 0.03 μc (0.3 μg) Dig-^{3}H was added to a 1:640 dilution of antiserum DB-6, approximately 90% of the Dig-^{3}H was protein bound. This binding of radioactivity by anti-Dig antibody was inhibited almost completely by 4 μg of nonradioactive digoxin. As little as 0.01 to 0.1 μg of nonradioactive digoxin exhibited detectable inhibition of binding of radioactivity, and it is hoped that this inhibitory capacity of nonradioactive digoxin may prove useful in the development of an immunological assay for plasma digoxin. It is also hoped that digoxin-specific antibodies may prove to be of value in the amelioration of severe digitalis toxicity.

Ineffective Atrial Systole in Mitral Stenosis. RICHARD A. CARLETON AND JOHN S. GRAETTINGER,* Chicago, Ill.

Our previous studies showed slight changes in cardiac output when patients with mitral disease converted from atrial fibrillation to sinus rhythm. Subsequent studies in patients without valvular disease (non-MS) showed progressively greater atrial contributions and shorter hemodynamically optimal P-R intervals with increasing heart rate. The optimal P-R interval of 0.15 second for a rate of 100 per minute produced a nearly maximal atrial contribution of 27%. In this study, the atrial contribution in mitral stenosis (MS) has been investigated. ¶ Pressures and Fick outputs were measured during dual pacing of atria and ventricles, with controlled P-R intervals, at a rate of 100 per minute, in four non-MS patients and four MS patients. In each group, sixteen sets of measurements were made, alternating between an ineffective P-R of 0.01 second and a 0.15 second optimal P-R. ¶ Eight comparisons in non-MS patients revealed changes attributable to atrial systole (AS): an increase in output of 0.72 (range, +0.17 to +1.62) to an average of 3.40 L per minute per m^2 ($p < 0.01$), of 0.04 second (+0.02 to +0.06) in left ventricular systolic ejection period (LVSEP) to 0.28 second ($p < 0.05$), of 285 (+70 to +450) in LV dP/dt to 1,550 mm Hg per second ($p < 0.05$), and an increase in LV end-diastolic pressure (EDP). ¶ Eight comparisons in MS patients revealed no significant changes attributable to optimally timed AS: output changed by +0.09 (−0.40 to +0.65) to 3.42 L per minute per m^2, LVSEP by +0.005 (0.0 to 0.01) to 0.23 second, LV dP/dt by +60 (−20 to +200) to 1,160 mm Hg per second; LVEDP was unchanged. ¶ The atria of non-MS persons made important contributions to LV end-diastolic volume and stroke output. Conversely, in rheumatic heart disease patients with MS, optimally timed AS was rendered ineffective by the stenotic valve, by the reduced translation of wall tension into pressure in their large left atria, and possibly by atrial myocardial disease.

Immunologic Tolerance Associated with Congenital Murine Reovirus Infections. MARY M. CARRUTHERS, ALI A. HASHIMI, AND A. MARTIN LERNER, Detroit, Mich. (introduced by Carl Harford †).

The deficient ability of fetal and newborn animals to respond to antigenic stimuli has long been recognized. That immunologic tolerance in the mouse may be initiated by intrauterine reovirus infection and maintained during a subsequent prolonged virus infection in the progeny is illustrated by the following experiment. ¶ Pregnant ICR Swiss albino mice were inoculated intraperitoneally at various days of gestation with 20,000 hemagglutinating units (HA) of reovirus, type 2, strain 988. Infant mice born of these mothers showed illness, first within several days and then at 2 to 5 weeks of age with decreased activity, failure to gain or loss of weight, and occasional proptosis and conjunctivitis. Reovirus was isolated in high titer from blood and many organs of both sick and apparently healthy animals sacrificed at both of these times. Virus was found in at least one organ up to 53 days after initial inoculation. ¶ Mice studied at 3 weeks of age had hemagglutinating inhibiting antibody against reovirus, type 2 in titers approximating those of their mothers. However, animals from the same group studied at 3 and 9 months of age failed to show antibody, whereas mothers continued to have high antibody titers. ¶ Virus infection and immunologic tolerance apparently did not persist indefinitely because animals reinoculated at 3 months with 20,000 HA of reovirus showed high homotypic antibody titers some months later. ¶ This work adds reovirus to Rous-associated and lymphocytic choriomeningitis viruses as a cause of infection without demonstrable antibody formation in young animals.

Regional Distribution of Acid Mucopolysaccharides in the Kidney. C. WILLIAM CASTOR,* JAMES A. GREENE,† AND MARGARET J. HAZELTON, Ann Arbor, Mich.

In earlier studies the dog renal medulla was found to be much richer in acid mucopolysaccharides (AMPS) than cortical tissue, the major medullary AMPS resembling hyaluronic acid (HA), the predominant cortical AMPS resembling heparitin sulfate (HMS). The present study extends previous data concerning the identification of AMPS components and differences of compositional pattern in medulla and cortex. Kidneys were carefully dissected and AMPS isolated with cetylpyridinium chloride after proteolytic digestion. Characterization of types of AMPS was based on identification and measurement of the hexosamine, hexuronic acid, and sulfate moieties, viscous characteristics, paper chromatography, and carbazole:orcinol ratios. Medullary tissue contained 11.0 mg AMPS per g dry weight. High molecular weight HA ($[\eta] = 19.1$) accounted for 70% of the medullary AMPS, the remainder being nearly evenly divided into HMS and chondroitin sulfate B (CS-B). Renal cortex

contained 1.3 mg AMPS per g dry weight, of which 12% was HA of lower molecular weight ($[\eta] = 10.1$). Cortical AMPS was predominantly (88%) of a sulfated type. Approximately 90% of this sulfated AMPS was heparitin sulfate, the remainder being CS-B. Analyses on a single human kidney revealed a distributional pattern similar to that in the dog. Clearly, the proportions of HA (and its $[\eta]$), HMS, and CS-B differ in cortex and medulla. In neither area do the findings match values reported for other vascular tissues. Although the physiologic implications of these chemical data are not certain, it is noteworthy that medullary HA, if uniformly distributed in extratubular water, would exceed the concentrations found in joint fluid by two to four times. This might be expected to provide a gelatinous support for tubular structures analogous to that offered by Wharton's jelly for the vascular structures of the umbilical cord. The role of medullary AMPS as a sodium trap is also under study.

The Identification of Two New Components Common to a Variety of Amyloid Tissues. EDGAR S. CATHCART AND ALAN S. COHEN,* Boston, Mass.

An α-globulin has recently been separated from amyloid fibrils isolated from the spleen of a patient with secondary amyloidosis. The potential significance of this finding in relating different types of amyloid to one another and in obtaining a soluble component for further immunochemical assay prompted the following study. Fibrils were prepared as previously described from amyloid laden spleens of four patients with "primary," three with "secondary," and one with myeloma-related disease. By electron microscopy the resultant samples consisted of homogeneous suspensions of laterally aggregated amyloid filaments of 70 to 75 A diameter. ¶ The following results were obtained from the eight tissue preparations. *1)* Although solubility of each patient's fibrils varied somewhat, two bands (a slow component, P, and a fast component, T, were discerned on disc electrophoresis of each pH 9.5 extract. *2)* T-component did not react with antiserum to whole human serum, nor was it antigenic after repeated immunization of several rabbits. T-component was also present in extracts of nonamyloidotic spleens. *3)* Antiserum prepared against one of the alkaline amyloid extracts reacted with P-component and formed identical precipitin lines against each of the eight amyloid extracts. *4)* When added to the amyloid extracts, the antiserum formed precipitates possessing classical green birefringence after Congo red staining. *5)* This antiserum did not react with nonamyloidotic spleen extracts, nor did antiserum to normal spleen extracts react with any of the amyloid extracts. *6)* The P-component reacted with antiserum to whole human serum and by immunoelectrophoresis was an α-globulin. *7)* Immunoassay of P-component revealed no significant differences between amyloidotic and control bloods. Cord blood, however, contained minimal or zero levels of P-component. ¶ In summary, two fractions were identified in extracts of purified amyloid filaments from all eight amyloid tissues. One was characterized as an α-globulin present in normal and pathologic plasma (P-component), the other as a nonplasma or tissue derivative (T-component) present in normal and amyloidotic spleens.

The Electrolyte Response of the Gastric Mucosa to Instilled Acid: A Search for "Leaky" Stomachs. MARK L. CHAPMAN, J. LAWRENCE WERTHER, FRANKLIN HOLLANDER, AND HENRY D. JANOWITZ,† New York, N. Y.

The gastric mucosa of man and dog presents a barrier to the passage of H^+ from lumen to blood and of Na from the blood to lumen. Experimental chemical injury to this barrier allows increased H^+ absorption and concomitant Na output. Thus the apparent hyposecretion of gastric ulcer patients may result from loss of H^+ through an altered mucosa. ¶ To study this possibility, we investigated the electrolyte response to instilled HCl in seven normal volunteers, five patients with benign gastric corpus ulcers, two patients with pernicious anemia (PA), and four patients with other gastric disorders. Volumes of 200 ml of isotonic HCl (160 mEq per L) (test solution) were instilled into the stomach via nasogastric tube, withdrawn completely after 15-minute intervals during six successive periods, and replaced by fresh stock solution. With phenol red as a dilution indicator, residual volume, secreted volume, and transpyloric loss were calculated. All samples were measured for Na, K, Cl, hexosamine, protein, osmolality, and volume, and from these, gains in Na and losses of H^+ calculated. ¶ The mean [Na] in the test solution after 15 minutes was 12.8 ± 2.8 (SD) mEq per L in controls, 12.8 ± 2.3 mEq per L in gastric ulcers, and 23.1 ± 4.9 mEq per L in PA. Mean Na output, per 15-minute period, was 3.2 ± 0.8 mEq in controls, 3.1 ± 0.7 mEq in gastric ulcers, and 5.4 ± 1.6 mEq in PA. Mean $[H^+]$ of the test solution had fallen to 132.7 ± 7.2 mEq per L in controls, 133.9 ± 5.4 mEq per L in gastric ulcers, and 117.8 ± 2.8 mEq per L in PA. H^+ loss, per 15 minutes, attributable to absorption by the mucosa, was calculated to be 2.4 ± 1.6 mEq in normals, 2.3 ± 1.2 mEq in gastric ulcers, and 2.9 ± 0.7 mEq in PA. Osmolality fell from 310.0 to 289.0 ± 7.6 mOsm per kg in normals, 289.3 ± 8.9 mOsm per kg in gastric ulcers, and 272.2 ± 9.7 mOsm per kg in the PA stomachs. ¶ These results indicate that small amounts of H^+ are regularly absorbed by the gastric mucosa. The H^+ loss and concomitant greater Na gain in gastric ulcer stomachs were similar to those occurring in control stomachs. The mucosa of PA patients was considerably more "leaky" for Na and somewhat more so for H^+ than both other groups.

Osmolality of Distal Tubular Fluid in the Dog. JAMES R. CLAPP AND ROSCOE R. ROBINSON,* Durham, N. C.

Previous renal micropuncture observations in rodents have shown that late distal tubular fluid is always isosmotic to plasma during hydropenia and osmotic diuresis. Our recognition of distal convoluted tubules on the surface of the dog kidney has now made it possible to de-

termine the contribution of distal nephron segments to urinary concentration and dilution in this species. Accordingly, tubular fluid osmolality was measured in 10 proximal and 57 distal samples from normal dogs during antidiuresis or water diuresis and during chlorothiazide administration in antidiuresis. Proximal fluid was essentially isosmotic to plasma during all experiments. However, during antidiuresis (osmolal U/P ratio: 5.3 ± 2.1), distal fluid was markedly hypotonic along the entire length of the tubule (osmolal TF/P ratio in early distal tubule: 0.40 ± 0.18; in late distal tubule: 0.41 ± 0.11). Distal fluid also remained dilute (osmolal TF/P: 0.48 ± 0.10) along the entire tubule after chlorothiazide administration (osmolal U/P: 2.6 ± 0.2). During water diuresis (osmolal U/P: 0.39 ± 0.08), the osmolal TF/P ratio in distal fluid (12 samples) averaged 0.26 ± 0.09, a figure which was significantly lower ($p < 0.01$) than that observed during antidiuresis. In contrast to previous observations in rodents, these data demonstrate that the diluting segment of the dog nephron includes the entire distal convoluted tubule. The maintenance of marked hypotonicity along the entire length of surface convolutions of the dog distal tubule indicates that this nephron segment exhibits either less permeability to water or more efficient solute extraction (or both) than in the rat, and provides a logical explanation for such findings as the excretion of hypotonic urine by man and dog during osmotic diuresis. Furthermore, in the dog, the lower osmolality of early distal fluid during water diuresis suggests strongly that the site of action of antidiuretic hormone must include the thick ascending limb of the loop of Henle.

Kinins and Granulocytes: A Model of Inflammation. MARTIN J. CLINE AND KENNETH L. MELMON, San Francisco, Calif. (introduced by Richard J. Havel *).

Five major actions have been attributed to plasma kinins; smooth muscle stimulation, increase in vascular permeability, vasodilatation, leukotaxis, and pain production. The kinins have been implicated in the inflammatory response to tissue injury. Studies were undertaken to examine the interaction between this system and leukocytes. ¶ Human leukocytes were incubated in solutions (3 to 30×10^6 cells per ml) containing plasma bradykininogen (BKG) or synthetic bradykinin (BK). BK activity or BKG level was determined by bioassay. ¶ Leukocytes contained high levels of kininase activity: 3×10^6 cells destroyed more than 9 μg of BK, approximately 500 times the usual plasma concentration, in 2 hours. Granulocytes accounted for most of this activity; lymphocytes had little or no ability to destroy BK. Kininase activity was detected in the postmitochondrial and lysosomal fractions of disrupted granulocytes and in intact cells. It was not affected by 4×10^{-5} M puromycin, 2×10^{-2} M NaF, or 6×10^{-5} M hydrocortisone. ¶ Kinin-forming (kallikrein) activity was demonstrated by a two- or threefold increase in kinin concentration and a corresponding decrease in BKG within 5 minutes of the addition of granulocytes, but not of lymphocytes, to plasma. During incubations in excess of 1 hour, kininase activity appeared to predominate, and plasma kinin concentration fell to or below base line. Approximately 1.2 μg of BKG was converted to BK by 3×10^6 cells in 2 hours. ¶ Synthetic BK in an initial concentration of 50 μg per ml had no effect on granulocyte acid-phosphate activity, lactate production, RNA or protein synthesis, or the phagocytosis of polystyrene particles. ¶ These observations of the ability of granulocytes to sequentially activate BKG and destroy BK are the basis for a model of inflammation: Granulocytes are attracted to sites of BK production and initially activate more BKG. Ultimately, however, they accumulate and destroy BK without undergoing significant metabolic alterations or impairment of phagocytosis. The character of a local inflammatory reaction would thus undergo a gradual transition from the manifestations of BK action to those of leukocyte accumulation.

Antibacterial Activity of the Urinary Bladder. C. GLENN COBBS AND DONALD KAYE, New York, N. Y. (introduced by Edward W. Hook *).

Activity of the rat urinary bladder against *Escherichia coli* and *Proteus mirabilis* was investigated. Rats were anesthesized, the peritoneal cavity was exposed, and the ureters were doubly ligated and divided to isolate the bladder from urine flow. The bladder was washed by alternately injecting and aspirating saline solution through the bladder wall. Varying numbers of bacteria in a small volume of broth were then injected into the empty bladder, and the abdomen was closed. Rats were killed at intervals, and the number of viable bacteria was determined in washouts of the bladder lumen and in homogenates of the bladder wall. After inoculation of 10^6 to 10^7 *E. coli* in male or female rats, the number of bacteria in the bladder decreased rapidly, and by 24 hours the lumina and homogenates of most bladders contained less than 10^4 bacteria (a 100- to 1,000-fold decrease). In contrast to the results with *E. coli, P. mirabilis* in an inoculum of 10^5 usually multiplied in the bladder. Studies with ^{51}Cr-labeled *E. coli* demonstrated that during the first 4 to 8 hours after inoculation the number of viable bacteria in the lumen of the bladder had decreased significantly without a proportional decrease in radioactivity. With obstruction of the urethra or ligation of the bladder neck, *E. coli* multiplied in the bladder. These studies demonstrate that *1*) there is an antibacterial activity in the rat bladder, *2*) this activity is more effective against the strain of *E. coli* than the *P. mirabilis* strain, and *3*) the antibacterial activity is eliminated by obstructing the urethra or ligating the base of the bladder.

An Inherited Alpha$_1$-Lipoprotein Variant. LOUIS COHEN AND JULIANA DJORDJEVICH, Chicago, Ill. (introduced by W. R. Barclay *).

In a previous report the development of methods of lipoprotein staining and electrophoresis that permit the discrimination of three common human serum alpha$_1$ lipoprotein (alpha$_1$LP) patterns was described. During

the survey of several hundred sera, an unusual pattern was noted in one healthy male; it was also found in his father and one daughter, but not his mother, wife, or three other daughters. ¶ The characteristics of this variant lipoprotein were investigated. Serum alpha$_1$LP was isolated from each family member by ultracentrifugal flotational methods, delipidated, and electrophoresed in pH 3.2 Al lactate 8 M urea starch gels. In this system a variant protein was seen only in the affected family members noted above. Previously, with this gel, normal alpha$_1$LP-protein was shown to contain two polypeptide varieties, one reactive with thiols and one not. In each affected individual two differences from normal were found: The single band, previously shown reactive with reagent thiols was present but reduced in amount; in addition two new bands were seen, and one of these was found also to be reactive with the reagent thiol. ¶ These findings are consistent with the interpretation that each reactive band is a polypeptide under genetic control. Each affected person is heterozygous for this trait, and each unaffected person, homozygous. The inheritance of this variant appears to be determined by a single autosomal gene difference. ¶ These observations *1*) represent additional proof of alpha$_1$LP-protein polypeptide heterogeneity; *2*) are the first demonstration of an inherited polypeptide variant of alpha$_1$LP; *3*) underscore the value of the new serum lipoprotein electrophoretic and staining methods for detecting such variants; and *4*) suggest a new approach toward the ultimate classification of the dyslipidemias on a genetic basis.

Histologically Proved Liver Disease and the *In Vitro* Metabolism of Dehydroepiandrosterone-^{3}H. GEORGE L. COHN,* AUSTIN R. BRENNEMAN, DAVID L. YOUNG, AND ROBERT L. SCHEIG, New Haven, Conn.

The metabolism of tracer amounts of dehydroepiandrosterone-^{3}H by human liver slices (7.2 to 14 mg wet weight) was studied in an attempt to correlate enzymatic activity with microscopic liver structure and liver function tests. The tissues were obtained with a Menghini needle from 26 patients with a variety of liver diseases. The incubation was carried out for 60 minutes in Krebs-Ringer bicarbonate buffer at 37° C in an atmosphere of 95% oxygen, 5% carbon dioxide. After chloroform extraction and paper chromatography, dehydroepiandrosterone-^{3}H was isolated. Measurement was performed by liquid scintillation spectrometry and the tissue analyzed by a microtechnique for nitrogen. There was 0.21 to 0.35% per μg of nitrogen of dehydroepiandrosterone-^{3}H metabolized in the incubations of liver tissue from patients with a variety of histologically proved liver disease that included cirrhosis, hepatitis, sarcoidosis, schistosomiasis, and fatty infiltration. These data indicated that these forms of liver disease were not associated with evident changes in dehydroepiandrosterone metabolism. However, striking decreases of dehydroepiandrosterone-^{3}H metabolism, 0.09 and 0.12% metabolized per μg of nitrogen, occurred in the incubations of liver slices from two patients with obstructive jaundice secondary to carcinoma of the pancreas. ¶ Strip scanning of the developed paper chromatogram after chloroform extraction revealed two major areas of radioactivity corresponding to dehydroepiandrosterone-^{3}H and a compound identified as Δ^5-androstenediol-^{3}H. The amounts of the Δ^5-androstenediol-^{3}H varied with different liver disorders. No attempts were made to identify two minor areas of radioactivity on the paper chromatogram. The technique affords a simple and direct micromethod for enzyme investigations.

The Persistence of Cholesterol-4-^{14}C in Atherosclerotic Aortas of Animals Treated with a Diet High in Polyunsaturated Fat. WILLIAM E. CONNOR,* MARK L. ARMSTRONG, CARL S. JACKSON, AND M. AMIR ALI, Iowa City, Iowa.

A crucial question about the dietary treatment of atherosclerosis is whether established lesions might be improved. Twenty-six rabbits were fed cholesterol-4-^{14}C in a 0.5% cholesterol and 2.5% peanut oil diet. After 14 weeks the blood and tissues of 5 animals (control) were analyzed for total cholesterol and cholesterol-4-^{14}C, and the aortas were graded 0 to 4 for atherosclerosis. Of the remaining 21 rabbits, 11 were treated with a diet containing 18.5% corn oil (CO) at 40% of total calories and 10 rabbits a low-fat diet (LF). Neither diet contained cholesterol or cholesterol-4-^{14}C. After 14 and 40 weeks of these diets, groups of animals were autopsied. ¶ The cholesterol feeding produced hypercholesterolemia in all animals (mean, 1,634 ± 109 mg per 100 ml). In treated animals, serum cholesterol levels were, at 14 weeks, 275 mg per 100 ml for CO and 55 for LF animals, and at 40 weeks, 223 for CO and 35 for LF rabbits. Serum specific activity (dpm per mg cholesterol) declined from 842 to 80 (CO) and 76 (LF) at 40 weeks. ¶ Aortic atherosclerosis was graded 2.4 (control), at 14 weeks 2.7 (CO) and 2.1 (LF), and at 40 weeks 3.0 (CO) and 2.7 (LF). Aortic cholesterol was 87 mg per g (control), at 14 weeks 120.6 (CO) and 91.4 (LF), and at 40 weeks 103.5 (CO) and 85.4 (LF). Aortic specific activity persisted. The control was 644. At 14 weeks it was 641 (CO) and 701 (LF), and at 40 weeks 652 (CO) and 616 (LF). In other organs (liver, intestine, spleen, kidney, eye), the cholesterol content and specific activity declined greatly in the treatment periods. ¶ Conclusion: No improvement of aortic atherosclerosis and no net outward flux of aortic cholesterol occurred after 40 weeks of corn oil or low-fat diets despite the restoration of normal serum cholesterol levels in the majority of animals for 6 months.

Contraction of the Mitral Valve: An Intrinsic Neuromuscular Basis for Valve Motion. THEODORE COOPER,* WILLARD M. DAGGETT, AND EDMUND H. SONNENBLICK, St. Louis, Mo., and Bethesda, Md.

The mitral valve leaflets are generally regarded as endothelial reduplications, motion of which follows passively upon the genesis of atrioventricular pressure gradients

during cardiac contraction. Unexpectedly, in the present study, 10 canine mitral valve leaflets, which were excised distal to the annulus and studied in a myograph, actively contracted upon electrical stimulation. Along with resting tension, actively developed tension increased with greater initial lengths, the latter reaching a maximum up to 1 g at 50% extension. Postextrasystolic potentiation, and the administration of norepinephrine (0.1 μg per ml), tyramine (1.0 μg per ml), and strophanthidin (1.0 μg per ml) increased developed tension, demonstrating the capability of the mitral valvar muscle to respond to positive inotropic interventions. Study of the structure of the mitral valve leaflets from 12 dogs by light and electron microscopy revealed a multilayered mass of striated muscle subtending the endothelium of the ventricular surface of the leaflets. The muscle cells were arranged perpendicular to the annulus, were connected by intercalated discs, and extended to the outer third of the leaflet. Nonmyelinated axons were found in the muscular and nonmuscular portions of the valve. The neural elements apparently consist of both efferent and afferent fibers, since some persisted after total extrinsic denervation of the heart, and since catecholamines could be measured fluorometrically in the excised leaflets (16 dogs, averaging 0.11 ± 0.02 μg per g). Small blood vessels and collagen were also present in the leaflets. The origin, orientation, and contractile capability of the mitral valvar muscle suggest that mitral valve closure could be initiated with the onset of ventricular systole by activation of the intravalvar muscle. These observations suggest a need to reassess the basic mechanisms of mitral valve function in normal and pathological states.

Gamma Globulin Synthesis by Lymphocytes from Normal and Agammaglobulinemic Individuals. SIDNEY R. COOPERBAND, FRED S. ROSEN, SIDNEY KIBRICK, AND CHARLES A. JANEWAY,† Boston, Mass.

Human lymphocytes in culture have been shown to proliferate in the presence of antigen and to synthesize γ-globulin. We have investigated circulating lymphocytes from normal and agammaglobulinemic patients to determine whether cells from both have similar competence. The character of the proteins synthesized in these cultures was studied after leucine-^{14}C incorporation. The proliferation was assessed by determining the quantity of ^{32}P incorporated into DNA. ¶ Lymphocytes from 12 normal and 13 agammaglobulinemic individuals were cultured without stimulus, and with *a*) antigens after immunization (diphtheria and tetanus toxoid), *b*) allogeneic lymphocytes, and *c*) phytohemagglutinin (PHA). The quantity of ^{32}P incorporated into DNA varied slightly among individuals, but was not significantly different between cultures of agammaglobulinemic and normal lymphocytes ($p < 0.05$). Incorporation of ^{32}P into RNA was similar in both populations. ¶ Both normal and agammaglobulinemic lymphocyte cultures demonstrated linear accumulation of leucine-^{14}C into extracellular protein for 10 days. With PHA, there was a threefold increase in the quantity of newly synthesized protein. All the lymphocyte cultures synthesized proteins with similar characteristics as determined by paper electrophoresis, DEAE-cellulose chromatography, and density gradient ultracentrifugation. ¶ In both unstimulated and PHA-stimulated normal cultures, $20.8 \pm 16.4\%$ of the extracellular protein had the physical, biochemical, and immunologic characteristics of γG-globulin. With PHA, normal lymphocytes produced 0.508 ± 0.205 μg of immunologically isolated γG per 24 hours per 10^6 cells; without stimulation, 0.175 ± 0.041. In agammaglobulinemic cultures, a similar proportion and quantity of the newly synthesized supernatant protein were γG. Agammaglobulinemic cells produced 0.502 ± 0.484 μg of γG per 24 hours per 10^6 cells in the presence of PHA, and 0.161 ± 0.132 without stimulus. ¶ Agammaglobulinemic lymphocytes thus appear similar to normal lymphocytes. The genetic defect in agammaglobulinemia, therefore, appears not to involve the structural or regulatory genetic loci necessary for synthesis of γ-globulin in lymphocytes, or the genetic apparatus necessary for antigenic recognition and subsequent proliferation of these cells.

Experimental and Theoretical Considerations of the Molecular Basis for Cardiac Muscle Contraction. LAMAR CREVASSE, H. DIAZ DE ARCE, AND DARWIN SMITH, Gainesville, Fla. (introduced by S. P. Martin †).

The major activity of 2-oxo-acid carboxylase (E.C. 4.1.1.1.) of isolated myocardial proteins has been localized in the F-actin fraction with only slight activity in myosin and none in tropomyosin. In 40 mM Tris-nitrate, 10 mM NaCl, 10 mM NaH_2PO_4, 0.05 mM $CaCl_2$, 0.25 mM pyruvate-1-^{14}C, 100,000 g of F-actin decarboxylates 0.8 mole of pyruvate per hour. This is a first order reaction being both pH, temperature, and concentration dependent. This decarboxylase activity in myofibrils is calcium and thiamine dependent and directly proportional to ATP-induced contraction as correlated by phase contrast micrometry. A mechanico-molecular model is proposed that relates ATPase activity in actomyosin with thiamine phosphorylation and decarboxylase activity. Changes from the helical to linear polymer of actin can account for contraction and implicate both the sliding and folding theories.

Sodium Phenobarbital-induced Decrease in Serum Bilirubin in an Infant with Congenital Nonhemolytic Jaundice and Kernicterus. JOHN F. CRIGLER, JR., AND NORMAN I. GOLD, Boston, Mass. (introduced by Louis K. Diamond †).

Sodium phenobarbital (15 mg, twice daily, orally), testosterone propionate (TP, 0.1 mg per day, intramuscularly), and *l*-triiodothyronine (T_3, 50 to 100 μg per day, orally) were administered between the ages of 3 and 12 months to an infant with congenital nonhemolytic jaundice and marked kernicterus to determine their effect on serum bilirubin and on steroid hormone metabolism. Bilirubin determinations were obtained daily and 24-hour

urine and stool collections made after the simultaneous administration of ^{3}H- and ^{14}C-labeled cortisol and testosterone. The following changes in serum bilirubin (mean value [range, number of determinations]) and urinary steroid glucuronides were observed. *1)* On phenobarbital and TP, serum bilirubin decreased continuously over 42 days from 17.9 mg per 100 ml (16.1 to 20.7, 46) in control periods (9 days TP alone preceded by 18 days without drugs) to 6.0 mg per 100 ml (4.7 to 7.3, 9). Phenobarbital alone maintained serum bilirubin for 30 days at 10 mg per 100 ml (7.7 to 13.8, 54). When phenobarbital administration was stopped, serum bilirubin increased continuously over 30 days to 23 mg per 100 ml. Previously, T_3, 50 μg per day for 18 days, produced no change in serum bilirubin, although on 75 μg per day for 18 additional days, bilirubin concentrations increased from 18.4 mg per 100 ml (15.8 to 22, 16) to 25 mg per 100 ml (20.4 to 32, 17). *2)* The per cent of the total urinary radioactive metabolites of labeled cortisol and testosterone excreted as glucuronides was 39 and 42 during two periods without drugs, 62 on T_3, and 52 on phenobarbital. The per cent of ATHF, THF, and THE excreted as glucuronides was, respectively, 70, 75, and 64 on TP, and 92, 88, and 85 on phenobarbital and TP. ¶ The marked decrease in serum bilirubin and clinical jaundice with phenobarbital administration may involve more than enhanced glucuronide formation.

Left Ventricular Emptying in Outflow Tract Obstruction. J. MICHAEL CRILEY, W. STANLEY WILSON, AND RICHARD S. ROSS,* Baltimore, Md.

The rate and degree of left ventricular (LV) emptying were studied by 60 fps cineangiography in eight patients with hypertrophic subaortic stenosis (HSAS) and seven patients with valvular aortic stenosis (VAS). Eleven patients with hemodynamically normal LV function were used as controls. Four of the HSAS patients were studied before and after the administration of isoproterenol, an agent that is said to increase the degree of obstruction in HSAS. Significant pressure gradients between the body and outflow tract of the left ventricle were present at rest or provoked by isoproterenol in each patient with HSAS. The area of the LV silhouette, in the right anterior oblique projection, was measured throughout systole by planimetry, and the systolic decrease in area (%ΔA), LV emptying time in milliseconds (LVET), and rate of area change (%ΔA/LVET) were compared in the three groups. ¶ The following significant differences were noted: The %ΔA was greater in the HSAS (58%) and VAS (65%) groups than in the control group (48%). The LVET was shorter than normal in the HSAS group and longer than normal in the VAS group. The rate of emptying (%ΔA/LVET) was highest in the HSAS group. Isoproterenol, while increasing the LV pressure gradient, increased the degree and rate of LV emptying. Separate measurement of the areas of the outflow tract and body of the ventricle revealed no disproportionate decrease in the outflow tract area. ¶ It is concluded that patients with HSAS empty the left ventricle more rapidly and completely than normal and that isoproterenol enhances rather than hinders emptying. The presence of significant outflow tract obstruction in HSAS is, therefore, questioned.

The Galactonic Acid Pathway: A New Route of Galactose Metabolism in Mammals. PEDRO CUATRECASAS AND STANTON SEGAL,* Bethesda, Md.

Galactose oxidation in mammals is currently thought to proceed only through the pathway: galactose→ galactose-1-P→ UDPgalactose→ UDPglucose→ glucose-1-P. A new pathway has been found in mammalian liver. It shares none of the intermediates of the conventional pathway, does not involve phosphorylated or nucleotide-linked intermediates, and joins the general pool of intermediary metabolism in the pentose phosphate shunt. ¶ An NAD-dependent enzyme (galactose dehydrogenase) capable of oxidizing galactose has been found in soluble preparations of liver of 9 mammalian species, including man. It has been purified 100-fold from rat liver and the product identified as galactonolactone. The latter is converted to galactonic acid by a delactonizing enzyme. Galactonate is converted to *d*-xylulose, probably in two steps, the first of which is an NAD requiring oxidation to yield an intermediate, β-keto-D-galactonic acid, which is then decarboxylated to D-xylulose. An ATP and Mg requiring xylulokinase can form xylulose-5-P, which enters the pentose phosphate shunt. ¶ Liver was the only tissue of 11 tested that had significant galactose dehydrogenase activity. Measurements of $^{14}CO_2$ evolution after incubation of liver slices and homogenates with labeled intermediates confirmed the presence of the pathway. Erythrocytes and leukocytes were unable to oxidize galactonate to CO_2. ¶ Measurements of expiratory $^{14}CO_2$ in rats after administration of galactose and galactonate labeled in C1 and C2 indicate that the new pathway is operative *in vivo*. This could explain the residual metabolism of galactose in patients with galactosemia and may provide insight into the toxicity of galactosemia and of animals given galactose diets. A 40% galactonate diet given to 18-day-old and newborn rats results in severe illness and death.

Paradoxical Effects of Microemboli and of Asystole on Pulmonary Diffusing Capacity in Dogs. WALTER J. DALY, Indianapolis, Ind. (introduced by Joseph C. Ross *).

Abrupt changes in 10-second breath-holding CO diffusing capacity ($D_{L_{10}}$) are often used as an index of altered effective pulmonary capillary blood volume. An apparent paradox was found in this study of microembolic ($BaSO_4$) pulmonary vascular obstruction. In 17 anesthetized dogs, $D_{L_{10}}$ was determined before and just after sufficient intravenous $BaSO_4$ to increase mean pulmonary arterial pressure (PA) from 14 ± 3 to 36 ± 8 mm Hg. This microembolization did not decrease $D_{L_{10}}$, 11.2 ± 3.3 and 12.9 ± 3.0 ml per minute × mm Hg, respectively. Electrically induced exercise ($\Delta \dot{V}_{O_2} = 500$ ml per minute) increased PA to 31 ± 12 mm Hg before em-

boli and to 50 ± 17 mm Hg after emboli, whereas D_{L10} increased to 22.5 ± 11.0 before emboli and 20.4 ± 6.6 after emboli. Thus, microcirculatory obstruction sufficient to double PA did not decrease CO absorption at rest or the potentiality for large increases in CO absorption during exercise. ¶ During 60-second breath-holding periods, D_L decreased from 12.2 ± 3.4 (D_{L10}) to 7.3 ± 1.4 ml per minute × mm Hg (D_{L60}) before emboli and from 13.2 ± 3.2 (D_{L10}) to 5.0 ± 1.9 (D_{L60}) after emboli ($p = 0.005$ that the decrease was greater after emboli). This suggests that after microemboli, a portion of the capillary bed contains blood available for CO absorption but flowing too slowly to prevent CO back pressure, which locally limits CO absorption. Thus, D_{L10} is preserved in microembolized lungs by CO uptake in capillaries filled at decreased flow rates from *1*) incompletely obstructed arterioles, *2*) bronchial collateral circulation, or *3*) retrograde filling. That D_{L10} may indeed be relatively independent of blood flow per se was further demonstrated by measurements in 5 dogs in which asystole was produced by aortic root injection of acetylcholine. D_{L10} measured before asystole averaged 15.3 ± 4.7 ml per minute × mm Hg and 16.9 ± 3.3 at the onset of asystole. ¶ This study suggests that D_{L10} is relatively unaffected by blood flow per se and that longer periods of breath holding or other methods of measuring diffusing capacity may better detect abnormalities with gross inhomogeneity of blood flow.

Abnormal Plasma Insulin Response with High Plasma Triglycerides Independent of Clinical Diabetes or Obesity. PAUL DAVIDSON AND MARGARET ALBRINK,* Morgantown, W. Va.

Elevated plasma insulin levels are observed with the onset of diabetes and with obesity. Increased plasma insulin is considered to be an indication of peripheral insulin resistance with functioning insular tissue. ¶ The plasma insulin response to 100 g of oral glucose was measured in 49 males by immunoassay. The subjects were selected to eliminate those greater than 20% above ideal weight and those with definite diabetes by the criteria of Wilkerson. The group was divided into 34 with plasma triglyceride fatty acids of less than 5.5 mEq per L and 15 with higher plasma triglycerides. The degree of obesity and the ages in the two groups were not significantly different. ¶ The insulin levels were higher in the high triglyceride group at each interval. The mean values and significance in the euglyceridemic and hyperglyceridemic groups at each interval were, respectively, initial, 24 and 35 μU per ml ($p < 0.05$); ½-hour, 100 and 168 ($p < 0.02$); 1-hour, 81 and 199 ($p < 0.001$); 2-hour, 63 and 134 ($p < 0.001$); and 3-hour, 43 and 77 ($p < 0.01$). The total plasma insulin elevation above the initial level during the postglucose meal response was twice as high in the hyperglyceridemic group as in the normal group, 18,207 vs. 9,326 μU-minutes per ml ($p < 0.02$). ¶ The insulin levels in the high plasma triglyceride group were comparable to the levels in noninsulin-dependent diabetics or obese nondiabetics. ¶ The ratio of plasma insulin to plasma glucose concentration for the entire area under the response curve or at each interval was not significantly different in these two groups, in contrast to significantly lower ratios in nonobese diabetics. ¶ Insulin resistance, demonstrated by abnormally high plasma insulin but normal blood sugar response to oral glucose, appears to be a feature of hyperglyceridemia in the absence of clinical diabetes or obesity.

A New Pathway of Serotonin Metabolism in Man and Its Accentuation by Ethanol. VIRGINIA E. DAVIS, HAROLD BROWN,† JAMES A. HUFF, AND JESSE A. CASHAW, Houston, Texas.

5-Hydroxyindoleacetic acid (5-HIAA) is normally the major metabolite of serotonin (5-HT) in man. Recently, 5-hydroxytryptophol (5-HTOH) has been identified as a 5-HT metabolite in experimental animals. ¶ In our laboratory, both free and conjugated 5-HTOH has been identified and quantitated in the urine of patients with carcinoid tumors. 5-HTOH was excreted as free, 34 to 180; sulfate-conjugated, 311 to 477; and glucuronide-conjugated, 249 to 1,314 (total, 594 to 1,971) μg per day. Quantitation was achieved by solvent extraction of free 5-HTOH from urine before and after hydrolysis with γ-glucuronidase and Glusulase with subsequent enzymatic conversion of the isolated 5-HTOH to 5-HIAA. ¶ The effect of ethanol ingestion on the metabolism of 5-HT-^{14}C was studied in these patients and in normal subjects. After ingestion of 13 μc 5-HT-^{14}C, the average urinary excretion of administered radioactivity in 8 hours in all subjects with or without ethanol pretreatment was 83%. No differences in the pattern of 5-HT metabolites were found between normal subjects and patients with carcinoid tumors. ¶ After 5-HT-^{14}C administration, the per cent of excreted radioactivity was as follows: 5-HIAA, 87; free 5-HTOH, 0.4; 5-HTOH-O-sulfate, 1.2; and 5-HTOH-O-glucuronide, 0.5 (total 5-HTOH, 2). When 60 ml of ethanol was ingested 1 hour before 5-HT-^{14}C administration, the per cent of excreted radioactivity was as follows: 5-HIAA, 44; free 5-HTOH, 0.6; 5-HTOH-O-sulfate, 11; 5-HTOH-O-glucuronide, 34 (total 5-HTOH, 46). ¶ These studies indicate that: *1*) 5-HTOH and its conjugates are regular although minor metabolic products of 5-HT in man; and *2*) the conversion of 5-HT to 5-HTOH is enhanced to major proportions at the expense of the 5-HIAA pathway after ethanol ingestion. ¶ These observations raise the possibilities that other biogenic amines are subject to the same alteration of metabolism and that certain neuropharmacological effects of alcohol are attributable to the aldehyde or alcohol metabolites of endogenous monoamines.

Peroxidation of Membrane Lipids: A Mechanism for Cell Damage in Acanthocytosis (β-Lipoprotein Deficiency). JAMES T. DODGE, GERALD COHEN, HERBERT J. KAYDEN, AND GERALD B. PHILLIPS,* New York, N. Y.

Patients with acanthocytosis recently were shown to be deficient in vitamin E, the principal lipid-soluble anti-

oxidant in mammals. Since membrane lipid peroxidation has been implicated as a mechanism for some of the manifestations of vitamin E deficiency in experimental animals, this hypothesis was tested in acanthocytosis by employing red blood cells (RBC) as a representative tissue membrane. Washed RBC (4 ml of a 10% suspension in isotonic saline-phosphate buffer, pH 7.4) were exposed to minute amounts of H_2O_2 (4 μmoles per hour) added by continuous gaseous diffusion at 37° C. Lipid peroxidation products were measured in trichloroacetic acid extracts by the 2-thiobarbituric acid (TBA) reaction for malonaldehyde and in washed lipid extracts by iodimetry and UV absorbancy at 234 and 268 mμ (conjugated dienes and conjugated trienes, respectively). RBC from two patients with acanthocytosis averaged 90% lysis during 4 to 5 hours of incubation (normal, 2% lysis after 7 hours). The TBA reaction provided an average malonaldehyde to lipid phosphorus molar ratio of 0.033 (normal, 0.004 ± 0.001). Iodimetry and UV absorbancy measurements confirmed the presence of lipid peroxidation products. Addition to the incubation flask of 10 μg DL-α-tocopherol, 8 mg glucose, 0.5 mg sodium nitrite, or 15% carbon monoxide in air (vol/vol) blocked lipid peroxidation and prevented hemolysis. Incubation of defibrinated blood for 48 hours at 37° C without H_2O_2 (the autohemolysis test) produced an average of 55% lysis (normal, 3%). After treatment with D-α-tocopherol succinate, 750 mg per day by mouth for 2 months, measurements of lipid peroxidation and hemolysis in response to H_2O_2 reverted to normal in both patients, and the autohemolysis test reverted nearly to normal, 5.5% lysis. These studies indicate that red cell membrane lipid peroxidation may be a mechanism for hemolysis in acanthocytosis and support the hypothesis that membrane lipid peroxidation may contribute to certain manifestations of this disease.

Suppression of Granuloma Formation in Schistosomiasis by Immunosuppressive Drugs. ERNESTO O. DOMINGO, RICHARD B. T. COWAN, AND KENNETH S. WARREN, Cleveland, Ohio (introduced by Leslie T. Webster, Jr.*).

Schistosomiasis is a disease characterized by formation of granulomata around schistosome eggs trapped in host tissues. The function of the granuloma, as suggested for tuberculosis, is to limit the multiplication and dissemination of the tubercle bacillus. As the schistosome egg neither multiplies nor disseminates, granuloma formation in schistosomiasis may be primarily harmful to the host by causing both tissue destruction and alteration of tissue architecture. One way of assessing the role of granulomata in the pathogenesis of schistosomiasis would be to prevent their formation in infected animals, followed by evaluation of the development of chronic hepatosplenic disease. Experiments were therefore initiated to determine whether granuloma formation could be suppressed by immunosuppressive drugs. Purified viable schistosome eggs were isolated from the livers of infected mice and injected via the tail vein into the lungs of 6 groups of 32 uninfected mice. One group were control animals, and five groups were treated from 2 days prior to egg injection to 32 days after injection with each of 5 immunosuppressive drugs: actinomycin D, azathioprine, fluocinolone acetonide, methotrexate, and thioguanine. At 1, 8, 16, and 32 days after egg injection, lungs of 8 animals in each group were removed for histological study. The diameter of each egg-containing granuloma was estimated with an image splitting microscope eyepiece. The average diameter of the eggs alone as determined at day 1, when there was no host reaction, was $58 \pm 15\,\mu$ (SD). By 16 days the reaction reached its peak in control animals ($206 \pm 73\,\mu$). Each of the drugs suppressed granuloma formation significantly ($p = < 0.0005$) at 16 days. The most effective was the steroid, fluocinolone acetonide, for which the granuloma size was $80 \pm 38\,\mu$. Long-term studies are now in progress concerning the effect of immunosuppressive drugs on the development of hepatosplenic disease in mice infected with *Schistosoma mansoni*.

Studies of Intestinal Microvilli: Intrinsic Factor-mediated Attachment of Vitamin B_{12} to Brush Borders and Microvillous Membranes. ROBERT M. DONALDSON, JR.,* IAIN L. MACKENZIE, AND JERRY S. TRIER, Madison, Wis.

Epithelial cell brush border preparations from proximal and distal hamster intestine were incubated with ^{57}Co-labeled vitamin B_{12} ($^{57}CoB_{12}$) in order to investigate site and mechanism of intrinsic factor (IF) action. Hamster and rat, but not human, gastric juice markedly enhanced attachment of $^{57}CoB_{12}$ to distal brush borders but inhibited uptake by proximal brush borders. When brush borders were further fractionated by the method of Eichholz and Crane, IF stimulated $^{57}CoB_{12}$ uptake by microvillous membranes isolated from distal but not proximal intestine. IF-mediated uptake by distal microvillous membranes was 15-fold greater and by distal brush borders was 10-fold greater than uptake by whole homogenates of distal mucosa. When distal brush borders were incubated with crude sources of IF at pH 7.4, uptake of $^{57}CoB_{12}$ diminished as time of incubation was increased. This was associated with marked disintegration of brush borders as demonstrated by light and electron microscopy and by release of tissue nitrogen into incubation media. Brush border destruction was caused by macromolecular substance in gastric mucosal extract that could be separated from IF by gel filtration with Sephadex G-200. Partial purification of crude IF greatly reduced destruction of microvilli and increased tissue uptake of $^{57}CoB_{12}$. IF consistently inhibited attachment of $^{57}CoB_{12}$ to proximal brush borders. Yet when proximal brush borders were preincubated with aqueous extracts of distal brush borders, IF significantly enhanced rather than inhibited tissue uptake of $^{57}CoB_{12}$. These results demonstrate the following: *1*) IF-mediated attachment of $^{57}CoB_{12}$ to brush borders and microvillous membranes of distal but not proximal intestinal absorptive cells, *2*) disintegration of microvilli by a factor in crude sources of IF, which may explain the inhibitory effects of excessive amounts of crude

IF previously observed *in vitro,* and *3*) presence of a soluble "receptor" for IF-vitamin B_{12} complex that can be extracted from distal and transferred to proximal brush borders.

A Study of the Mechanism of Accumulation of Rhinovirus Nasal Secretion Antibody. R. GORDON DOUGLAS, JR., ROGER D. ROSSEN, THOMAS R. CATE, WILLIAM T. BUTLER, AND ROBERT B. COUCH, Bethesda, Md. (introduced by Vernon Knight †).

Previous studies have demonstrated that protection against rhinovirus infection is associated with the presence of neutralizing antibody in nasal secretions (NS). In this study the source of the antibody content of NS was assessed by investigating the rate of appearance of neutralizing antibody in serum, NS, parotid saliva, and tear specimens. Thirteen antibody-free volunteers became infected, and 12 developed colds after inoculation with rhinovirus NIH 1734. Fourfold antibody rises were detected 24 days after inoculation in serum from 13 volunteers, in NS from 9 volunteers, in saliva from 6 volunteers, and in tears from 9 volunteers. Virus was recovered from NS but not saliva or tear specimens. In 4 of these volunteers, testing of serial specimens revealed that neutralizing activity first appeared in serum specimens 14 to 17 days after inoculation, whereas neutralizing activity did not appear in NS, saliva, or tear specimens until 24 days. Density gradient ultracentrifugation studies revealed serum antibody in the 19 S (γM), 11 to 14 S (γA), and 7 S (γA and γG) regions, whereas neutralizing activity in all 3 secretions appeared predominantly in the 11 S region and was associated with γA-globulin. These data suggest a similar mechanism of antibody accumulation in all 3 secretions. Since antibody appears later in these secretions than in serum, and since uninfected secretions (tears, parotid saliva) accumulate antibody at the same time as the infected secretion (nasal), it is suggested that rhinovirus antibody is formed systemically and transported into these secretions from the plasma pool.

Studies on the Membranes Surrounding the Fat Globules of Milk. ROBERT M. DOWBEN * AND J. ROBERT BRUNNER, Cambridge, Mass., and East Lansing, Mich.

The butterfat in raw milk is dispersed as small globules approximately 2 μ in diameter that are surrounded by a protein envelope. The fat globules are secreted by the mammary epithelial cells by a sort of "reverse" pinocytosis. Upon reaching a critical size, they project into the duct pushing the acinar cell membrane out around them. Eventually, the cell membrane pinches off behind and completely encases the free fat globule. Very little cytoplasm is carried along in the fat globule, and consequently there is no ATP generating system. Ghosts, which resemble erythrocyte ghosts, can be prepared from the fat globules by freeze-thawing or sonication. Large quantities of membranes can be prepared from cream separated immediately from fresh, uncooled milk. The cream is washed three times with 3 vol of 0.25 M sucrose, cooled to 12 to 15° C, and churned. Approximately 1 g membranes are obtained per gallon of milk. Enzymes associated with cell membranes generally, including alkaline phosphomonoesterase, NADH-cytochrome c reductase, and Na^+-K^+-Mg^{++}-activated ATPase, are found in the fat globule membrane. This system lends itself to a study of cell membrane proteins. Two proteins that represent about half of the total protein of the membranes have been isolated and partially purified.

Blood and Cerebrospinal Fluid Relationships in Cheyne-Stokes Respiration. A. R. DOWELL, H. O. SIEKER,* W. H. BELL, AND H. A. SALTZMAN, Durham, N. C.

In Cheyne-Stokes respiration arterial blood gases and pH fluctuate remarkably with the cycles of breathing. These changes were correlated with carbon dioxide tension (P_{CO_2}), pH, and bicarbonate (HCO_3^-) in simultaneously collected samples of lumbar cerebrospinal fluid (CSF) and arterial blood in six patients during cycles of Cheyne-Stokes respiration. The mean arterial P_{CO_2} of 32.2 ± 6.08 mm Hg varied markedly during hyperpnea and apnea and differed significantly ($p < 0.001$) from the higher but variable CSF P_{CO_2} of 40.2 ± 12.21 mm Hg. The arterial pH of 7.502 ± 0.065 differed significantly ($p < 0.001$) from the mean CSF pH of 7.323 ± 0.020, but CSF pH remained constant during both hyperpnea and apnea despite wide variation of CSF P_{CO_2}. Four patients showing arterial hypocapnia and alkalosis and one in the eucapneic range had a mean arterial HCO_3^- of 18.32 $\pm$ 6.48 mmoles per L, and a mean CSF HCO_3^- of 14.02 ± 5.38 mmoles per L ($p < 0.001$). The remaining patient had metabolic alkalosis and maintained a higher HCO_3^- in CSF (29.98 mmoles per L). The data suggest that Cheyne-Stokes respiration occurs with a relatively constant CSF pH and either high or low HCO_3^-, but only cyclic changes in CSF P_{CO_2} and HCO_3^- reflect periodic ventilation.

Cellular Illness: A Prelude to Death? M. J. DUNN, E. K. M. SMITH, A. CZERWINSKI, H. J. GITELMAN, AND L. G. WELT,† Chapel Hill, N. C.

It has been demonstrated that among patients with uremia there are some with high concentrations of sodium in erythrocytes. This is accompanied by a defect in active transport. These particular uremic patients appear to be the most severely ill. For these reasons, patients without uremia but with advanced metastatic cancer have been sampled, and another group of individuals with elevated levels of sodium in erythrocytes (13.5 to 19 mmoles per L erythrocytes) has been found. The active components of efflux were defined as those that were sensitive to cardiac glycoside and ethacrynic acid. In each of four studies the rate constants for sodium efflux were clearly diminished in the patients compared with simultaneous evaluation of normal subjects. A new steady state appeared to have been achieved, however, since the

total effluxes (calculated as rate constant times intracellular concentration of sodium) were similar in the two groups. ¶ The steady state levels of erythrocyte ATP were normal and did not differ in the patients with tumors and their controls (unlike the reported uremics where the ATP levels were increased). The ouabain-sensitive component of the alkali metal ATPase of the erythrocyte ghosts was estimated at 10^{-3} and 10^{-5} M ATP, and no difference was found between patients and paired controls. This does not exclude differences in the characteristics of ATPase that are currently under study. ¶ These data coupled with those from patients sick with uremia raise the interesting speculation that one characteristic of severe illness may be a gradual failure of membrane transport with consequent changes in the intracellular environment which, if not reversed, disturb molecular mechanisms and lead to the death of the cell.

Human Plasma Kallikrein Esterase Associated with the α_2-Macroglobulin Binding Protein. BARBARA DYCE, SALLY HOWARD, NABEEL ADHAM, JOHN MEHL, AND BERNARD J. HAVERBACK,* Los Angeles, Calif.

We previously have reported a protein in human plasma that combines with trypsin and chymotrypsin in such a way that the enzyme is active and cannot be inhibited by certain inhibiting proteins, e.g., soy bean trypsin inhibitor. Mehl subsequently purified this plasma protein, which is an α_2-macroglobulin with a sedimentation coefficient of between 16 S and 20 S. During the isolation of this macroglobulin, it was discovered that an esterase was attached to it and could be separated from it by low ionic strength precipitation. ¶ The purpose of this study was to determine *1*) the esterolytic nature of the enzyme, *2*) whether it is a kinin former, *3*) which substances inhibit its esterolytic or kinin-forming activities, and *4*) whether it is different from the various other plasma esterases. This esterase hydrolyzed TAMe and BAPNA but not ATEE, casein, or gelatin, nor did it have clot-forming activity. The esterase activity was not inhibited by soy bean trypsin inhibitor, kallikrein trypsin inhibitor (Trasylol), or Kunitz's pancreatic juice trypsin inhibitor. It is a kinin former in that it liberated bradykinin from plasma. The kinin-forming activity, however, could be inhibited by soy bean trypsin inhibitor and Trasylol. Seventy per cent of the esterolytic activity (TAMe) could be destroyed by incubation for 1 hour at 56° C. The esterolytic activity was maximal at pH 8.4. These studies show that this esterase is different from serum ether esterase and C'1 esterase, trypsin-2-macroglobulin complex, kinin-forming substance, plasma amidase, human plasmin, plasma kallikrein, thrombin, and Hageman factor. ¶ We have found that changes occur in the level of α_2-macroglobulin and the esterolytic activity of this enzyme in plasma of patients with acute pancreatitis. Further work to elucidate the significance of these changes is warranted.

Cyanide-induced Pituitary Adrenal Activation. RICHARD H. EGDAHL,* Boston, Mass.

Previous studies from this laboratory have established that pituitary adrenal activation occurs in oligemic states despite the maintenance of mean blood pressure with vasoconstrictors. Localized anoxic activation of chemoreceptors was a possible explanation, and the following experiments were designed to test this idea. Intermittent samples of adrenal venous blood were obtained from dogs with chronic adrenal venous cannulas and were analyzed for content of corticosteroids. Injections of sodium cyanide, in dosages too small to result in either blood pressure or respiratory changes, or in venous blood lactate: pyruvate ratios, were made into the portal vein or femoral artery. Dosages ranged from 0.005 to 0.1 mg per kg. Increased corticosteroid secretion, occurring within 10 minutes and lasting for 15 to 60 minutes, took place after injection into either the portal vein or femoral artery. As a result of these experiments it was concluded that cyanide, thought to cause inactivation of the cytochrome system for aerobic oxidation, either directly affected vascular chemoreceptors or led to the production of metabolites which themselves were effective in that regard. An experimental preparation was then devised in which only an artery, vein, and nerve were left intact on one leg of a "chronic" animal with adrenal cannula. Injection of cyanide into the femoral artery with vein occluded, followed by arterial occlusion to minimize pooling of blood in the extremity, resulted in adrenal activation. It is concluded that small doses of cyanide activate chemoreceptors or other peripheral nerve endings, with resultant ACTH secretion via the hypothalamic-pituitary system.

The Relationship between Intestinal Plasma Cells and Serum Immunoglobulin A (IgA) in Man. SHMUEL EIDELMAN, STARKEY D. DAVIS, DAVID LAGUNOFF, AND CYRUS E. RUBIN,* Seattle, Wash.

Recent studies have suggested the gastrointestinal tract's immunologic importance. The major immunoglobulin in human gastrointestinal secretions and in its plasma cells is probably IgA. On reviewing 300 small bowel biopsies from normal and diseased adults, we saw plasma cells in all except five hypogammaglobulinemic subjects. We therefore studied the relationship between plasma cells in small intestinal suction biopsies and serum IgA levels in 3 groups: 21 normal controls, 6 celiac sprue patients, and 5 hypogammaglobulinemic subjects. ¶ The 3 groups differed distinctly in both parameters studied. Serum IgA levels, as per cent normal standard, were 72 ± 45 (mean $\pm$ SD) in the normals, 147 ± 20 in the sprues, and 3 ± 1.5 in the hypogammaglobulinemics. Intestinal plasma cells paralleled the serum IgA level, being markedly increased in the sprues and absent in the hypogammaglobulinemics. ¶ Two of the hypogammaglobulinemic subjects with almost no serum IgA, but normal IgG, had malabsorption and jejunal lesions similar to celiac sprue. They and 3 control subjects (1 normal, 2

celiac sprues) were studied further by multiple hydraulic biopsies of the jejunum and ileum. Plasma cells were present in all biopsies from the normal subject, increased in biopsies from the sprue patients, and absent in all biopsies from the hypogammaglobulinemics. ¶ Representative jejunal and ileal biopsies from these 5 subjects were quick-frozen and stained with monospecific fluorescein-conjugated, rabbit antihuman IgA serum. Specific fluorescence of plasma cells was demonstrated in all biopsies from the normal subject and the sprues. Fluorescent plasma cells were far more numerous in the sprue biopsies. No fluorescent plasma cells were seen in any of the biopsies from the 2 subjects lacking serum IgA. ¶ This positive relationship between IgA-containing intestinal plasma cells and IgA blood levels lends support to the contention that the gut may be a major site of synthesis of this immunoglobulin.

Gluconeogenesis in the Isolated Perfused Rat Liver: Role of the Adrenal Cortex. ALBERT B. EISENSTEIN * AND SYLVIA SPENCER, St. Louis, Mo.

Although adrenal glucocorticoid hormones stimulate hepatic gluconeogenesis, their site of action is not agreed upon. These experiments were conducted to determine the role of adrenal steroids in carbohydrate synthesis from alanine, pyruvate, and lactate by the isolated perfused rat liver. Livers of normal, adrenalectomized, and hypophysectomized rats were perfused for 4 hours with red cell-free medium containing substrate. Hepatic viability was assessed by the rates of perfusate flow, glucose formation, bile flow, and changes in liver weight. Glucose and urea concentrations of the perfusion medium were determined periodically, and glycogen levels were measured in biopsies taken before and after perfusion. Glucose production was determined by subtracting the glucose derived from glycogen from the total quantity that appeared in the medium. ¶ Normal rat liver formed glucose from pyruvate at a relatively constant rate (25.0 $\pm$ 2.0 μmoles glucose per g liver per hour) that was not altered by adrenalectomy or hypophysectomy. Addition of dexamethasone to the medium did not affect glucose synthesis from pyruvate. Liver of intact rats readily synthesized glucose from lactate (23.4 $\pm$ 1.0 μmoles per g per hour), and adrenalectomy did not influence this conversion. Glucose production from alanine by normal liver (18.1 $\pm$ 1.1 μmoles per g per hour) was significantly less than from pyruvate or lactate and was further reduced by adrenalectomy (6.8 $\pm$ 0.4). Addition of dexamethasone (2×10^{-5} M) to medium perfusing adrenalectomized liver increased glucose production from alanine to the level synthesized by normal liver (16.2 $\pm$ 1.2 μmoles per g per hour) and doubled the amount of urea formed. Gluconeogenesis and urea production from alanine by adrenalectomized liver were also returned to normal by intraperitoneal administration of dexamethasone before hepatectomy. ¶ These data demonstrate that glucocorticoid hormones have an important effect on conversion of alanine to pyruvate and indicate that a primary function of adrenal steroids in hepatic gluconeogenesis is to enhance conversion of amino acids to pyruvate.

Role of Acetylcholine in Alveolar Collapse during Systemic Hyperoxia. G. EMMANUEL, L. MIJARES, E. MEHLMAN, AND P. KOTTMEIER, Brooklyn, N. Y. (introduced by William Dock †).

The fall in arterial oxygen saturation frequently observed during infusion of acetylcholine into the pulmonary circulation has been attributed to release of pulmonary vasoconstriction and increase of blood flow to underventilated and hypoxic alveoli, or to opening of anatomical shunts. The alternative possibility that acetylcholine affects arterial oxygen tension not by its vasomotor effect on the pulmonary circulation but by constriction of terminal airways was investigated. Acetylcholine was infused into the pulmonary artery in five anesthetized dogs at a rate of 0.06 mg per kg per minute during inhalation of 100% oxygen, which produced a maximal rise in alveolar and capillary oxygen tension. Pulmonary and femoral arterial pressure, transairway pressure and airflow, and arterial oxygen tension (Pa_{O_2}) were continually monitored, the latter *in vivo* by an intra-arterial oxygen microelectrode. Since administration of 100% oxygen raises oxygen tension to nearly maximal even in underventilated alveoli in the normal lung, a fall in oxygen tension produced by acetylcholine infusion during systemic hyperoxia must be attributed to either collapsed but perfused alveoli or to openings of anatomical shunts. Within 45 seconds of acetylcholine infusion Pa_{O_2} fell from an average control value of 571 mm Hg to a final value of 476 mm Hg, which was maintained during the 5- to 10-minute infusion. Five to 8 minutes after cessation of the infusion, Pa_{O_2} averaged 498 mm Hg and increased to 593 mm Hg within 1 to 2 minutes after increase of tidal volume from 250 to 650 ml. The data suggest that atelectasis due to constriction of terminal air passages or changes in alveolar surface tension or both occurs during acetylcholine infusion. It is postulated that some of the effects of acetylcholine on the pulmonary circulation previously described may be partly attributed to focal atelectasis.

Carbonic Anhydrase Deficiency with Persistence of Fetal Hemoglobin. LIE-INJO LUAN ENG AND R. TARAIL, San Francisco, Calif. (introduced by H. H. Fudenberg *).

A new syndrome is reported in a 47-year-old Greek male with weakness, muscle cramps, evanescent dermal lesions, night sweats, episodes of partial loss of vision, and hepatosplenomegaly. Although he was not anemic, red cell abnormalities and immature leukocytes were found in the peripheral blood smear. Fetal Hb ranged between 60 and 70% and HbA_2 between 0.3 and 0.5% of the total hemoglobin. Marked deficiency of red cell carbonic anhydrase (both the B and C genetic variants) was demonstrated by qualitative as well as quantitative methods. His father is dead. His mother died with

an obscure blood disease; his two children, two brothers, and two maternal uncles had normal amounts of HbA_2, no increase of HbF, and no carbonic anhydrase deficiency. Beta-thalassemia major and homozygous hereditary persistence of fetal hemoglobin (HP-F) are excluded, since both children of the patient had normal amounts of HbF and HbA_2, and the level of HbF in the patient was too high for heterozygous HP-F. HP-F associated with δ-thalassemia as the cause for the increase of HbF and extremely low HbA_2 seems unlikely, since in this condition one would not expect HbF to increase above 50%. Further HP-F and β-thalassemia are not associated with deficiency of carbonic anhydrase. Carbonic anhydrase catalyzes the reaction $CO_2 + H_2O \rightleftharpoons H_2CO_3$; deficiency of the enzyme may result in slowing the reaction and possibly in carbon dioxide accumulation. It seems fortunate that the patient had a large amount of HbF, which is more suitable under anoxic conditions. It is possible that carbonic anhydrase deficiency is the primary defect and results in compensatory adjustments in the types of hemoglobin synthesized.

Activity of Phosphofructokinase in Human Erythrocytes: Relationship to Cell Age. R. Bennett Eppes, James V. McNamara, Robin D. Powell, and Paul E. Carson,* Chicago, Ill.

Available evidence indicates that the activities of a number of enzymes, including glucose-6-phosphate dehydrogenase (G-6-PD), hexokinase, and pyruvate kinase, decrease markedly in human erythrocytes as they age *in vivo*. Reactions catalyzed by hexokinase, pyruvate kinase, and phosphofructokinase have been considered by some workers to be rate-limiting steps of anaerobic glycolysis in mature human red cells. ¶ To determine the relationship between the activity of phosphofructokinase and the age of normal human erythrocytes, we separated blood from five normal men by a method of serial centrifugation into fractions containing relatively young and relatively old red cells. Reticulocyte, erythrocyte, leukocyte, and platelet counts of each fraction, and activities of pyruvate kinase, G-6-PD, glutathione reductase, and phosphofructokinase in hemolysates of each fraction were determined. ¶ Mean reticulocyte counts and mean activities of pyruvate kinase and G-6-PD were markedly greater in young compared to old cell fractions, confirming previous results. In contrast, the mean activity of glutathione reductase, as reported in earlier studies, was not significantly different in hemolysates of young compared to old cells. The mean activity of phosphofructokinase in hemolysates of young cells was 45% greater than that in hemolysates of old cells. The magnitude of the mean differences in enzyme activity between young and old cell fractions was: pyruvate kinase > G-6-PD > phosphofructokinase > glutathione reductase. In this sequence, the change in activity of phosphofructokinase is similar to that previously reported for hexokinase. ¶ Because glycolysis provides the major source of energy in mature human erythrocytes, decreased activities of certain glycolytic enzymes may be important determinants of red cell survival. The age-related diminution in the activity of phosphofructokinase, along with decreased activities of other key erythrocytic enzymes, including hexokinase, pyruvate kinase, and G-6-PD, may be of importance in senescence of the human red cell.

Intestinal Lactase Deficiency and Saccharide Malabsorption Produced by Oral Neomycin. W. W. Faloon, P. Searl, M. Rubert, and I. C. Paes, Syracuse, N. Y. (introduced by Eugene L. Lozner †).

Steatorrhea and excessive fecal loss of nitrogen and electrolytes occur with oral administration of neomycin, possibly as a result of widespread intestinal mucosal damage. Mucosal lactase activity, saccharide absorption, and fecal excretion have been studied in obese subjects receiving constant diets containing 50 to 150 g of lactose before, during, and after oral administration of neomycin, 12 g daily for 4 to 6 days. In four subjects jejunal mucosal lactase was decreased during neomycin: control, 79 (range, 37 to 149) U per g of wet tissue; neomycin, 18 U (range, 9 to 24). Addition of neomycin to mucosal homogenates *in vitro* did not alter lactase activity. Oral lactose tolerance (100 g) in five subjects revealed a decrease in average blood glucose rise during neomycin: control mean, 47 (range, 25 to 80) mg per 100 ml; neomycin, 21 (3 to 33) ($p < 0.01$). Similar decrease was found in the average blood glucose rise after glucose-galactose tolerance: control, 55 (30 to 76); neomycin, 31 (7 to 49) ($p < 0.01$). By thin-layer chromatography, lactose, glucose, and galactose were not found in feces during control periods, but were found in all subjects during neomycin. Addition of lactose to normal feces *in vitro* yielded glucose and galactose after 24 hours of refrigerated storage with or without added neomycin. Substitution of glucose for dietary lactose in two patients eliminated saccharide excretion in the feces. Thus, the fecal glucose and galactose found *in vivo* after neomycin and lactose may have been due to lactose splitting in an intestinal segment too distal for absorption. Use of a lactose-free diet failed to prevent steatorrhea in two patients receiving neomycin. ¶ It is concluded that among the absorptive defects produced by neomycin, mucosal lactase deficiency and saccharide malabsorption should be included.

Some Characteristics of the Renal Nuclear Receptors for Aldosterone. Darrell D. Fanestil and Isidore S. Edelman,* San Francisco, Calif.

The available evidence indicates that aldosterone regulates sodium transport by inducing *de novo* synthesis of proteins. The present report is on the first step in this process, namely the binding of aldosterone to receptors and the relationship of these binding reactions to the physiological action of this steroid. In the first three sets of experiments varying amounts of aldosterone-^{3}H were injected into adrenalectomized rats with and with-

out the competing steroids, 9α-fluorocortisol or 17β-estradiol (2×10^{-6} mole per rat). The kidneys were removed at varying times after the injections and fractionated by density gradient and differential centrifugation into nuclear, mitochondrial, microsomal, and supernatant fractions. The following results were obtained: *1*) Only the nuclear fraction contained saturable binding sites for aldosterone. *2*) The metabolites of aldosterone did not bind to the saturable sites of the nuclear fraction. *3*) The nuclear binding sites saturated at plasma concentrations of aldosterone that corresponded to the maximal antinatriuretic effect. *4*) The active mineralocorticoid, 9α-fluorocortisol, inhibited the binding of aldosterone to the nuclear receptors competitively. *5*) The inactive steroid, 17β-estradiol, did not inhibit nuclear uptake of aldosterone. To obtain information on the nature of the nuclear receptors, renal nuclei, labeled endogenously with aldosterone-^{3}H, were incubated *in vitro* either with control solutions or with a variety of lytic enzymes. Incubation with DNAase (0.25 mg per ml) solubilized 94% of the nuclear DNA, and incubation with RNAase (0.25 mg per ml) solubilized 75% of the RNA, but neither enzyme released the bound aldosterone. Similarly, lipase (0.25 mg per ml) and phospholipase (0.25 mg per ml) did not reduce the quantity of aldosterone-^{3}H bound to the nuclei. The proteases, papain (0.25 mg per ml), pronase (0.25 mg per ml), and chymotrypsin (0.25 mg per ml), released virtually all of the bound aldosterone, suggesting that the nuclear receptors for aldosterone are solely proteins.

A Multicompartmental Analysis of Glucose Kinetics in Man. JOHN W. FARQUHAR, GERALD R. CHASE, ROGER L. LERNER, AND GERALD M. REAVEN, Palo Alto, Calif. (introduced by Halsted R. Holman*).

Previous estimates in man of exchangeable glucose pool size (EG) and its turnover rate (EG-tr) have assumed that EG distributes in a single pool and turns over without recycling. Furthermore, irreversible disposal of glucose (EG-ID) and new glucose production were considered equal to EG-tr. We have given glucose-^{14}C as a single intravenous injection to fasted normal and diabetic humans and to normals during a steady state of hyperglycemia produced by glucose infusion. Disappearance of ^{14}C label from plasma was clearly not first order, indicating the inadequacy of the one pool model. The observed multiexponent plasma glucose-^{14}C removal rates were analyzed by a digital computer program for linear compartmental analysis that sought a least squares solution for a series of exponentials. A two or three pool model fit the experimental data equally well, but a one pool model could be readily rejected ($p < .001$). In eleven studies, glucose infused at a mean rate of 475 mg per minute resulted in a mean plasma concentration of 175 mg per 100 ml. Further evidence for the inadequacy of the one pool model was obtained by its prediction of a mean EG-tr and EG-ID of only 342 mg per minute in contrast to the 475 mg per minute infused. Comparisons of mean values (in mg glucose per kg body weight per hour) between fasted normals, glucose-infused normals, and diabetics revealed the following: *1*) EG-tr of 590 in fasted normals is due largely to recycling (480), whereas irreversible disposal (EG-ID) was only 110; *2*) new glucose production of infused normals was largely suppressed (20), but the EG-ID (412) was fourfold greater than that of diabetics (103) whose glucose concentrations averaged 226 mg per 100 ml. Although low EG-ID in diabetics (103) was expected, its comparability to fasted normals EG-ID (110) is of interest. In diabetics, recycling (1,030) was large in absolute quantity and comprised as large a fraction of the EG-tr (1,133) as in normals (480 of 590). Anticipated further refinement of these methods should allow more precise formulation of glucose transport and distribution in normal and disease states.

Spontaneous and Induced Alterations in the Anion-binding Properties of Human Albumin. RICHARD S. FARR,* ROBERT T. REID, AND PERCY MINDEN, La Jolla, Calif.

Although serum albumin levels are frequently altered during disease, it is not known whether the quality of albumin may sometimes be altered. More than 1,000 sera have been studied for their capacity to bind ^{131}I-labeled sodium acetrizoate (I* acetrizoate) with either equilibrium dialysis or anion exchange columns. Normal sera bind I* acetrizoate in direct proportion to the albumin content. Crystalline human albumin also binds I* acetrizoate, but normal α-, β-, and γ-globulins do not. In contrast, sera from some patients bind as much as five times more I* acetrizoate than can be accounted for on the basis of normal albumin content. This unusual affinity for I* acetrizoate, most often observed in sera from patients with rheumatoid arthritis, is due to a qualitative alteration of albumin. Elevated I* acetrizoate binding has not been observed in normal individuals. There is no correlation between I* acetrizoate binding, salicylate levels, the latex fixation test, or steroid medication. Some patients who take neither steroids nor salicylates have elevated I* acetrizoate binding, but albumin from normal individuals given 2.4 g acetylsalicylic acid per day for 21 days binds significantly more I* acetrizoate. Similarly, the capacity of normal albumin to bind I* acetrizoate can be enhanced by dialysis against acetylsalicylic acid *in vitro*. Thus, albumins sometimes undergo qualitative changes during disease, and some of the albumin alterations may be affected by exogenous chemicals such as aspirin. The presence of albumins with high affinity for sodium acetrizoate in some patients not taking medications suggests that albumin can be altered by endogenous products as well. These data indicate that the capacity of albumin to bind, and hence transport, biologically important anions may sometimes be altered in disease.

An Electron Microscope Radioautographic Study of Cytoplasmic Granule Formation in Rabbit Myelocytes. MARTHA E. FEDORKO AND JAMES G. HIRSCH,* New York, N. Y.

The cytoplasmic granules of mature polymorphonuclear leukocytes exemplify primary lysosomes, i.e., they are membrane-bound organelles containing hydrolytic enzymes that apparently have not yet been involved in digestion. Immature leukocytes in the bone marrow, which are actively forming cytoplasmic granules, are thus suitable for a study of primary lysosome formation. We have investigated granule genesis in rabbit heterophilic myelocytes by observing the intracellular flow of tritiated lysine as revealed by electron microscope radioautography. Label over the Golgi complex rose to a maximum of 37% of total cytoplasmic grains 30 minutes after pulse exposure to the tracer and fell to 11% by 3 to 4 hours of incubation. Coincident with decrease in label over the Golgi complex, grain counts over granules rose to 32% by 3 to 4 hours. The time sequence of incorporation and flow of tritiated lysine and the per cent distribution of label were similar in bone marrow myelocytes under *in vivo* and *in vitro* conditions. The results demonstrate a function of the Golgi complex in incorporating or packaging certain basic amino acids or proteins into cytoplasmic granules of heterophilic myelocytes.

The Relationship between Amphotericin B Action and Membrane Sterols. DAVID S. FEINGOLD, Boston, Mass. (introduced by A. Stone Freedberg †).

A body of evidence suggests that membrane sterols play a central role in the lethal action of the polyene antibiotics such as amphotericin B. Recently, additional strong support for this hypothesis has come from our laboratory in studies using *Mycoplasma laidlawii* Type A. The organism grown in sterol-free medium contains no sterol and is resistant to amphotericin B. When cholesterol is added to the medium, sterol is incorporated into the membrane by a temperature-dependent process, and the organism becomes sensitive to amphotericin B. ¶ The nature of the sterol-drug interaction has been further investigated with the following results: *1*) Cholesterol binds with amphotericin B, resulting in a shift in the absorption spectrum of the antibiotic; *2*) the maximal shift in absorption occurs at approximately equimolar concentrations of drug and sterol; and *3*) esterification of the 3-hydroxyl group of the sterol abolishes both the interaction with amphotericin B and the ability of the compound to convert drug-resistant to drug-sensitive organisms. ¶ A mutant of *Mycoplasma laidlawii* Type A isolated in the presence of amphotericin B is resistant to the drug even when grown in cholesterol. Although the mutant incorporates much less cholesterol-4-^{14}C into its cell membrane than the wild type, it takes up an amount adequate to render the wild type sensitive to amphotericin B. Thus the presence of an appropriate sterol in the membrane is essential but not sufficient for polyene sensitivity. The labeled cholesterol incorporated by the mutant can be recovered as free cholesterol. Therefore, these data suggest that in the mutant the membrane sterol is either inaccessible to amphotericin B or is oriented in such a way that its interaction with drug no longer results in membrane damage.

Prognosis by Computer: Based on Storage and Retrieval of Data for 5-Year Clinical Course of 691 Cases of Lung Cancer. ALVAN R. FEINSTEIN AND NEAL KOSS, New Haven, Conn. (introduced by J. W. Hollingsworth *).

To select routine treatment or to design clinical trials with precision, physicians must assess the quantitative details of previous therapeutic experience. The necessary details cannot be maintained in any single human memory and are not readily available in most contemporary techniques of record keeping, statistical charting, and "end results" surveys. ¶ Failure to analyze details of symptoms and of indications used for therapeutic decision has been a major source of nonreproducible results (and controversy) in cancer therapy. By classifying and analyzing these clinical and judgmental data in addition to the conventional laboratory and morphologic data, we have previously demonstrated the achievement of improved accuracy in the evaluation of prognosis and treatment for human cancer. ¶ The systems of classification developed in those investigations have served as a basis for storing, in an IBM 7094-7040 digital computer system, the complete natural and 5-year post-therapeutic course of 691 patients with cancer of the lung. The computerized details include all the standard morphologic and laboratory data, as well as such clinical features as iatrotropic stimulus, types of symptoms, pretherapeutic duration of symptoms, reasons for therapeutic decisions, and post-therapeutic events. These stored data are then used to predict the outcome of a new patient by comparing results of previous patients in appropriate categories of resemblance. ¶ Perhaps the greatest value of pretreatment predictions in lung cancer is to anticipate the "operable" patients who will die in less than 6 months and the inoperable cases who will survive more than 6 months. The computer system correctly predicted these 6-month survival distinctions before treatment in 80% of "new" cases whose data were analyzed "prospectively." Analogous predictions of 2-year survival distinctions were correct in 89% of these patients. ¶ This type of computer program can be an effective aid in designing investigative therapeutic trials in cancer, in choosing treatment for individual patients, and in evaluating natural history and therapeutic accomplishments.

Heterogeneity in the Inheritance of Glycogen Storage Diseases. JAMES B. FIELD * AND ADREINNE REMER, Pittsburgh, Pa.

Absent debranching enzyme activity (DE) has been reported in leukocytes of patients with type III glycogen storage disease (GSD). Normal activity averages 316

± 13 cpm per 10^7 leukocytes (range, 200 to 450). Three patients with this type GSD and their parents were studied. One patient had DE of 12 cpm per 10^7 leukocytes; her mother's activity was 155 and the father's 107, consistent with the heterozygote state. Another patient had DE of 5 cpm per 10^7 leukocytes; the mother's level was 111, but the father's value of 247 was in the normal range. The third patient had leukocyte activity of 128 ± 28. Very low hepatic DE and 14% hepatic glycogen content established the diagnosis. Hepatic glucose-6-phosphatase and phosphorylase were normal. The father's leukocyte DE was 167 ± 32, and the mother's was 211 ± 27. Thus, leukocyte DE is not always very low in type III GSD, and such studies may not clearly establish the diagnosis. Although the patient's muscle DE was 5% of normal, muscle DE in both parents was normal. The mother's hepatic DE was 60% of normal, whereas the father's was normal. This complex situation is quite different from that observed in four families with children having type I GSD (glucose-6-phosphatase deficiency). Type I patients have very low glucose-6-phosphatase activity in intestinal mucosa. Each parent of the four sets had intestinal glucose-6-phosphatase activity approximately 50% of normal. The maternal grandfather of one patient had about 50% reduction of enzyme activity, whereas the maternal grandmother had normal levels. One of the grandfather's brothers had a similar reduction, and the other had normal activity. These observations are consistent with inheritance as a recessive autosomal gene and intermediate expression in the heterozygote. The findings in type III GSD indicate a more complex genetic situation such as polygenic modification or inactivation of the type described by Lyon.

Immunoglobulin (Gamma Globulin) Formation by Human Cells in Continuous Culture. IRA FINEGOLD, ALAN S. RABSON, AND JOHN L. FAHEY,* Bethesda, Md.

Test tube production of antibody in continuously cultured cells has not yet been realized. As a step towards this goal, effects were made to establish human lymphoid cells in continuous culture. Investigations of immunoglobulin synthesis *in vitro* included lines of Burkitt lymphoma obtained from American and African patients. These tumor cells characteristically have ribosome rich cytoplasm and resemble transformed lymphocytes. When these cells were incubated in medium containing ^{14}C-labeled amino acids, synthesis of immunoglobulin was demonstrated in one of the cell lines that had been maintained in culture for 6 months. This immunoglobulin was characterized by physicochemical and immunochemical techniques and shown to be 7 S IgG (γG) with kappa type light polypeptide chains. Re-examination of the cell line in the succeeding 3 months showed continued production of the same protein. After these original observations, studies were extended to other lymphomas maintained in culture. Preliminary results indicate that additional forms of immunoglobulins are being synthesized by other human cell lines. As a result of these findings immunoglobulin formation in continuous culture of human cells is under investigation.

An Abnormality of Creatine Trapping by Dystrophic Skeletal Muscle. COY D. FITCH, LACKEY G. MOODY, AND M. RAHMANIAN, Little Rock, Ark. (introduced by Richard V. Ebert †).

Since *N*-phosphorylcreatine serves as an energy source to sustain contraction, the proper function and perhaps the viability of skeletal muscle depend on a uniquely high creatine content. Mediated entry and intracellular trapping are two of the mechanisms that compensate for creatine losses. Either abnormal entry or abnormal trapping of creatine could lower creatine content. To gain new information about one of the metabolic errors in hereditary muscular dystropy of the mouse, we have measured *in vitro* movements of creatine-1-^{14}C across the membranes of an intact skeletal muscle, the extensor digitorum longus (EDL). For a study of entry, EDL were incubated in Krebs-Ringer bicarbonate solutions containing a series of creatine concentrations ranging from 1 to approximately 300 times the physiologic serum creatine concentration. With each concentration, the rate of creatine entry per milliliter of intracellular water was greater in EDL from dystrophic than from paired control mice. This increased transport of creatine into skeletal muscle suggests that abnormal intracellular trapping rather than abnormal entry accounts for the previously demonstrated low creatine content of dystrophic skeletal muscle. Additional information indicative of abnormal creatine trapping was obtained from efflux studies. Efflux from the EDL was measured 1 week after labeling muscle creatine by giving creatine-1-^{14}C parenterally to mice. Under aerobic conditions no difference between dystrophic and control EDL was detected, but, under anaerobic conditions, a part of the intracellular creatine moved out of dystrophic EDL at a reduced rate in comparison to the movement of creatine out of control EDL. Thus, not only is less creatine trapped in dystrophic skeletal muscle, but also some of that which is trapped evidently is held in an abnormal way.

The Renal Response to Streptococcal Infection. PHILIP FREEDMAN, H. PETER MEISTER, BUN SIU CO, CLEM ASERON, ABRAHAM S. MARKOWITZ, AND ALVIN DUBIN, Chicago, Ill. (introduced by Harry F. Dowling †).

In the course of studies designed to explore the relationship between beta hemolytic streptococcal infection and the kidney, 304 adults were examined once weekly for 4 weeks after their infection. One hundred four of these were admitted on the basis of clinical or urinary abnormalities for study. Sixty patients were selected further for intensive investigation including renal biopsy. ¶ In this group of 60 patients, the latent period ranged between 2 and 32 days with a mean of 14. Edema occurred in 15 patients, significant hypertension in 6, and gross hema-

turia in 2. Thirty-nine patients were without significant symptoms. Urinary abnormalities were present in all 60 patients, including a mild to moderate proteinuria in 58. Proteinuria was absent in 2 patients. The sediment contained a preponderance of erythrocytes in 6, leukocytes in 22, and both features in 23. Creatinine clearances ranged between 36 and 113 with a mean of 68 ml per minute. ¶ The principal histological features present in varying degree in all the biopsies studied consisted of mesangial and cellular changes both epithelial and endothelial. Immunofluorescent studies have revealed no significant localization of immunoglobulins or β_{1c}-globulins in the glomerular lesion. Bacteriological studies so far in this group have revealed the streptococcus to be group A in 59, type 12 in 15 instances, and other than type 12 in 14. Follow-up studies to date in 15 patients have shown variable histological evolution with an appearance in 8 instances of chronic glomerulonephritis without change in their clinical status. ¶ It is considered on the basis of these studies that at least one-third of all patients after a beta hemolytic streptococcal infection have significant urinary abnormalities. This has been associated with a glomerulonephritic lesion in all the patients studied by biopsy. Follow-up studies are in progress to determine the status of the residual lesion.

The Enzymatic Synthesis of Wax. SAMUEL J. FRIEDBERG * AND RONALD C. GREENE, Durham, N. C.

In the course of a study in this laboratory of the metabolism of cetyl alcohol, it was found that this material was converted to another compound by an enzyme system present in both mammalian and shark liver. After isolation of the unknown by thin layer chromatography and hydrolysis, it was found to represent an ester of cetyl alcohol and long chain fatty acids (wax). The hydrolysis products were further identified by gas chromatography. Microsomal and supernatant fractions were active, and wax formation occurred in these systems without activation, but did require the addition of emulsifiers such as Triton X-100, Tween 20, or bile salts. (The reaction was not stimulated by ATP, CoA, magnesium, or CTP.) With a substantially delipidated enzyme preparation it was possible to demonstrate that wax synthesis required both long chain fatty acids and cetyl alcohol, thereby providing further confirmation for the identity of the compound. With ^{14}C-labeled wax it was shown that the rate of hydrolysis was much slower than the rate of formation. The enzyme was inhibited by DFP. In many ways the reaction resembles the synthesis of cholesterol esters from pancreatic cholesterol esterase. It is postulated that the synthesis of waxes from long chain alcohols and fatty acids without activation is possible in an environment from which water is essentially excluded and can be envisioned in the case of two nonpolar compounds and an enzyme that interact in a micellar state. The absence of water would favor synthesis rather than hydrolysis in an enzyme system that catalyzes an equilibrium reaction—in conformity with the law of mass action.

Hemodynamically Induced Reversal of the Sodium Retention Resulting from Acute Superhepatic Inferior Vena Caval Constriction. ROBERT M. FRIEDLER, LOUIS BELLEAU, AND LAURENCE E. EARLEY, Boston, Mass. (introduced by Maxwell Finland †).

In the dog constriction of the inferior vena cava (IVC) above the diaphragm increases tubular reabsorption of sodium (T_{Na}), prevents natriuresis during saline infusion, and leads to sodium retention and ascites. We previously reported that combined renal vasodilatation and increased arterial pressure may result in large decreases in T_{Na} in normal hydropenic dogs. The present studies were designed to determine if these hemodynamic factors affect T_{Na} in the presence of IVC constriction. In ten dogs the IVC was constricted after natriuresis had been established by infusing 1,500 ml of an isotonic Ringer's solution. Arterial pressure, filtration rate (GFR), and renal plasma flow (RPF) decreased during IVC constriction, and sodium excretion ($U_{Na}V$) decreased an average of 71% (−937 μEq per minute per kidney). Unilateral renal vasodilatation (renal arterial infusion of acetylcholine) restored RPF but not $U_{Na}V$. When arterial pressure was returned towards control by intravenous infusions of angiotensin, and in some experiments norepinephrine, $U_{Na}V$ in vasodilated kidneys increased to average 100% of control (preconstriction) rates, whereas GFR was 2 to 16 ml per minute below control. Increases in $U_{Na}V$ by nonvasodilated control kidneys during pressor infusions were less and were unpredictable. In six experiments caval constriction before saline loading decreased arterial pressure and prevented natriuresis. Vasodilatation produced ipsilateral increases in $U_{Na}V$ averaging +127 μEq per minute. Pressor infusions increased arterial pressure an average of 22 mm Hg above control and increased $U_{Na}V$ by vasodilated kidneys to an average of +747 μEq per minute and only +102 μEq per minute in nonvasodilated control kidneys. Filtered sodium increased an equivalent amount in only one experiment. These studies demonstrate that the sodium-retaining effect of acute IVC constriction may be reversed by combined renal vasodilatation and increased arterial pressure and that a reduction in these hemodynamic factors may be involved in limiting sodium excretion in the presence of IVC constriction.

Cardiac Participation in Renovascular Hypertension. EDWARD D. FROHLICH, MILOS ULRYCH, HARRIET P. DUSTAN,* AND IRVINE H. PAGE, Cleveland, Ohio.

Increased vascular resistance is considered the hemodynamic hallmark of diastolic hypertension; the heart is thought to play a secondary role, except in some labile hypertensive patients with elevated cardiac output. Labile blood pressure is often associated with renal arterial stenosis, suggesting a possible cardiac role in this renal hypertension. ¶ Hemodynamic functions were studied in ten normal subjects and ten patients each with untreated essential and renovascular hypertension; none had been in cardiac failure. Renal arteriography was

performed in all patients; only those with nonatherosclerotic types of stenosis were included. The essential hypertensives were selected from a large number because their mean arterial pressures matched those of patients with renal arterial disease. Duplicate cardiac output determinations and brachial arterial pressures were obtained. Cardiac indexes for each group were as follows: normal, 2,912; essential hypertension, 2,562; and renovascular hypertension, 3,367 ml per minute per m^2. Respective total peripheral resistances were 0.016, 0.028, and 0.022 mm Hg per ml per minute; and respective mean rates of left ventricular ejection were 151, 124, and 155 ml per minute per m^2. ¶ Cardiac indexes of patients with renovascular hypertension were higher than normal ($p < 0.01$), whereas those of essential hypertensives were lower ($p < 0.05$). The difference in cardiac index between the two hypertensive groups was highly significant ($p < 0.001$); this could not be attributed to arterial pressure, age, or sex. Vascular resistance was increased in the hypertensives, but was greater in essential than renovascular ($p < 0.01$). Mean rate of left ventricular ejection of essential hypertensives was less than occurred normally or in renovascular hypertensives ($p < 0.005$). ¶ Increased vascular resistance was not the sole hemodynamic abnormality in these hypertensive patients. The heart also participated in renovascular hypertension because cardiac output was significantly higher in this type even though in both hypertensions resistance was increased. The low cardiac indexes and left ventricular ejection rates may implicate the heart in essential hypertension, but more observations are necessary.

Effects of Epinephrine on Isolated Adipose Cells from Normal and Overweight Patients. DAVID J. GALTON AND GEORGE A. BRAY, Boston, Mass. (introduced by E. B. Astwood †).

Subcutaneous adipose tissue from man was treated with collagenase, and the liberated fat cells were isolated and incubated in an albumin-phosphate buffer. Several effects of epinephrine on the metabolism of these cells were examined, and the results were expressed on the basis of millimoles triglyceride of adipose cells per hour. ¶ In a series of ten normal subjects epinephrine (3 μg per ml) stimulated fatty acid release from 0.43 to 3.17 μmoles, and the oxygen uptake increased from 26 to 36 μl. When insulin (1 mU per ml) was added with epinephrine to the incubation medium, the increment in release of fatty acids was less than with epinephrine alone (from 0.53 to 2.40 μmoles). However, the oxygen consumption was stimulated to the same extent (25.7 to 35.3 μl). ¶ Adipose cells were then isolated from groups of five normal and five obese patients weighing over 300 pounds. Cells from the normal group showed the following responses to epinephrine (3 μg per ml): a release of fatty acids from 0.17 to 3.30 μmoles, a release of glycerol from 0.47 to 3.40 μmoles, and an increase in oxygen consumption from 30 to 43 μl. Cells from the obese patients behaved in a similar manner: the release of fatty acids rose from 0.43 to 4.5 μmoles, glycerol release increased from 0.61 to 3.7 μmoles, and an increment in oxygen utilization of 12 μl was observed. ¶ We conclude that epinephrine stimulates lipolysis in subcutaneous adipose cells isolated from normal and overweight patients and that in cells from both sources the amount of fatty acids re-esterified, as judged from the glycerol release, is approximately equal to the amount of fatty acids released into the medium.

Renal Excretion of Acetoacetate and Beta-Hydroxybutyrate in Experimental Ketosis. R. DENNIS GALVIN, JANET A. HARRIS, AND ROBERT E. JOHNSON,† Urbana, Ill.

The individual ketone bodies in the blood and urine have been studied in experimental ketosis produced in seven young men and four young women by *a*) a walk of 2.5 hours in the postabsorptive state followed by fasting and resting; *b*) a fast of 3 days followed by a walk with subsequent fasting and resting; or *c*) a diet providing 900 kcal fat and 100 kcal carbohydrate daily. Blood plasma was prepared from venous blood drawn during timed periods of collecting urine. Acetoacetate (plus acetone) and total ketone bodies were measured chemically and beta-hydroxybutyrate calculated as the difference. Endogenous creatine was measured and glomerular filtration rate calculated. For ketosis of increasing severity, plots for the blood plasma concentration of acetoacetate against beta-hydroxybutyrate and against the rate of excretion of acetoacetate were both linear. By contrast, the ratio of urinary beta-hydroxybutyrate to acetoacetate became progressively greater in an exponential relation, and a plot of beta-hydroxybutyrate concentration in the blood plasma against its rate of excretion was also curvilinear. Clearance values for both acetoacetate and beta-hydroxybutyrate were never higher than 20% of the glomerular filtration rate. A plot of the renal clearance for acetoacetate against its concentration in the blood plasma was hyperbolic with an asymptote around 15 ml per minute, but beta-hydroxybutyrate gave a relation of catenary type. At blood plasma values of beta-hydroxybutyrate around 1 mmole per L there was a minimal clearance at around 2 ml per minute. Above and below this point clearance values up to 25 ml per minute were reached. Our data are consistent with two hypotheses. First, there may be tubular reabsorption of both acetoacetate and beta-hydroxybutyrate. Second, the kidney may convert acetoacetate into beta-hydroxybutyrate.

Relationship of Blood Glucose (BG) and Serum Immunoreactive Insulin (IRI) during Repeated Intravenous Glucose Tolerance Tests (IVGTT) in Normals. M. J. GARCIA, J. S. SOELDNER, R. E. GLEASON, AND R. F. WILLIAMS, Boston, Mass. (introduced by A. Marble †).

Preliminary studies suggested a significant positive correlation between BG and IRI during IVGTT (0.5 g per kg) in normals. Seven nonobese volunteers, aged 19 to

35 years, received duplicate IVGTT performed 2 to 9 months apart. Although glucose disappearance rate (K) ranged from 5.60 to 1.34% per minute, the K of the two tests on the same subject did not differ significantly. IRI was maximal 1 minute after the end of the rapid (2 to 4 minutes) glucose infusion in 13 of 14 tests. BG and IRI were significantly correlated in all 14 tests ($p < 0.001$). Linear regression coefficients of IRI (y) on BG (x) were calculated for each test and regression lines compared. A sixfold variation between individuals was noted; however, regression coefficients for duplicate tests in each subject did not differ significantly. The linear regression equation of pooled data from all first tests ($y = 0.49x - 23.2$) was almost identical to that calculated from the second tests ($y = 0.49x - 18.3$). The predicted value of BG when IRI was set to 0 μU per ml was 47.3 and 37.3 mg per 100 ml, respectively. An index of insulin release was derived from the area under a graph of IRI versus time. A highly significant correlation ($p < 0.001$) was noted between K and the fraction of insulin released in the 0- to 10-minute interval, but no significant correlation was present between K and total (0 to 60 minutes) insulin release. Thus, the major determinant of glucose disappearance appears to be the immediate release of available insulin.

Erythropoietin Potentiation by Serum Proteins. Edward Gardner, Claude-Starr Wright,† Jasper P. Lewis, and Russell R. Moores, Augusta, Ga.

In the course of investigating the production of antisera to human urinary erythropoietin, it was found that antisera, incapable of neutralizing erythropoietin, paradoxically enhanced the erythropoietic effect twofold or more when assayed in polycythemic mice. Normal rabbit and human sera also enhanced the activity. The human urinary erythropoietin fraction (obtained from a patient with severe anemia) was the same Fraction II + III obtained by DEAE-cellulose column chromatography as previously described by Lewis. ¶ A highly purified α_1 acidic glycoprotein ("orosomucoid") also enhanced the activity of the purified urinary erythropoietin. Human albumin did not enhance the activity. ¶ A 20 × urine concentrate (same urine from which Fraction II + III was extracted) showed a twofold increase in erythropoietin activity when mixed with normal human serum before injection. Whole sera from the same patient (and other patients with anemia) were slightly, although consistently, enhanced by normal human serum. ¶ The enhancing effect was not found when Standard B (National Institute for Medical Research, London) was substituted for Fraction II + III. The difference was believed to be in the degree of purification of the two human urinary erythropoietin concentrates. ¶ These findings are in keeping with those of Kuratowska and associates, who implicated an α_1-globulin as the enhancing factor, and Contrera and associates. ¶ Possible hypotheses include: The enhancing protein *1)* provides a protective barrier, *2)* neutralizes an inhibitor to erythropoietin, or *3)* activates an erythropoietin precursor.

Pituitary Regulation of Bilirubin Excretion by the Liver. Lawrence M. Gartner and Irwin M. Arias,* New York, N. Y.

Hepatic uptake, conjugation, and excretion of bilirubin have been studied in normal and hypophysectomized male Sprague-Dawley rats. Total hepatic uptake of unconjugated bilirubin was calculated from the sum of conjugated bilirubin excreted in bile during a 90-minute intravenous infusion of unconjugated bilirubin, and total hepatic bilirubin and conjugated bilirubin in serum at the end of the infusion. Glucuronyl transferase activity was estimated in liver homogenates *in vitro* with unconjugated bilirubin as a receptor and excess uridine diphosphate glucuronic acid. Hepatic excretory maximum for conjugated bilirubin (E_mCB) was determined in bile obtained during continuous intravenous infusion of unconjugated bilirubin. Uptake of bilirubin by the liver was the same in normal rats [41.4 ± 7.4 (SD) μg per 100 g body weight per minute] and hypophysectomized rats (38.6 ± 11.2, $p > 0.10$). Hepatic glucuronyl transferase activity was the same in normal rats [0.120 ± 0.024 (SD) μmole bilirubin glucuronide formed per g of liver per 40 minutes] and hypophysectomized rats (0.127 ± 0.013, $p > 0.10$). E_mCB was significantly reduced in hypophysectomized rats [16.29 ± 5.45 (SD) μg conjugated bilirubin excreted per 100 g body weight per minute] as compared with normal rats (37.31 ± 9.61, $p < 0.01$). At the end of the infusion, the concentrations of conjugated bilirubin in serum and liver were four times greater in hypophysectomized rats than in normal rats. None of these results could be accounted for by differences in liver weight or blood volume between normal and hypophysectomized rats. The results suggest that after hypophysectomy, there is defective transfer of conjugated bilirubin from the liver cell into bile. ¶ Subcutaneous administration of porcine growth hormone (1.0 or 2.0 mg per day for 6 days) to hypophysectomized rats restored E_mCB to normal. In hypophysectomized rats E_mCB was significantly increased by oxytocin and thyroxine, unaffected by insulin and vasopressin, and decreased by diethylstilbestrol. Enzyme cytochemical studies of liver from hypophysectomized rats revealed changes in bile canaliculi and lysosomes, which also returned to normal after growth hormone administration.

The Contractile State of the Human Left Ventricle as Determined by Myocardial Tension-Velocity Relations. James H. Gault, John Ross, Jr.,* James W. Covell, Edmund H. Sonnenblick, and Eugene Braunwald,* Bethesda, Md.

It has been shown in the intact dog heart that the relation between instantaneous wall tension (WT) and shortening velocity (V_{CF}) provides a means of examining the contractile state of the intact left ventricle. Previously, however, it has not been possible to quantify this important relation in the human left ventricle. In six patients, left ventricular (LV) pressure was

measured continuously with a catheter tip transducer, while radiographic contrast material was injected into the left atrium. The true radius (r) of the minor LV circumference (circ) was determined at 0.017-second intervals from cineangiograms, and this measurement was correlated with instantaneous LV pressure, the tracing of which was recorded directly on cine film. V_{CF} was calculated as 2π dr/dt. WT in the corresponding slice of muscle was computed as P.r/wall thickness. In two patients without LV, the maximal V_{CF} were 1.60 and 1.70 circ per second, the corresponding WT being 350 and 565 g per cm², respectively. At maximal WT (360 and 616 g per cm²) the corresponding V_{CF} were 1.21 and 0.98 circ per second, respectively. In the remaining four patients, all of whom had other hemodynamic evidence of LV disease, the maximal V_{CF} were consistently lower, ranging from 0.33 to 1.05 circ per second, whereas the range of WT present at the time of maximal V_{CF} was similar to that observed in the other patients (230 to 670 g per cm²). The V_{CF} at maximal WT (240 to 670 g per cm²) in these four patients were also low, ranging from 0.30 to 0.91 circ per second. It is concluded that the instantaneous relations between tension and velocity can be determined in the human left ventricle by these techniques and that this type of analysis may permit detection as well as quantification of LV myocardial dysfunction.

Effect of Luminal pH on Ion Transport across the Reptilian Bladder. D. GENTILE, C. F. GONZALEZ, Y. SHAMOO, AND W. A. BRODSKY,† Louisville, Ky.

Previous work has shown that reduction of the pH of ambient mucosal fluid of the isolated turtle bladder causes a systematic reduction in transbladder potential (PD) and short circuit current (I_{sc}). Mucosal surfaces were bathed by buffer-free isosmotic solutions of $NaCH_3SO_4$, NaCl, or choline chloride and serosal surfaces by Na-rich or Na-free (choline) Ringer's. Addition of H^+ (H_2SO_4 or HCl) to the Na-rich system (serosa electropositive) caused decreases in PD, I_{sc}, and in net sodium current, I_{Na} (estimated from fluxes of labeled Na), and caused increases in d-c resistance (R) as mucosal fluid pH decreased from 8.0 to 3.5. The plot of PD vs. mucosal pH formed a pattern resembling the titration of an electronegative macromolecule with an iso-electric point of pH 3.8. With $NaCH_3SO_4$ as mucosal fluid, $I_{sc} = I_{Na}$ at high and at low levels of I_{sc} (corresponding to a range of mucosal pH from 8 to 4, respectively). This suggests that H^+ interferes with the affinity of Na for cationic transport sites. Addition of H^+ to Na-free system (serosa electronegative) caused decreases in PD, I_{sc}, and in net chloride current, I_{Cl} (estimated from fluxes of labeled chloride), and caused increases in R as mucosal pH decreased from 8 to 3.5. The pattern of PD vs. pH was in the direction of that expected from diffusion of H^+ from mucosal to serosal fluid, but the slope of the line was only 4 to 5 mv per tenfold change in concentration of free H^+. With choline chloride as mucosal fluid, $I_{Cl} \geq I_{sc}$ at pH 8; and $I_{Cl} = 4.01\ I_{sc}$ at pH 4, suggesting that H^+ either increases the affinity of choline for cationic transport sites, or that H^+ competes successfully with choline for occupation of cationic sites.

Hb $C_{Georgetown}$; First Abnormal Hemoglobin Due to Two Different Mutations in the Same Gene. PARK S. GERALD * AND CHARLES E. RATH, Boston, Mass., and Washington, D. C.

Hb $C_{Georgetown}$ (Hb C_G) is an unusual hemoglobin that exhibits sickling properties indistinguishable from those of Hb S but has the electrophoretic mobility of HB C. A composition of $\alpha_2\beta_2^{7\ 17s}$ was previously proposed for this hemoglobin on the basis of incomplete chemical studies combined with the assumption that only a single mutation will be found in any given hemoglobin gene. This assumption was based on the experience available at the time. A recent re-examination of Hb C_G has disclosed that it represents the first known departure from this assumption. On electrophoresis in urea-starch gel the β chain of Hb C_G, as expected, migrates parallel to the β chain of the usual Hb C. The 2-U charge difference between Hb A and Hb C_G thus resides solely in the β chain. When the isolated Hb C_G is exposed to cyanogen bromide (which cleaves β chains specifically at the single methionyl residue, no. 55) and the fragments are subjected to electrophoresis, both β fragments exhibit abnormal electrophoretic mobility. The abnormal electrophoretic mobility in each corresponds to a 1-U charge difference. This is incontrovertible evidence of two distinct point mutations, each resulting in a single charge alteration, at different points in the β-chain gene. Of the soluble tryptic peptides, only the amino terminal peptide appears to be abnormal (it has the composition of the peptide from Hb S). The second point mutation, therefore, resides in the "core" (residues 83 to 120). This doubly mutated gene may have arisen either *1*) by a second mutation superimposed upon a β^s gene or *2*) by a crossover between a β^s gene and a second β gene that had a mutation in the core region.

Evidence for an Effect of Deoxycorticosterone on the Proximal Renal Tubule. JOHN R. GILL, JR., AND FREDERIC C. BARTTER,† Bethesda, Md.

Sodium-retaining steroids promote reabsorption of sodium in the distal renal tubule; it has not been demonstrated that they affect reabsorption of sodium in more proximal portions of the nephron. In the present study, the effect of infusion of 4% fructose solution, 14 ml per minute, on clearance of solute-free water was determined in six subjects (five normal, one a patient with diabetes insipidus), receiving a sodium intake of 140 mEq per day or greater. Renal clearances were measured twice, once as control and once on the second day of treatment with deoxycorticosterone (DOC), 20 mg

per day. Mean control maximal free water clearance was 9.6 ± 0.7 (SEM) ml per minute; maximal free water clearance with DOC was significantly less, 7.6 ± 0.7 ml per minute ($p < .05$). Clearances of inulin and of para-aminohippurate were comparable in each pair of studies. In the studies with DOC, sodium excretion ($U_{Na}V$) was lower by 40 to 63% and potassium excretion (U_KV) higher by 90 to 418% than in the corresponding control studies. The decrease in free water with DOC was associated with a decrease of 25 to 34% in solute excretion ($U_{Na}V + U_KV$) in five studies. These findings suggest an effect of DOC proximal to the distal tubule and probably proximal to the ascending limb of the loop of Henle.

The Incorporation of Urea-^{15}N into Serum Proteins of Uremic Patients on Low Nitrogen Diets. CARMELO GIORDANO, CARLO DE PASCALE, CIRO BALESTRIERI, DOMENICO CITTADINI, AND ADA CRESCENZI, Naples, Italy (introduced by Eugene C. Eppinger †).

The diet of five uremic subjects and one normal volunteer on nitrogen balance study was supplemented with 38 mg per kg of ^{15}N in the form of urea. The specific activity of ^{15}N in the serum proteins and the constituent amino acids was studied by mass spectrometry. The ^{15}N content of blood ammonia was estimated in all of the subjects including two patients whose intestinal flora was suppressed with Humatin. Uremic patients on a diet with a nitrogen source of 11 g of L-essential amino acids incorporated about 80 mg of ^{15}N into the serum proteins. Isotopic ^{15}N was greater in the nonessential amino acids although some essential acids were labeled *in vivo*. The normal volunteer incorporated 7 mg of ^{15}N into protein on a 76-g protein diet and 9 mg on a 21-g protein diet. This patient remained in negative nitrogen balance during a 41-day low protein intake. Azotemic patients incorporated more than 50 mg of ^{15}N into serum proteins and showed positive nitrogen balance. The patients receiving antibiotics had no ^{15}N in their ammonia. These same patients, however, did incorporate ^{15}N into their amino acids. All of the other patients were noted to have labeled blood ammonia. These data support the hypothesis of the anabolic role of urea in uremic patients on low nitrogen diets.

The Protein Requirement for Platelet Surface Reaction. M. F. GLYNN, M. A. PACKHAM, J. HIRSH, AND J. F. MUSTARD,* Bethesda, Md., and Toronto, Canada.

Adherence of platelets to surfaces is the initial event in hemostasis, in thrombus formation, and in platelet phagocytosis. Following this interaction, platelets aggregate and they release nucleotides (including adenosine diphosphate), serotonin, and lysosomal enzymes. In a model system when polystyrene particles are added to platelets in plasma the platelets aggregate and release constituents; when the particles are added to once washed platelets in a suspension, no release or aggregation occurs. If the polystyrene particles are first coated with plasma and then added to the suspension, the entire sequence of events takes place. Similarly, none of the other surfaces tested, including glass beads, antigen-antibody complexes, intact viruses, and bacteria, was able to induce aggregation unless first coated with this plasma factor. Albumin and fibrinogen, which readily coat the surfaces, are not able to render them effective. Starch gel electrophoresis, immunoelectrophoresis, and immunodiffusion have shown that the activity is in the γ-G-globulin fraction. Collagen is the only substance tested that appears to have a self-contained ability to induce platelet release and aggregation, but after partial denaturation it was possible to elute a protein that, when adsorbed onto polystyrene particles, was able to induce release and aggregation. This material shared some of the characteristics of the above protein. ¶ Salicylaldoxime, a compound that inhibits the action of complement, and DFP, an inhibitor of esterase activity, were examined. Both blocked the action of collagen, antigen-antibody complexes, and γ-globulin–coated polystyrene. ¶ The importance of the specific protein in eliciting the release reaction is related to fibrinogen. If collagen or the other surfaces are coated with fibrinogen, release of platelet constituents is suppressed. This suggests that the interplay between fibrinogen and this protein influences the platelet-surface reaction in the body.

Response of Renal Diluting Sites to Saline Loading in Man. MARTIN GOLDBERG,* BARRY R. WALKER, AND VARDAMAN M. BUCKALEW, JR., Philadelphia, Pa.

To evaluate the hypothesis that urinary dilution begins in the loop of Henle (site 1), and maximal dilution occurs more distally (site 2), we studied four patients with diabetes insipidus. Three per cent NaCl was infused until solute clearance (C_{osm}) reached values of 15 to 30 ml per minute [fractional solute excretion (C_{osm}/GFR) of 15 to 20%]. At low C_{osm} free water excretion (C_{H_2O}) rose initially 2 to 3 ml per minute and then reached a plateau while C_{osm}/GFR rose from 4% to 15%. Further increments of C_{osm}/GFR were associated with a sharp secondary rise in fractional free water excretion; C_{H_2O} rose to values as high as 28 ml per minute, with no evidence of an upper limit as urine osmolality/plasma osmolality (U_{osm}/P_{osm}) approached a maximal limiting value of 0.6. The plateau in the C_{H_2O} vs. C_{osm} curve might be explained by the net effect of simultaneous increases in sodium reabsorption (T_{Na}) at site 1 and decreases in T_{Na} at site 2. To eliminate the effects of changes in T_{Na} at site 2, we gave infusions of maximal doses of chlorothiazide that initially decreased C_{H_2O} 1 to 3 ml per minute, after which the infusion of 3% NaCl now produced a progressive rise in C_{H_2O}, with no evidence of a plateau even at C_{osm}/GFR exceeding 15%. In contrast, ethacrynic acid (which acts at site 1) administered before saline loading markedly lowered C_{H_2O}, which could not be raised to control levels with saline. Data indicate two

sites of dilution in man with the following functional characteristics: site 1 in the loop of Henle progressively increases T_{Na} with saline loading to maintain a constant U_{osm}/P_{osm} at 0.6, and site 2 in the distal nephron produces minimal U_{osm}/P_{osm} only at low C_{osm} and decreases T_{Na} with saline loading.

The Relationship between Urinary H^+ and K^+ Concentrations during Hydropenia in Man. CARL GOLDSMITH AND GEORGE E. SCHREINER,* Washington, D. C.

The relationship between urinary H^+ and urinary K^+ in dogs was studied by Berliner and associates, who showed by acute infusion of Diamox that there was competition for tubular secretion between H^+ and K^+ probably in exchange for Na^+. More recently this mechanism has been challenged by micropuncture studies which indicate that secretion of K^+ and H^+ may, in part, be passive in response to changes in intraluminal potential differences. Neither possibility has been explored in man. We have studied six normal young men who were fed diets that varied independently in K^+ and H^+ content. After subjects had been on each diet for 4 days, urine collections were made during hydropenia. Under these conditions there is an inverse relationship between H^+ and K^+ concentrations in the linear form $H^+ = 118 - 0.82\ K^+$ ($r = 0.72$, $p < 0.001$). The relationship between H^+ and K^+ excretion rates is likewise inverse and significant. In those patients whose urine was alkaline during dehydration because of diet, oral NH_4Cl diminished urinary K^+ as urinary H^+ concentration rose. Conversely in those patients whose urine was acid, the infusion of hypertonic $NaHCO_3$ or the oral administration of KCl again produced inverse changes in urinary H^+ and K^+. The administration of aldosterone during a low H^+ diet produced a greater increase in H^+ than in K^+ concentration as Na^+ concentration fell, but because there was simultaneously a further decrease in urine flow, K^+ excretion actually diminished inversely as H^+ excretion rose. These studies indicate that in man there is a linearly inverse relationship between urinary H^+ and K^+ in many different circumstances. This inverse relationship strongly supports the concept of competition for secretion between H^+ and K^+ in the distal nephron.

Effects of Oral Phosphate Supplements in Demineralizing Disorders of Bone. RALPH S. GOLDSMITH AND SIDNEY H. INGBAR,* Boston, Mass.

Previous studies from this laboratory have indicated that in a variety of disorders associated with hypercalcemia, supplements of inorganic phosphate (1 to 3 g P daily) promptly decrease both serum calcium concentration and urinary calcium excretion. To evaluate the mechanism of this effect and to determine whether phosphate supplements have therapeutic utility in other disorders of mineral metabolism, we have conducted additional studies in ten patients with demineralizing disorders of bone. Of these, four were hypercalcemic (hyperparathyroidism, malignancy, multiple myeloma) and six were normocalcemic (paraplegic immobilization, multiple myeloma, and Cushing's syndrome). Except in the patient with Cushing's syndrome, severity of illness precluded formal balance studies. Phosphate was administered in divided oral doses (1 to 2.5 g P per day) as a buffered mixture of Na_2HPO_4 and KH_2PO_4 for periods as long as 6 weeks. In hypercalcemic patients, phosphate restored normocalcemia; in normocalcemic patients, serum calciums were not significantly changed. Phosphate concentrations measured in sera obtained before the first daily dose were also unaffected. In all patients, phosphate induced a prompt and sustained decrease in urinary calcium excretion. In the patient with Cushing's syndrome, strongly negative calcium and phosphorus balances were reversed. In each of the ten patients, urinary excretion of hydroxyproline, considered an index of bone resorption, decreased during phosphate administration. The mean decrease of 46% was highly significant ($p < 0.01$). In four of five patients, excretion of hydroxyproline increased after phosphate withdrawal. Bone pain, when present, was moderately or strikingly ameliorated during phosphate therapy. ¶ The findings suggest that phosphate supplementation has a fundamental effect of decreasing bone resorption, an effect that is manifest in a variety of demineralizing disorders. Long-term studies to evaluate the therapeutic efficacy of phosphate in remineralizing bone in such disorders are in progress.

The Lack of an *In Vivo* Effect of Synalbumin upon Glucose Metabolism in the Rat. CHARLES J. GOODNER AND MAYER B. DAVIDSON, Seattle, Wash. (introduced by J. Thomas Dowling*).

In 1958 Vallance-Owen showed that albumin from human plasma antagonized the effect of insulin on rat diaphragm. This *in vitro* observation took on potential clinical significance when he further demonstrated that plasma from untreated diabetics had higher than normal concentrations of this antagonist. However, *in vitro* studies on the mechanism of this insulin antagonist in our laboratory caused us to question its postulated physiological role. Thus, with rat diaphragm, antagonistic albumin showed a noncompetitive pattern of insulin inhibition, blocked only two of three sites of insulin action (transport and RNA synthesis but not glycogen synthesis), and depressed oxygen consumption. ¶ To search directly for a physiologic effect, we tested antagonistic albumin for its capacity to alter glucose homeostasis in highly anesthetized rats. Antagonistic albumin (human Fraction V) or nonantagonistic albumin (bovine Fraction V) was infused intravenously for 3 hours. The concentrations of foreign albumin achieved with infusion rates of 180 or 450 mg per hour averaged 3.4 and 5.5 g per 100 ml, respectively. The antagonist did not prevent establishment of a constant blood glucose in nonfasted normal rats or alter disposal of exogenous glucose in fasted normal rats. To limit insulin reserve, we used partially pancreatectomized rats in further studies. Again, the antagonist had no discernible effect on glucose regulation. Furthermore, the hypoglycemic response

to exogenous insulin was not altered by the antagonist. Diaphragms removed from infused rats showed complete insulin sensitivity when tested *in vitro*. And finally, plasma collected from infused rats did not antagonize the effect of added insulin on normal diaphragms. This inability to alter glucose homeostasis *in vivo* and the failure of both the tissues and the plasma from infused rats to demonstrate insulin antagonism provide direct evidence that the albumin antagonist is not active in the living animal and therefore probably has no pathogenetic significance in diabetes mellitus.

Studies of Uterine Renin. PHILLIP GORDEN, THOMAS F. FERRIS, AND P. J. MULROW,* New Haven, Conn.

A renin-like enzyme in the uterus of the rabbit has been demonstrated. This finding is of special significance since the plasma renin activity is elevated in human pregnancy and the renin concentration of amniotic fluid and umbilical cord blood is higher than in maternal plasma. ¶ Our studies have shown that extracts of pregnant rabbit uterus have renin concentrations equal to extracts of whole rabbit kidney. Pregnant rabbit uterus has a higher renin concentration than nonpregnant uterus ($p < 0.025$). There is no diminution in uterine renin concentration 48 to 72 hours postnephrectomy in the pregnant rabbit. The administration of DOCA and NaCl to the pregnant animal lowers renal renin concentration ($p < 0.005$) but does not diminish uterine renin concentration. Uterine renin concentration falls rapidly 24 hours postpartum ($p < 0.05$). ¶ Plasma renin activity is higher in pregnant than nonpregnant animals. Plasma renin activity is unchanged or higher 48 hours postnephrectomy in the pregant animal, whereas it disappears from the plasma in the nephrectomized nonpregnant animal. The administration of DOCA and NaCl to the pregnant rabbit does not diminish plasma renin activity. Thus, plasma renin activity correlates best with the uterine renin concentration. ¶ A renin-like substance has also been detected in the pregnant human uterus. Incubation of pregnant uterine extracts with renin-free human plasma forms small amounts of antiotensin II. ¶ In summary, these data suggest that the maintenance of uterine renin concentration and plasma renin activity in the pregnant rabbit is not solely dependent upon the kidney and does not respond to the same physiologic stimuli that suppress renal renin. Since the half-life of renin is short, these studies suggest that the rabbit uterus is capable of producing renin. Whether there is any analogy between the rabbit and human tissue with respect to uterine renin remains to be determined. A uterine source of renin, however, may explain the high concentrations found in amniotic fluid and umbilical cord blood in human pregnancy.

Transperitoneal Water Transport during Peritoneal Dialysis. ARTHUR GORDON, LESLIE R. BENNETT, AND MORTON H. MAXWELL,* Los Angeles, Calif.

Radioactive isotope tracer techniques have been applied to peritoneal dialysis in an attempt to evaluate the dynamics of transperitoneal transport of water. When radioiodinated human serum albumen (RIHSA) was added to dialyzate and introduced into the peritoneal cavity of normal and uremic dogs and uremic humans, insignificant quantities (0.5 to 3%) of the administered isotope were lost from the peritoneal cavity. Therefore, changes in intraperitoneal RIHSA concentration accurately reflected changes in intraperitoneal volume. Isotopically determined intraperitoneal volumes varied only 1 to 4% from the volumes ascertained by complete mechanical drainage of the peritoneal cavity. Dialyzing solutions of varied osmolality were utilized. In dogs, when hypotonic dialyzate (145 mOsm per L) was used, intraperitoneal volume decreased at a rate of 500 ml per 2 hours, indicating that the peritoneal cavity is a potential route for the parenteral administration of hypotonic solutions at a rapid rate. In humans and dogs, isosmotic dialyzing solutions resulted in little or no net transperitoneal water movement. Hyperosmolar dialyzing solutions resulted in increased intraperitoneal volumes in both humans and dogs. The rate of this net fluid transport remained maximal for 30 to 60 minutes and ranged from 7.6 ml per minute for an osmotic gradient of 50 mOsm per L to 42.3 ml per minute for an osmotic gradient of 350 mOsm per L. The rate of this increase was dependent upon transperitoneal osmotic gradient and the surface area of the peritoneal cavity. There was no difference in transperitoneal transport of water in normal and uremic subjects. Because of the reproducibility of transperitoneal water movement curves, for any given osmolality of dialyzate formulae can be derived that permit prediction of water movement in any subject. These formulae also serve to determine optimal equilibration time for clinical application of hypertonic dialysis in the treatment of edematous states.

Hydrogen Transport into Mitochondria: A Study with Intact Ehrlich Ascites Tumor (ELD) Cells. EDWIN E. GORDON,* LARS ERNSTER, AND GUSTAV DALLNER, New York, N. Y., and Stockholm, Sweden.

Several proposals have been advanced to account for the high rate of aerobic glycolysis and low respiratory activity of ELD cells. A deficiency in the mitochondrial electron transport system is unlikely because the concentrations of the respiratory components are adequate to support a far higher respiratory rate. Studies of the regulatory role of ADP and the efficiency of transport of extramitochondrial hydrogen to the mitochondrial respiratory chain have been hampered because of the necessity to study these mechanisms in the intact cell. In the present experiments, endogenous respiration (Clark O_2 electrode) was almost completely blocked with specific inhibitors. Under these conditions, the contribution of extramitochondrial hexose metabolism to respiration could be assessed. Addition of glucose to cells in which the tricarboxylic acid cycle was inhibited with fluoroacetate, fluorocitrate, or arsenite resulted in considerable lactate production, but failed to initiate respiration. Thus, the reducing equivalents generated during glycolysis were

unable to gain access to the mitochondria. However, in the presence of vitamin K_3, addition of glucose resulted in a 6- to 30-fold stimulation of O_2 consumption and substantially diminished lactate production. Evidence was obtained that the vitamin K_3-induced respiration proceeded via DT diaphorase and involved the transfer of hydrogen from the extramitochondrial compartment to the mitochondrial respiratory chain at the level of cytochrome b. Iodoacetate (5×10^{-3} M) completely inhibited lactate formation and reduced the glucose-vitamin K_3-induced respiration by about 50%. Thus, glucose oxidation via the pentose phosphate shunt and the Embden-Meyerhof pathway contributed TPNH and DPNH, respectively, for the DT diaphorase-mediated respiration. ¶ These experiments indicate that the endogenous respiration of ELD cells can be dissociated from respiration supported by extramitochondrial metabolism. The data are consistent with the concept that the high rate of lactate accumulation under aerobic conditions results from an inability of these cells to transfer hydrogen from extramitochondrial reduced pyridine nucleotides to the mitochondrial respiratory chain.

Role of Sympathetic Nervous System in Mediating Renal and Adrenocortical Secretory Responses to Upright Posture. R. D. Gordon, O. Kuchel, D. P. Island, and G. W. Liddle,* Nashville, Tenn.

Previous studies have demonstrated that the assumption of upright posture leads to increases in renal secretion of renin and adrenocortical secretion of aldosterone. Other studies have shown that upright posture is associated with increased catecholamine excretion and that catecholamine administration is associated with increased renal vascular resistance. The present studies were performed to determine whether the sympathetic nervous system plays an important role in mediating renal and adrenocortical secretory responses to upright posture. ¶ Four patients with sympathetic nervous system disease leading to postural hypotension were found to have subnormal or negligible increases in plasma renin activity in response to upright posture. However, the intravenous infusion of norepinephrine:epinephrine (10:1) at a rate just sufficient to prevent postural hypotension corrected the defect in renin secretion, so that the assumption of upright posture led to normal brisk increases in plasma renin activity. Aldosterone excretion was also higher when postural hypotension was corrected by catecholamine infusions than when it was uncorrected. Thus, a fall in systemic blood pressure in the absence of adequate sympathetic activity appears to be relatively ineffective in bringing about a rise in renin secretion. ¶ Further studies were performed in normal subjects to determine whether sympathetic nervous system activity would again be correlated with renin secretion. When three healthy individuals on liberal sodium diets repeatedly immersed their hands in ice water for 90 minutes, they exhibited elevations of plasma renin activity despite simultaneous elevation of arterial blood pressure. The usual posturally induced rises in plasma renin activity were consistently prevented by compression of the carotid sinus by a cuff (25 mm Hg) or by bandaging the legs before subjects assumed an upright posture. ¶ It is concluded that upright posture leads to pooling of blood in the legs. This leads to reflex sympathetic nervous activity and catecholamine release. This then mediates (possibly through renal arteriolar constriction) increased renin secretion, which in turn leads to increased aldosterone secretion.

Correlation of B_{12}-Binding Proteins with Disorders of B_{12} Metabolism: Relation to Hypo- and Hyperleukocytic States and Leukocyte Turnover. C. W. Gottlieb, F. P. Retief, P. W. Pratt, and V. Herbert,* New York, N. Y.

The high B_{12}-binding α-globulin seen in chronic myelogenous leukemia (CML) and low B_{12}-binding globulin in aplastic anemia suggest serum B_{12}-binding globulins may derive in part from leukocytes. In this study, four parameters [B_{12} (pg per ml), unsaturated B_{12} binding capacity (UBBC, pg per ml), and per cent of UBBC on α and β] were (number of subjects) as follows: *a*) normals (15): 604, 1,186, 19, 81; *b*) untreated pernicious anemia (PA) (20): 56, 1,447, 32, 68; *c*) treated PA (15): 476, 1,429, 21, 79; *d*) untreated, non-PA, B_{12} deficient (9): 71, 1,868, 31, 69; *e*) pregnancy (11): 358, 2,188, 23, 77; *f*) cirrhosis (24): 884, 1,449, 23, 77; *g*) polycythemia vera (PV) (31): 827, 2,162, 28, 72; *h*) spent PV (5): 764, 2,804, 25, 75; *i*) myeloid metaplasia (8): 1,284, 3,496, 46, 54; *j*) CML (20): 3,331, 5,104, 74, 26; *k*) erythroleukemia (3): 826, 766, 35, 65; *l*) acute myelogenous leukemia (8): 2,583, 4,895, 24, 76; *m*) chronic leukopenia (7): 1,161, 617, 17, 83; *n*) chronic leukocytosis (4): 526, 1,915, 23, 77. This study confirms reduced total serum B_{12} binding capacity in untreated PA. Despite frequent associated leukopenia, UBBC was reduced relatively slightly compared to non-B_{12}-deficient leukopenic states, suggesting total leukocyte pool may be increased to compensate for reduced leukocyte turnover. Philadelphia (Ph^1) chromosome did not correlate with α-globulin abnormalities since three Ph^1 negative patients had high α-globulin binders, as did all untreated Ph^1-positive patients. B_{12}-binding α- and β-globulins do not have reduced ability to bind B_{12} after preincubation with antibody to α- or β-glycoprotein. Quantity and distribution of B_{12} binders were normal in β-lipoprotein deficiency (acanthocytosis) and acute porphyria (elevated thyroxine binding); there was low UBBC but normal α:β ratio in one case of α-lipoprotein deficiency (Tangier disease).

Cholera Toxin and Nonionic Diffusion of Ammonia. George F. Grady, Morton A. Madoff, Edward W. Moore, and Thomas C. Chalmers,† Boston, Mass.

Contrary to the popular concept that cholera toxin produces diarrhea by specifically inhibiting active sodium transport, the following data suggest that inhibition of the frog skin sodium pump occurs by a mechanism that is unrelated to the effect of such toxins upon intestinal

mucosa. This conclusion was reached during assays of comparative toxicity using two common test systems, the isolated short-circuited frog skin and the diarrhea-susceptible infant rabbit. Bacteria-free filtrates were prepared from 24-hour aerated shake cultures of virulent *Vibrio cholerae,* strain 569B, and as controls, avirulent *V. cholerae,* normal enteric flora, and uninoculated media, 3% peptone or 1% casamino broths. Frog skin toxicity was defined as the net inhibition of transmembrane potential (PD) or current (I_{sc}) produced within 45 minutes after replacement of Ringer's solution in the double chamber by test or control filtrates. Infant rabbits were observed for diarrhea and histological alterations of intestine after intragastric instillation of up to 2 ml of filtrate per 100 g body wt hourly four times. Frog skin PD or I_{sc} inhibition was produced by filtrates of either *V. cholerae* or normal flora and was proportional to alkalinity. Total ammonia was measured by the Nathan-Rodkey method, and nonionic ammonia concentration [NH_3] was calculated using a pK'_a of 9.37. The PD and I_{sc} inhibition was found to be directly proportional to log [NH_3] regardless of the inoculum or media used. Furthermore, inhibition did not exceed that found with Ringer's solution containing equal amounts of NH_4^+ salts, and toxicity of filtrates was abolished by removal of NH_3. However, the infant rabbit model was not susceptible to any filtrate except virulent cholera, regardless of [NH_3] or pH. In summary, the toxic effects of *V. cholerae* and other bacterial filtrates upon frog skin membrane can be explained by nonionic diffusion of NH_3, but the effect upon intestine is specific for virulent *V. cholerae.*

Inhibition of the Bactericidal Activity of Alveolar Macrophages by Cigarette Smoke. GARETH M. GREEN AND DIANA CAROLIN, Boston, Mass. (introduced by Edward H. Kass †).

Recent evidence has demonstrated that alveolar macrophages are primarily responsible for the clearance of inhaled bacteria. When alveolar macrophages were obtained by tracheobronchial lavage of rabbits' lungs and layered on the surface of tissue culture flasks, 90% of an added bacterial culture of *Staphylococcus albus* was killed in 3 hours. Introduction of cigarette smoke depressed this *in vitro* killing activity in a dose-related fashion. Five ml of smoke in the culture flask produced about 50% inhibition, and 10 ml complete suppression of bacterial killing with resultant bacterial multiplication. Several standard length, king size, and filtered brands of cigarettes produced virtually identical results. One brand with a charcoal filter had a toxicity of one-third to one-half that of other brands. ¶ The toxic action of cigarette smoke was not removed by filtration of the visible particles. However, after a single bubbling through an aqueous buffer, the smoke became nontoxic for the cells. Furthermore, aqueous extraction of the smoke showed virtually a quantitative transfer of the toxic action to the aqueous phase. ¶ Preliminary mechanistic studies showed that the toxic action did not depend on the presence of oxygen, as the effect was equally demonstrable aerobically and anaerobically. There was no regular reversal of the toxic effect with addition of the substrates glucose, acetate, succinate, or citrate. Attempts to reproduce the toxic action with acetaldehyde, formaldehyde, and cyanide were unsuccessful in concentrations similar to those found in smoke. ¶ The finding that phagocytosis of bacteria by alveolar macrophages is inhibited by cigarette smoke suggests a possible direct pathogenic relationship between cigarette smoking and bacterial infection in such cigarette-associated diseases as chronic bronchitis. The ability to remove the toxic material indicates a potentially useful preventive approach.

The Role of Endotoxin during Typhoid Fever and Tularemia in Man. SHELDON E. GREISMAN,* RICHARD B. HORNICK, WILLIAM E. WOODWARD, AND THEODORE E. WOODWARD,† Baltimore, Md.

When bacterial endotoxins are infused intravenously at constant rates into healthy volunteers, the febrile and toxic responses return to base line within several hours despite the continuing infusion. Such rapid refractoriness appears to represent specific desensitization to endotoxin, since endotoxin-refractory rabbits exhibit unimpaired pyrogenic responsiveness to influenza virus, tuberculin, and staphylococcal enterotoxin. If sustained endotoxemia plays an important role in pathogenesis of typhoid fever and tularemia, the capacity for rapid desensitization to endotoxin should be drastically suppressed during illness. Eight volunteers with induced typhoid fever and seven with induced tularemia were infused intravenously with 18×10^{-6} μg per minute *Salmonella typhosa* endotoxin. After an initial hyperreactive pyrogenic and toxic response, a rapid decline to, but not below, the base-line febrile levels ensued. In several subjects, this endotoxin-refractory state was interrupted by a protracted rise in temperature coincidental with the expected increase in late afternoon fever. These observations during typhoid fever and tularemia indicate that *1*) refractoriness to continuous endotoxemia develops as readily as in normal subjects, *2*) despite such refractoriness, fever and toxemia are not mitigated, and *3*) late afternoon temperature increases occur in the expected fashion during the endotoxin-refractory state. ¶ It is concluded that sustained endotoxemia cannot be primarily responsible for the continuous febrile and toxic course of typhoid fever and tularemia in man. Rather, other mechanisms, presumably similar to those responsible for sustained fever and toxemia during infection with nonendotoxin-containing microbes, must be operative. It is emphasized that although endotoxemia cannot account for sustained illness, release of a relatively small bolus of endotoxin into the circulation during illness (or upon appropriate antibiotic administration) could readily produce an acute febrile and toxic spike, including shock. Such an event, however, would be sharply circumscribed and would be superimposed upon the more basic mechanisms responsible for the characteristic prolonged febrile and toxic state.

Effect of Glucose "Pulse," Glucagon, and the Cations Ca^{++}, Mg^{++}, and K^{+} on Insulin Secretion *In Vitro*. GEROLD M. GRODSKY AND LESLIE L. BENNETT, San Francisco, Calif. (introduced by Peter H. Forsham †).

The isolated perfused preparation of rat pancreas has been modified *1*) to employ oxygenated albumin-buffer as perfusate thereby allowing precise addition of ions and *2*) to permit "pulsing" of the pancreas with various agents while collecting total pancreatic eluate at 30-second periods. Thus, controlled evaluation of glucose, hormone, or cation effects on insulin secretion has been facilitated. Tissue viability was evaluated by oxygen consumption, glucose uptake, and valine-^{14}C incorporation into total protein. ¶ *Glucose* pulse experiments produced a graded insulin response within 30 seconds after glucose entry, terminating within 30 seconds after glucose disappearance. Thus a glucose-stimulated pancreas has no "memory," secretion occurring only during stimulation. *Glucagon* stimulated insulin secretion directly in the absence of glucose. Secretion paralleled within 30 seconds a glucagon pulse (measured with glucagon-^{131}I). A species-discriminating immunoassay confirmed that the insulin was derived from rat pancreas rather than impurity in the glucagon. Casein, an insulinase inhibitor, had no effect on insulin release, nor did glucagon affect degradation of perfused trace insulin-^{131}I. *Ca^{++}* is required for insulin secretion; in its absence, tissue remained viable, but glucose stimulation of insulin secretion was totally blocked. Inhibition could be reversed by addition of Ca^{++} at 20 or 40 minutes after deprivation. *Mg^{++}* was nonessential and could not substitute for Ca^{++}. *K^{+}*, when raised to 8 mEq per L, proved a direct stimulant of insulin release in the absence of any glucose. Thus insulin secretion is sensitively controlled by glucose concentration, there being essentially no pancreatic "memory." However, other biologic substances such as glucagon, Ca^{++}, and K^{+} may act at more primary sites in the release mechanism and, regardless of glucose concentration, may be major controlling factors in subjects with diabetes, islet tumors, or aldosteronism.

Mechanism of Action of Emetine: The Demonstration of Its Inhibitory Action on Protein Synthesis. ARTHUR P. GROLLMAN, New York, N. Y. (introduced by David Hamerman *).

Despite the established efficacy and wide clinical use of emetine in amebiasis, the mechanism of its amebicidal action remains obscure. We have found that emetine, at concentrations of 10^{-7} mole per L, instantly and irreversibly inhibits protein synthesis in mammalian cells without directly affecting the synthesis of RNA or DNA. It had no effect on bacterial protein synthesis *in vivo* or *in vitro* but prevented the growth of poliovirus and vaccinia virus in HeLa cells. The inhibitory effect of emetine has been demonstrated in cell-free systems prepared from rabbit reticulocytes, rat liver, or HeLa cells and has been shown to be due to its action on the enzymes that transfer amino acids from aminoacyl-sRNA to the polyribosome in the assembly of the polypeptide chain. ¶ The structural requirements for these inhibitory effects of protein synthesis by emetine are highly specific; thus, cephaline and dehydroemetine are active at concentrations of 10^{-7}, whereas the closely related ipecac alkaloids, O-methylpsychotrine, emetamine, isoemetine, (+)-emetine, and other stereoisomers are inactive at concentrations of 10^{-3} mole per L. There is a good correlation between the capacity of emetine and its isomers to inhibit protein synthesis, as demonstrated in these studies, with their relative amebicidal activities. In addition to its amebicidal properties, emetine has recently been reported to have antiviral activity and to prevent the growth of granuloma tissue in man. These effects, as well as the cardiovascular, neuromuscular, and gastrointestinal complications of emetine therapy, are readily explicable on the basis of the presently reported effects of emetine on protein synthesis. The present findings offer a molecular basis for the pharmacologic and therapeutic action of emetine and its isomers.

Human Cholesterol Synthesis Is Regulated by Bile Acids. S. M. GRUNDY, A. F. HOFMANN, J. DAVIGNON, AND E. H. AHRENS, JR.,† New York, N. Y.

Utilizing chromatographic techniques developed here for measuring cholesterol balance in man, we have evidence that bile acids within the enterohepatic circulation regulate human cholesterol biosynthesis. This observation complements recent findings by others (confirmed by us) that, in normo- and hypercholesteremia, 2 to 3 g of dietary cholesterol has little effect on sterol synthesis, probably because intestinal absorption is limited. ¶ The present study involved eight sterol balance studies in five formula-fed hypercholesteremic patients who were given radioactive cholesterol intravenously. Cholesterol synthesis was quantified *1*) by measurement of total fecal neutral plus acidic steroids of endogenous origin (since in the steady state, synthesis equals excretion), and *2*) by calculations based on specific activities of fecal steroids and plasma cholesterol. Qualitative changes in synthesis were signified by changes in slopes of die-away curves of plasma radioactive cholesterol. ¶ Two lines of evidence indicated that bile acids inhibit the rate of cholesterol synthesis. First, expansion of the bile acid pool by oral administration of sodium taurocholate produced decreased rates of cholesterol turnover (shown by significantly decreased slopes in die-away curves). Sterol balance data indicated no increase in miscible body pool of cholesterol; therefore, decreased slopes of isotope decay signified decreased cholesterol synthesis. Conversely, interruption of the enterohepatic circulation of bile acids by ileal bypass or by feeding cholestyramine resulted in increased cholesterol synthesis (shown in the steady state by increased excretion of cholesterol products and by accelerated cholesterol turnover). ¶ The regulatory function of bile acids extends also to control of conversion of cholesterol to bile acids. Interruption of enterohepatic circulation was accompanied by a fourfold (±) increase in bile acid synthesis, and expansion of the bile acid pool

resulted in inhibition of cholesterol conversion to bile acids, without causing increases in serum cholesterol. Examples of these negative feedback control mechanisms will be presented.

Hypothalamic Obesity—A Biologic Bank Holiday? HERBERT A. HAESSLER AND JOHN D. CRAWFORD,* Boston, Mass.

The demonstration of a relative increase in palmitic acid and alterations in the distribution of other fatty acids in epididymal fat of hypothalamic obese rats has acquired greater significance since studies of human subcutaneous fat have shown significant differences between lean and obese children. To investigate mechanisms underlying these changes, we studied synthesis and release of individual fatty acids *in vitro* with epididymal fat from rats with ventromedial hypothalamic lesions. Total fat synthesis from acetate was accelerated early, but the distribution of synthesized fatty acids was unaltered. At 2 weeks, total fat synthesis was normal, but percentage synthesis of palmitate had declined and that of oleate increased relative to proportions in control tissue. Differential synthesis, therefore, failed to explain the alterations in tissue composition that were now fully developed and opposite to changes predicted from synthesis. ¶ Total free fatty acid release due to epinephrine from fat of animals with lesions declines rapidly after operation. Since altered tissue composition in these rats destined to develop obesity might be caused by this disturbance in lipolysis, studies were undertaken that showed changes in distribution of the individual released fatty acids predisposing to the characteristically altered distribution of obese animals' tissue fatty acids. For example, their fat released less palmitate compared to tissue content than did fat from controls. Since total synthesis is near normal and mobilization diminished during bulk fat accumulation, both obesity and the compositional change could reflect the animals' inability easily to withdraw calories from the adipose tissue energy bank. ¶ In fat of obese children, the alteration pattern differs from that of obese rats, the most consistent changes being elevation of palmitoleate and depression of stearate. Accordingly, these findings in animals should not be directly extrapolated to clinical obesity but may provide a prototype for similar studies of metabolic activity in human fat.

Fluctuating Patterns of Blood Clotting in Patients with Coronary Artery Disease. JAMES W. HAMPTON, PAUL COSTILOE, AND EDWARD N. BRANDT, Oklahoma City, Okla. (introduced by Francis J. Haddy *).

In vitro tests of blood coagulation have been repeated at frequent intervals in subjects presumed to have a high degree of susceptibility to coronary thrombosis and in controls to establish a pattern of the balance between clot-promoting and clot-destructive blood substances. The "high susceptibility" group consisted of 58 patients who had sustained one or more well-documented myocardial infarctions in the past. An equal number of presumably healthy individuals matched for sex, age, height, and weight served as controls. The data included intermittent measurements over a period of 53 months of silicone clotting time, prothrombin time with both whole and dilute plasmas, estimations of platelet numbers by phase microscopy, and plasma fibrinogen levels (micro-Kjeldahl method). An estimator for the common variance was derived for each of the tests measured in both groups. The variance was then used as a measure of fluctuation of these *in vitro* clotting tests. It was found that the platelet counts and the fibrinogen levels in the patients fluctuated significantly more than the same tests in the controls ($p < 0.01$ for both tests). Episodes of infection and bleeding could not account for the greater fibrinogen fluctuation in the patients. Although anticoagulation of 12 patients increased the fluctuation of their clotting and prothrombin times, it did not reduce their greater platelet and fibrinogen variances. Five of six patients not on anticoagulants who later died suddenly showed mean variances of their silicone clotting time after 6 tests greater than the maximal variance in the controls and accounted for five of the top ten variances of the patients. It is suggested that the fluctuation of clotting measurements in patients with coronary artery disease represents an unstable balance of their coagulation mechanism and may have prognostic significance for the recurrence of myocardial infarction.

The Role of Phosphorylase and Phosphofructokinase in the Regulation of Glycolysis in Toad Bladder. JOSEPH S. HANDLER *AND JERZY ROGULSKI, Bethesda, Md.

The rate of glycolysis of the toad bladder *in vitro* is known to be accelerated by anaerobiosis and by vasopressin and reduced by removal of sodium from the incubation medium. The enzymatic regulatory mechanism under these circumstances has been investigated by measuring the concentration of glycolytic intermediates, certain cofactors, and high energy compounds in perchloric acid extracts of the intact tissue. Stimulation of a rate-controlling enzymatic step should result in a lower steady state concentration of substrate and a higher concentration of product. The reverse will occur with inhibition. When the toad bladder was incubated anaerobically, glycolysis was accelerated, and there was a decrease in the concentration of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P) and a rise in the concentration of fructose 1,6-diphosphate (FDP) and triose phosphates (triose-P). These results are indicative of stimulation of phosphofructokinase, an effect of anaerobiosis described in other tissues. When the sodium in oxygenated Ringer solution was replaced by Tris, glycolysis was depressed and an increase in G6P and F6P and a fall in FDP and triose-P observed. These changes indicate inhibition of phosphofructokinase by removal of sodium. During aerobic or anaerobic incubation in sodium Ringer solution, vasopressin stimulated glycolysis and caused a rise in G6P and F6P and a relatively greater rise in FDP and triose-P. These results have been

interpreted as indicating stimulation of both phosphorylase and phosphofructokinase. The stimulation of phosphorylase activity by vasopressin is presumably related to activation by 3',5'-AMP. The link between sodium transport or vasopressin and phosphofructokinase activity is unknown. However, changes observed in the concentration of creatine phosphate, ATP, ADP, 5'-AMP, and inorganic phosphate can account for the stimulation of phosphofructokinase during anaerobiosis.

The Relationship between Mitochondrial Respiration and Steroid Hydroxylation, Competitive or Supportive. B. W. HARDING, L. D. WILSON, S. OLDHAM, AND D. H. NELSON,* Los Angeles, Calif.

With the demonstration of the P-450 cytochrome in adrenal mitochondria and its involvement in 11β-hydroxylation, an interrelationship between this system and the respiratory enzymes of the adrenal cortex was suggested. Correlation of measurements of dehydrogenase activity, oxygen tension, and shifts in oxidation-reduction steady states of electron carriers in intact mitochondria and isolated mitochondrial components have demonstrated the existence of a cyanide, uncoupled insensitive TPN-linked malate oxidase and an energy controlled, uncoupled sensitive pyridine nucleotide transhydrogenase. Both of these enzymes support electron transport to the hydroxylation enzymes at 10 to 20 times the rate supported by extramitochondrial TPNH. In the presence of suitable steroid substrates the rate of oxygen utilization by the hydroxylation pathway approaches that of the respiratory chain and suggests a potentially high level of competition between hydroxylation and respiration in bovine adrenal cortical mitochondria for oxygen and reducing equivalents. A similar situation exists in normal human adrenal mitochondria, but in this tissue marked preference for TPNH as an electron donor is shown. It appears likely that steroid hydroxylation is an energy-linked function of mitochondria supported by coupled respiration, but a nonenergy-dependent intramitochondrial TPNH-generating system supporting hydroxylation does exist that could compete with respiration for reducing equivalents.

Collagen Content, Solubility, and Cross-Linking in Human Dermis. EDWARD HARRIS, JR., AND ALBERT SJOERDSMA,* Bethesda, Md.

Collagen abnormalities in disease states remain poorly defined. In the present work a direct approach to the chemistry of tissue collagen was undertaken in biopsy specimens of skin from 30 normal subjects (aged 19 to 60 years) and 45 patients (aged 19 to 65) with conditions appearing to involve cutaneous collagen. The effects of long-term treatment with penicillamine were also examined in 5 patients because of recent evidence that this drug solubilizes collagen, as do lathyrogens (e.g., β-aminopropionitrile). ¶ Variables studied and normal values were as follows: *1*) total collagen (as hydroxyproline, HOPr) = 95 to 130 μg HOPr per mg dry weight (dw); *2*) soluble collagen (per cent extractable in 0.5 M acetic acid) = 1.2 to 3.2%; *3*) degree of cross-linking in soluble collagen expressed as α:β ratio (monomer:dimer by disc gel electrophoresis) = 0.8 to 1.2; and *4*) water content = 59 to 69%. Keloids and young scars (<6 months) contained increased soluble collagen (4.6 to 9.7%) with decreased cross-linking (α:β = 1.4 to 2.0) and had a high water content (74 to 80%). Older scars resembled normal skin. Specimens from 5 of 6 patients with scleroderma had low soluble collagen (0.6 to 1.0%), and 3 of 6 yielded low total collagen (72 to 83 μg HOPr per mg dw). Other findings included the following: dwarf, low soluble collagen; mycosis fungoides, low total but high soluble collagen; and Marfan's syndrome and osteogenesis imperfecta, chemically normal. Four of 5 patients on penicillamine had increased soluble collagen (3.8 to 9.8%) that was poorly cross-linked (α:β = 1.4 to 2.1); similar findings were noted in a case of homocystinuria. ¶ In addition to increasing understanding of pathologic mechanisms, these studies suggest that drugs which affect the cross-linking process may have therapeutic potential.

Cell-fusing Activity of a Virus That Causes a Demyelinating Disease. DONALD H. HARTER AND PURNELL W. CHOPPIN,* New York, N. Y.

Visna virus causes a slow demyelinating disease of sheep affecting both brain and spinal cord. The interactions of visna virus with sheep choroid plexus (SCP) cells and baby hamster kidney (BHK21-F) cells in culture have been studied. The virus multiplies in SCP cells but not in BHK21-F cells. However, virus particles have been found to alter the cell membranes of both cell types. After inoculation of SCP cells at a multiplicity of approximately 2 tissue culture infective doses ($TCID_{50}$) per cell, the latent period was 15 to 20 hours, and cytopathogenic effects that included giant cell formation appeared after 48 hours. No multiplication of virus and no cytopathic effects were detected in BHK21-F cells inoculated at low multiplicity. Cell-fusing activity of visna virus was directly demonstrated by inoculation of SCP or BHK21-F monolayers at virus multiplicities > 20 $TCID_{50}$ per cell. By 4 to 6 hours nearly all cells in each monolayer had fused to form large syncytia which then degenerated. That this activity is associated with the virus particle was shown by purification of the virus by potassium tartrate density gradient centrifugation. Cell-fusing activity and virus infectivity both came to equilibrium at a buoyant density of 1.19 g per ml. Antibody to visna virus prevented giant cell formation. Virus that had lost its infectivity after ultraviolet irradiation retained the ability to cause giant cell formation. The results suggest that the visna virus particle possesses a component that can alter the plasma membranes of both SCP and BHK21-F cells, leading to cell fusion and eventual disintegration, and that virus multiplication is not necessary for this effect. Since myelin is a membranous structure and essentially an extension of the cell membrane, it is possible that there is a relationship between the ability of visna virus to alter cell membranes

and the demyelination that is prominent in visna-infected neural tissue.

Studies on the Mechanism of the Urinary Concentrating Defect in Sickle Cell Anemia. FRED E. HATCH, L. W. DIGGS, AND JAMES W. CULBERTSON,† Memphis, Tenn.

Since the consistent renal concentrating defect in sickle cell anemia remains unexplained, the effects of 10% mannitol and 3% saline diuresis were compared in six patients with SS-hemoglobin and six normal subjects after hydropenia. During rapid mannitol infusion free-water reabsorption ($T^c_{H_2O}$) progressively rose with increasing osmolar clearance (C_{osm}) and reached a maximal level ($T^cM_{H_2O}$) in both groups (mean values: control, 5.29 ml per minute; SS-H, 4.14 ml per minute). During saline diuresis $T^c_{H_2O}$ progressively rose in the normal subjects and exceeded the maximal levels attained during mannitol diuresis as C_{osm} increased, with no evidence of $T^cM_{H_2O}$. Contrariwise, no saline curve exceeded the mannitol curve in SS patients; both tended to coincide at comparable rates of C_{osm}. ¶ Since $T^c_{H_2O}$ is an index of both sodium transport in the ascending limb of Henle's loop and accumulation of sodium in the medullary interstitium, the difference in $T^c_{H_2O}$ curves might represent a defect in either. Therefore, tubular sodium transport was examined during both water and osmotic diuresis. Free water formation (C_{H_2O}), which occurs in the ascending limb and is dependent on sodium reabsorption without water reabsorption, was measured in all subjects during hydration. Fraction of filtered sodium load reabsorbed in the ascending limb and distal tubule, fraction of filtered sodium load excreted (C_{Na}/GFR), fraction of urinary solute composed of sodium (U_{Na}/U_{osm}), potassium excretion (U_KV), and C_{H_2O} were comparable in both groups during water diuresis, indicating normal sodium transport at diluting sites in SS patients. C_{Na}/GFR, U_{Na}/U_{osm}, and U_KV were similar in both groups during mannitol diuresis, suggesting normal tubular sodium transport during osmotic diuresis. ¶ Thus, the concentrating defect is not due to insufficient loop sodium transport but probably to lack of sufficient sodium accumulation in the medullary interstitium to support unlimited passive water diffusion from the collecting ducts. This failure to maintain a sufficiently hypertonic medulla may reflect supernormal medullary blood flow.

Hemoglobin $M_{Hyde\ Park}$: A New Variant of Abnormal Methemoglobin. PAUL HELLER, RICHARD D. COLEMAN, AND VINCENT YAKULIS, Chicago, Ill. (introduced by Mark H. Lepper †).

The hemoglobin M variants are characterized by amino-acid substitutions that interfere with normal heme function. In Hb M_{Boston} ($\alpha_2^{58tyr}\beta_2$) and $M_{Saskatoon}$ ($\alpha_2\beta_2^{63tyr}$) the distal histidines are replaced, whereas in Hb M_{Iwate} the proximal histidine 87α is substituted by tyrosine ($\alpha_2^{87tyr}\beta_2$). ¶ Hb $M_{Hyde\ Park}$ was discovered in a 77-year-old Negro bachelor who was hospitalized because of mild congestive heart failure. The brownish hue of a blood sample led to the finding of otherwise undetected cyanosis of the mucous membranes and nail beds. Hematologic findings were normal. Diaphorase had normal activity. Starch block electrophoresis of the ferricyanide-treated hemolysate at pH 7.0 yielded normal hemoglobin and a more anodic greenish-brown fraction of 40%. Since the oxygen capacity of the total hemolysate was shown to be 80%, only half of the abnormal fraction was capable of combining reversibly with oxygen. The visible spectrum of acid methb $M_{Hyde\ Park}$ resembled that of M_{Iwate}, whereas the cyanmethemoglobin spectrum was not significantly abnormal. Chromatographic separation of the chains from total globin yielded two β peaks. The soluble peptides of the tryptic digest of the abnormal β chain were normal, but the fingerprint of the aminoethylated chain was abnormal: peptide β T-10, comprising aminoacid residues 83 to 95, was shifted slightly anodically, had a higher R_f, and contained tyrosine normally absent in this peptide. ¶ From these findings it appears that in Hb $M_{Hyde\ Park}$ histidine 92, the site of the main heme-globin bond of the normal β chain, is replaced by tyrosine. It is likely that in this hemoglobin histidine 63β functions as the proximal histidine and the phenol of tyrosine 92β forms a bond with heme-iron, a molecular abnormality similar to that of the α chain of Hb M_{Iwate}.

On the Action of Steroid Hormones on the Central Nervous System in Man. ROBERT I. HENKIN, ROBERT L. DALY, AND GEORGE A. OJEMANN, Bethesda, Md., and Washington, D. C. (introduced by Robert W. Berliner †).

Patients with untreated adrenal cortical insufficiency (ACI) exhibit increased detection sensitivity for taste, smell, and hearing. Sensitivity does not change after treatment with deoxycorticosterone acetate (DOCA) but returns to normal after treatment with carbohydrate-active steroid (CAS). Because hearing offers a convenient system for the study of the manner in which sensory processes change, a battery of standardized auditory tests was administered to four patients with ACI under treatment with *1*) nothing, *2*) DOCA, and *3*) CAS. Peripheral nerve conduction velocity and evoked cortical potentials for light flashes and clicks were also measured. With no treatment or with DOCA, average tonal sensitivity was significantly increased above normal; middle ear muscle reflex thresholds decreased an average of 23 db, demonstrating significant compression in the dynamic range of the auditory system. Errors in judgments of equal loudness were three times normal. Average word discrimination decreased significantly: that for undistorted speech by more than 5%, that for distorted speech by more than 15%. Axonal conduction velocity increased by more than 25%; average evoked response latencies for both light flashes and clicks increased by more than 15 msec, and amplitude increased above normal. With CAS results of auditory tests returned towards normal as did peripheral nerve conduction velocity and latencies and amplitude of evoked responses. The results demonstrate that although detection thresholds are improved off treat-

ment or with DOCA, integrative functions of the central nervous system, e.g., loudness judgments and speech discrimination, are significantly worse. The data indicate that when CAS is absent from the nervous system, normal time patterning of sensory signal transmission is significantly altered, resulting in a significant loss of information.

Asynergy of Ventricular Contraction: An Important Cause of Cardiac Failure in Coronary Heart Disease. MICHAEL V. HERMAN, JAY M. SULLIVAN, ROBERT A. HEINLE, HARVEY G. KEMP, STEVEN WOLFSON, AND RICHARD GORLIN,* Boston, Mass.

Clinical congestive heart failure (CHF) occurs in 25% of patients with coronary artery disease (CAD). Laboratory hemodynamic findings in CAD, however, are characterized almost invariably by decreased cardiac and stroke output, frequently by increased left ventricular end diastolic pressure (EDP) (70%), but often with normal or near normal LV end-diastolic volume (EDV) (66%). The degree or location of coronary artery lesions does not explain the difference between failure and no failure. Postmortem studies rarely delineate why CHF occurs in one patient and not in another. Currently, there is no good explanation. Studies by cine left ventriculography performed at 60 frames per second have provided a possible kinetic answer to this important question. Following life size calibration, frame by frame analysis of multiple points on the ventricular inner surface yielded sequential information concerning regional ventricular motion during systolic contraction. The normal synergic (working together) progression in contraction was readily defined. Thirty-five patients (60% of a series with angiographically documented CAD) had marked regional disturbances (asynergy) in this normal pattern and could be categorized: paradoxical systolic expansion, total lack of motion (akinesis), inadequate motion (asyneresis), and disturbed temporal sequence of contraction (asynchrony). Such asynergy led to dissipation of contractile effort even with normal EDV and EDP and resulted in uniformly reduced cardiac output. The asynergic zones bore a uniform relationship to sites of CAD and therefore to potentially ischemic zones. Direct inspection at postmortem or surgery showed the asynergic zones often to be viable muscle and not always fibrotic scar, suggesting that the associated CHF may be due to poor functional performance of local areas of living muscle. The various types, degrees, and extent of asynergy (often living muscle) adversely affect over-all ventricular contractile effort and thus represent an important functional basis for cardiac failure in coronary heart disease.

Effect of Hydrocortisone on Embryonic Duodenal Tissue. J. C. HIJMANS, K. S. MCCARTY, AND W. O. DOBBINS III, Durham, N. C. (introduced by Malcolm P. Tyor*).

The activities of alkaline phosphatase, invertase, and lactic dehydrogenase were examined in duodenum of 14-, 16-, and 19-day-old chick embryos before and after culture for 3 to 4 days on Millipore membranes in Eagle's medium with 10% calf serum. Alkaline phosphatase and invertase but not lactic dehydrogenase activity increased with age. Hydrocortisone (0.5 μg per ml) added to the medium increased the activities of alkaline phosphatase and invertase but not lactic dehydrogenase, larger increases being seen in tissues from 16- and 19-day-old embryos than from 14-day-old embryos. The enzyme response of alkaline phosphatase and invertase to hydrocortisone induction was examined after 3.5 hours, 15 hours, and 4 days of *in vitro* incubation. The results of these experiments suggest that prolonged exposure to steroid hormone at certain developmental stages may be critical to the induction of duodenal invertase and alkaline phosphatase. After 4 days in culture, the tissues showed little histologic change to light and electron microscopy. When cultured in the presence of hydrocortisone, dilatation of the endoplasmic reticulum, mitochondrial enlargement, and numerous large lysosomes were noted, but the microvilli remained unaltered. Preliminary experiments have been performed to elucidate the mechanism of cortisone stimulation. Duodenum of 16- and 19-day-old embryos showed a 24% and 150% increase, respectively, in RNA synthesis when exposed to hydrocortisone for 3.5 hours *in vitro*. The nature of this RNA is under investigation to test the hypothesis that the effect of hydrocortisone on duodenal enzymes is mediated through a synthesis of messenger RNA.

Ribosomal Turnover in Tissues of Fed, Fasted, and Tumor-bearing Rats. CARL A. HIRSCH AND HOWARD H. HIATT,* Boston, Mass.

In multiplying bacteria, rapid quantitative alterations in protein and ribonucleic acid (RNA) content promote efficient regulation of metabolism. These changes are effected by alterations in differential rates of synthesis, continuing growth serving to dilute out those components no longer essential after environmental change. However, in the differentiated tissues of higher organisms, this dilutional mechanism is not available. In most nonproliferating mammalian cells, macromolecular synthesis continues at a rapid rate and normally is balanced by catabolism. Synthetic reactions are regulated in part by the mechanisms of induction and repression that have been elucidated in microorganisms; very little is known about the catabolic process. To study both synthetic and catabolic aspects of macromolecular turnover and to investigate the possibility that regulation of these processes might be abnormal in tumors and in tumor-bearing animals, we have examined turnover of ribosomes in normal and hepatoma-implanted rats during feeding and starvation. The steady-state kinetics of hepatic ribosomal turnover were determined by labeling the ribosomal protein and RNA components in a series of normal rats and measuring changes with time in specific radioactivity of the components. The kinetics were first order; protein and RNA specific radioactivities fell in parallel, with half-lives of 5 days. During starvation, synthesis was

markedly slowed, but the rapidity of decrease in hepatic ribosomal content indicated accelerated catabolism as well. Ribosomal depletion during starvation was also observed in the kidney, heart, and skeletal muscle of normal and tumor-bearing animals, but not in the tumor. Evidently, both synthetic and catabolic aspects of ribosomal turnover are subject to independent regulatory mechanisms, and these may function abnormally in neoplastic tissue.

Cell Lipid Content and Cell Number in Obese and Nonobese Human Adipose Tissue. JULES HIRSCH,* JEROME L. KNITTLE, AND LESTER B. SALANS, New York, N. Y.

Fragments of subcutaneous adipose tissue were obtained by needle aspiration from 25 hospitalized subjects of average or elevated (94 to 185 kg) body weight. The tissues were fixed with osmium tetroxide under conditions allowing all adipose cells to be dislodged from the tissue matrix. Cells were counted automatically by a Coulter counter. The amount of lipid in a known fraction of the aspirate was used to calculate the average lipid content per cell. Total body fat (from water space determination) and cell lipid content were used to estimate the total number of adipose cells in each subject. ¶ Cells in different subcutaneous sites contained the same quantity of lipid. The cells of nonobese adults contained 0.6055 ± 0.0439 μg of lipid, and the cell number was $(26.83 \pm 1.77) \times 10^9$. The cell lipid of obese subjects was 0.9088 ± 0.0828 μg. In contrast to this slight increase of cell size in obesity, cell numbers showed a threefold elevation to $(77.02 \pm 13.47) \times 10^9$. Nine infants and children who were studied showed increasing cell sizes and numbers with age. Apparently, maximal values are not reached until adolescence or early adult life. Once established, only cell number tended to remain fixed, since cell size could vary greatly with weight changes. Thus, a reduced obese individual was found to have smaller than average cells with persistent "hypercellularity." ¶ If metabolic parameters such as glucose conversion to CO_2 or glycerides, studied by *in vitro* adipose tissue incubation, were expressed in the customary way (by lipid content or wet weight), there were differences in the activity of children, normal adults, and obese adults. Such differences were largely eradicated by calculating the activity on a per-cell basis, again suggesting that cell number rather than cell size or individual cell metabolism was the major defect in the obese subjects.

Immunoglobulin and Antibody Production by Human Peripheral Lymphocytes *In Vitro*. KURT HIRSCHHORN * AND CAROLYN S. RIPPS, New York, N. Y.

Human peripheral blood lymphocytes were shown to produce and contain γG-, γA-, and γM-immunoglobulins when cultured for 3 days in the presence of phytohemagglutinin (PHA) or specific antigens to which the donor had been sensitized. This was demonstrated by immunofluorescence, by specific coprecipitation of newly formed radioactively labeled protein, and by immunoelectrophoresis and radioautography. Seventy-five to 90% of cells from normal individuals showed specific immunofluorescence after PHA culture, whereas only 5 to 35% were positive after culture with specific antigens. The specificity of fluorescence was determined by blocking experiments with identical anti-γ-globulins without fluorescein. Controls for nonspecific adsorption of the conjugated antibodies included the use of fluorescein-conjugated antirabbit γ-globulin and antigoat γ-globulin. Cells from eight agammaglobulinemic patients showed no specific fluorescence after PHA culture, whereas those from five mothers of patients with sex-linked agammaglobulinemia were divided into two populations in each individual, 50 to 65% fluorescence positive and the rest negative. The specificity of coprecipitation was controlled by comparison with radioactivity precipitated with unrelated antigen-antibody complexes. Cells from four patients sensitive to penicillin and demonstrating an immediate skin reaction to penicillin were shown to produce antibodies capable of binding penicillin and benzyl-penicilloyl-polylysine (BPO). Antibody production occurred during culture with either penicillin or PHA. The antibody was demonstrated by detecting cells capable of binding penicillin with the use of fluorescein-conjugated anti-BPO. Unconjugated anti-BPO blocked the fluorescence. Cells from nonallergic individuals showed no fluorescence, again indicating the absence of nonspecific staining.

Sodium-Potassium–activated ATPase in Human Small Intestine: Reversible Depression in Cholera and Acute Gastroenteritis. NORBERT HIRSCHHORN, J. R. SAHA, AND IRWIN H. ROSENBERG, Dacca, Pakistan, and Boston, Mass. (introduced by Charles S. Davidson †).

Sodium-rich fluid losses in cholera and other diarrheal diseases may reflect, in part, a transient defect in the reabsorption of sodium by the intestine. Evidence implicating sodium-potassium–activated, ouabain-inhibited adenosine triphosphatase (Na-K ATPase) in active sodium transport in other tissues prompted us to look for a similar enzyme in the human intestine and to examine its activity in diarrheal disease. ¶ A brush-border rich fraction of homogenates of surgically excised human small intestine was found to contain Na-K ATPase activity. Aging with deoxycholate made possible the separation of Na-K ATPase from a magnesium-requiring, sodium-, potassium-, and ouabain-insensitive ATPase (Mg ATPase) present in the same homogenate fraction. These enzymes were characterized, and the assay was applied to peroral biopsy specimens of human jejunum. Seven patients with cholera and fifteen patients with acute gastroenteritis (no bacterial pathogen isolated) were studied during acute disease and after recovery. Biopsy ATPase activity during disease was compared in each case with repeat values obtained after recovery. In cholera mean Na-K ATPase activity during disease was significantly lower than the mean recovery activity ($p < 0.05$). The mean individual depression during dis-

ease was 30%. Patients with gastroenteritis were found to have an even more significant ($p < 0.01$) lowering of mean Na-K ATPase during disease; mean individual depression was 22%. Mg ATPase, assayed simultaneously in the same biopsy specimens, did not fall during disease in either group. No consistent morphologic changes were noted in these biopsies, and mucosal damage was not found during acute disease. ¶ The observed depression of intestinal Na-K ATPase in cholera and acute gastroenteritis suggests that this enzyme may be involved in the cation losses seen in diarrheal disease.

Apparently Independent Actions of Insulin on Na, K, and Glucose in Gastric Fistula Dogs. B. I. HIRSCHOWITZ AND G. SACHS, Birmingham, Ala. (introduced by T. R. Harrison †).

In nine conscious dogs with gastric cannula secreting in response to a 4.5-hour constant iv infusion of histamine (50 μg base per kg per hour), a single injection of insulin in doses of 0.45 to 0.9 U per kg inhibited gastric electrolyte secretion (H, K, Cl) by 60 to 100% for 3 to 4 hours independently of hypoglycemia or atropinization. The iv injection of KCl (1 mEq K^+ per kg) produced a rapid (5 minutes) and sustained (90 minutes) complete reversal of the inhibition, without affecting blood glucose levels. The same dose of RbCl was about 70% as effective as KCl, but NaCl, $MgCl_2$, glucose, and glucagon were inactive. Inhibition was apparently not due to a single lowering of plasma K since equivalent depression of K to 2.5 to 3.4 mEq per L occurred with smaller doses of insulin (0.15 to 0.3 U per kg) or with 2-deoxy-glucose (a glucose analog that produces cytoglucopenia) without inhibition of gastric secretion. This action of insulin is interpreted as a redistribution of K^+ in secretory cells resulting in depletion of K^+ at sites essential for the normal secretion of electrolytes. ¶ In the course of these studies a different electrolyte effect was also noted. With both inhibitory and noninhibitory doses of insulin, plasma Na rose by 2.5 to 6 mEq per L and with 2-deoxyglucose by 8 to 12 mEq per L within 30 to 60 minutes. Whereas under these conditions there was a log-linear relation between loss of K from, and gain of Na into ECF, the same experiments repeated after atropine (0.08 mg per kg) showed the same decrease of plasma K but no rise in plasma Na. ¶ Thus the injection of K or Rb distinguished between hypoglycemia and the gastric electrolyte effect of insulin, and atropine dissociated the effects of insulin on Na and K in the plasma.

Independence of Selenomethionine Pathways from Those of Methionine in Mammalian Protein Metabolism. JAMES F. HOLLAND,* SANDRA PETERS, BRADLEY BRYANT, AND MONTE BLAU, Buffalo, N. Y.

L-Selenomethionine-^{75}Se, the γ-emitting structural analog of methionine, was fed to female Swiss mice from conception through gestation and lactation. The labeled offspring, upon weaning, were fed 18% casein (approximately 200 μmoles of methionine per mouse per day) or isocaloric protein-free synthetic diets. Total body radioactivity disappeared at identical rates ($t_{\frac{1}{2}}$ 20 days). Additional methionine did not alter this slope, but feeding approximately 0.25 μmole nonisotopic selenomethionine markedly accelerated ^{75}Se disappearance ($t_{\frac{1}{2}}$ 4 days), suggesting that methionine and selenomethionine were in different metabolic pools. ¶ Upon intravenous or intraperitoneal injection of selenomethione-^{75}Se to rats, the simultaneous administration of 0.05 or 0.5 μmole selenomethionine produced significant dose-related decrease in ^{75}Se incorporation into serum proteins. An equimolar dose of methionine, or greater amounts of a mixture of 12 amino acids essential for mammalian cell growth, did not impair ^{75}Se incorporation, however. ¶ The ^{75}Se label in serum proteins is not decreased by exhaustive dialysis in selenite or selenomethionine or by trichloroacetic acid precipitation in the presence of nonradioactive selenomethionine. ¶ Methionine-^{35}S and selenomethionine-^{75}Se injected simultaneously result in different specific activities of serum and tissue proteins and different distribution between mouse carcass and tumor and between human serum albumin, globulins, and tumor. The nonidentity of selenomethionine with methionine or other amino acids essential in mammalian metabolism and the ready incorporation of selenomethionine-^{75}Se into mouse, rat, and human proteins suggest that selenomethionine is a physiologic trace amino acid.

The Anemia of Hypothyroidism. CHARLES S. HOLLANDER, RONALD H. THOMPSON, PETER V. O. BARRETT, AND NATHANIEL I. BERLIN,* Bethesda, Md. (introduced by Samuel P. Asper †).

The anemia of hypothyroidism is not completely understood. The erythropoietic effect of thyroid hormone has been viewed as secondary to its over-all stimulation of oxygen need. More recent studies suggest that the stimulus for erythropoiesis is a primary effect of thyroid hormone. ¶ Sixteen dogs were rendered hypothyroid with a dose of radioiodine insufficient to cause bone marrow depression. Twenty-four-hour mean metabolic rates were determined in a specially designed chamber that permitted continuous indirect calorimetry. ¶ Hypothyroid dogs had mean metabolic rates of 24.7 kcal per kg day, low thyroxine by column values, low free thyroxine iodine levels, and a strikingly abnormal lipid profile. Hypothyroidism was achieved within 3 months of radioiodine administration. Red cell volumes fell more gradually to a mean of 21.8 ml per kg at 6 months and remained stable for a 2-year period. The anemia was of a normochromic, normocytic variety, and only a single population of cells was present. Red cell life spans were normal, but ferrokinetic studies revealed diminished turnover of plasma iron and a decreased incorporation of radioiron by red cells. The anemia was resistant to iron, copper, B_{12}, thiamine, and cobalt. ¶ Four hypothyroid dogs, treated with 0.04 mg per kg of *l*-thyroxine parenterally daily for 3 months, responded with an increase in red cell volume to normal (38.3 ml per kg). This dose of thyroxine also reverted the metabolic rate to normal levels (45.6 kcal

per kg day) and corrected the abnormalities in serum lipids. Three dosage regimens of recrystallized, α-dinitrophenol administered to six hypothyroid dogs for 3 months corrected the metabolic rate and serum lipid abnormalities but failed to alter the anemia. Dinitrophenol also did not stimulate erythropoiesis in six normal dogs. The data are compatible with the view that thyroid hormone stimulates erythropoiesis through a primary mechanism.

The Metabolism of Acid Mucopolysaccharides and Lipoproteins in Normal and Diseased Human Arteries. WILLIAM HOLLANDER,* DIETER KRAMSCH, AND GOSUKE INOUE, Boston, Mass.

Previous studies have indicated that lipids accumulate in atherosclerotic plaques mainly as low density lipoproteins. Accordingly, the metabolism of lipoproteins in normal and diseased atherosclerotic human arteries was studied particularly in relationship to changes in arterial acid mucopolysaccharides (AMP), which may influence the transport of lipoproteins. The AMP were extracted and isolated by the method of Schiller and Dorfman, and the lipoproteins were extracted in saline and separated by differential density ultracentrifugation. ¶ The AMP content was found to be increased in fatty-streaked intima but reduced in the advanced plaques as compared to normal arterial intima. Incubated fatty streaks incorporated more $^{35}SO_4$ and acetate-^{14}C into sulfated and nonsulfated AMP than normal intima. The incorporation of the AMP precursors by incubated plaque was reduced. ¶ The extractable lipoprotein content of the fatty streak and plaque was two to five times greater than that of the normal arterial intima. The ratio of low density lipoproteins (D < 1.019, D 1.019 to 1.063) to high density lipoproteins (D 1.063 to 1.210) averaged 12:1 in diseased intima, but only 1.5:1 in normal intima. The incorporation of leucine-^{14}C and acetate-^{14}C into the lipoprotein fractions of incubated diseased and nondiseased intima was proportional to the lipoprotein content of each fraction. ¶ The influx of intravenously administered cholesterol-^{14}C into fatty streaks was increased, whereas the influx into the plaques was decreased as compared with normal intima. The incorporation of the injected cholesterol-^{14}C into lipoprotein fractions of diseased and nondiseased intima was generally proportional to the cholesterol content of each fraction with over 90% of the cholesterol-^{14}C being in the low density fractions. ¶ The findings suggest that the deposition of low density lipoproteins in early atherosclerotic lesions is associated with an increased content and *in vitro* synthesis of acid mucopolysaccharides and an increased influx of plasma cholesterol into the lesion.

Regulation of Adipose Tissue Triglyceride Structure. C. H. HOLLENBERG,* Montreal, Canada.

There is as yet incomplete understanding of the factors which regulate the distribution of fatty acids among the many triglyceride species of adipose tissue and which determine the relative quantities of the various triglycerides present in the tissue. As transfer of fatty acids between adipose triglyceride molecules occurs very slowly, triglyceride structure is determined at the time of triglyceride formation and is not appreciably affected by subsequent intermolecular exchange of triglyceride fatty acids. Hence the proportions of the various adipose triglycerides may also be determined coincident with triglyceride synthesis. To explore this possibility, radioactive glucose was administered intravenously to fed rats and lumbar fat removed 15 minutes later. By thin layer chromatography with silver nitrate impregnated silica gel, ten classes of tissue triglycerides were obtained differing in degree of unsaturation and fatty acid composition. The distribution among these classes of triglycerides formed during the 15-minute interval, represented by the distribution of radioactive triglyceride-glycerol, was found to be almost identical to that of the total tissue triglycerides. The distribution of radioactive saturated fatty acids was also determined; these newly synthesized acids were present in all triglyceride species containing saturated acids, including those having both saturated and diunsaturated fatty acid components. These results indicate that before triglyceride formation in adipose tissue, mixing occurs between acids synthesized in the tissue and dienoic acids derived from plasma or tissue triglycerides. The identity of the pattern of new triglycerides synthesized during the 15-minute interval with that existent in the tissue indicates that the proportions as well as the structures of the various adipose triglycerides are established at the time of triglyceride formation. It is thus unnecessary to postulate that these proportions result from extensive intermolecular exchange of triglyceride fatty acids or from differences among triglyceride classes in turnover rates.

How the Platelet Factor 3 of Blood Platelets Enters into Coagulation Reactions. HERBERT I. HOROWITZ, Bronx, N. Y. (introduced by Edward E. Fischel *).

Blood platelets are unique in being able to provide platelet factor 3 (PF-3) for blood coagulation. PF-3 of intact platelets is low. As citrated or native platelet-rich plasma clots, PF-3 activity, measurable as shortening of the Stypven time, increases markedly. PF-3 activation of platelets has been attributed to release from the platelets of particulate or soluble material, or alternatively, to changes in the platelet membrane. Our studies indicate that the bulk of activated PF-3 remains associated with platelet and platelet fragments, but that 6 to 25% of total PF-3 is transferred to serum in soluble form. Nonsedimentable PF-3 appears to complex with Factor X, protecting the latter from heat inactivation. PF-3 activation is independent of coagulation Factors XII, XI, VIII, X, and V. Incubating intact citrated PRP with 1 to 10 μg ADP per ml induces PF-3 activation. Other substances that aggregate platelets also induce PF-3 activation: thrombin, trypsin, norepinephrine, serotonin, and

Escherichia coli endotoxin. PF-3 activation by these compounds is inhibited by AMP, suggesting that a common pathway for platelet PF-3 activation is mediated by ADP. Approximately 15% of total PF-3 is released to plasma in nonsedimentable form by ADP. Deykin has found PF-3 increase but absence of nonsedimentable PF-3 on incubation of platelets with ADP, perhaps because his washing procedure removed a labile pool of potential nonsedimentable PF-3. Hemostatic reactions are initiated by platelet adhesion to collagen leading to release of ADP, platelet aggregation, and formation of a platelet plug. PF-3 activation induced by ADP may be an important step leading to thrombin evolution. This would promote fibrin in proximity to platelet membranes. Through release of nonsedimentable PF-3 fibrin would also form in the surrounding plasma.

Demonstration of More than a Single Thyroxine Binding α-Globulin (TBG) on Starch Gel Electrophoresis. MITSUO INADA AND KENNETH STERLING,* New York, N. Y.

Besides the three principal serum protein carriers of thyroxine, *1*) thyroxine-binding α-globulin (TBG), *2*) thyroxine-binding prealbumin (TBPA), and *3*) albumin, recent studies have suggested an additional binding protein. ¶ On starch gel electrophoresis of serum with added thyroxine-^{131}I, TBPA moved well ahead of the albumin band, and the major TBG radioactivity was immediately behind albumin on radioautographs and histograms made by counting serial segments of gel. The radioactivity in the broad albumin band invariably exceeded the known binding capacity of human serum albumin, as has been the case in many other laboratories. ¶ Paper electrophoretic studies of material eluted from the starch gel revealed radioactivity with the mobility of α-globulin throughout the albumin-staining area of the starch gel. An α-globulin had a leading edge within the broad albumin area, as shown by cold thyroxine loading studies, and most strikingly by displacement with diphenylhydantoin (DPH). At serum concentrations of DPH that displaced thyroxine from TBG on paper electrophoresis, the starch gel studies revealed not only absence of radioactivity in the major postalbumin TBG area, but also diminution throughout the broad albumin zone, and absence of radioactivity in the leading edge, confirming the existence of one or more rapidly migrating TBG components. The displaced labeled hormone was albumin" or "slow" TBG had a much greater affinity for thyroxine than the newly observed α-globulin ("fast" bound by TBPA. ¶ The data indicated that the "post-TBG). The maximal binding capacities for thyroxine, however, were reversed; approximately 7 μg per 100 ml for slow TBG, and approximately 12 μg per 100 ml for the fast component. ¶ Current protein fractionation work with preparative column chromatography also supports the existence of more than a single TBG.

Fatty Acid Composition of Human Adipose Tissue: Relationship to Body Weight, Age, and Serum Cholesterol. WILLIAM INSULL, JR., HAROLD B. HOUSER, AND ARTHUR S. LITTELL, Cleveland, Ohio (introduced by Olof H. Pearson†).

The increased proportion of cholesterol oleate dominating atherosclerotic lesions may be related to increasing proportions of oleic acid in adipose tissue with age. To study factors influencing composition of adipose tissue, we have measured, in nonhospitalized subjects with multiple sclerosis, 27 men and 32 women aged 29 to 63 years, the proportions of 14 fatty acids in adipose tissue, serum cholesterol, body weight, and triceps and subscapular skinfolds. Weights averaged 101%, range 75 to 135%, of ideal. Cholesterol values and composition of adipose tissue were similar to those of populations without multiple sclerosis. Relationships between subject variables and proportions of acids were determined by linear regression. ¶ Lauric and stearic acids had negative regressions ($p < 0.02$) on weight both as per cent of ideal weight and as per cent of average weight for age. Palmitic acid ($p < 0.05$) and cholesterol ($p < 0.01$) had positive regressions and stearic acid a negative regression ($p < 0.05$) on skinfold thickness. When each subject's weight was expressed as per cent of his weight at age 25, palmitic acid had a negative regression ($p < 0.01$), oleic acid a positive regression ($p < 0.01$), and, in males, cholesterol a negative regression ($p < 0.01$). ¶ These data indicate that individuals with increasing amounts of adipose tissue in relation to the population average will have decreasing proportions of adipose lauric and stearic acids and increasing levels of cholesterol. Individuals having greater weight increases from their reference weight at age 25 will have higher proportions of oleic and lower proportions of palmitic acid. The converse occurs with weights lower than weight at age 25. We suggest that the proportion of cholesterol oleate in atheroma may likewise be associated with the amount weight has changed after age 25. When weight is considered in studies of lipid metabolism, evaluation of an individual by his weight history would appear more reasonable than evaluation by comparison with a population standard.

Rapid Effect of Thyrotropin on Thyroidal Organification of Iodide. G. H. ISAACS AND I. N. ROSENBERG,† Boston, Mass.

Thyroidal accumulation of iodine involves both the trapping of circulating iodide and its subsequent organification. The rapidity with which TSH affects organification was studied in dogs by sequential measurements of glandular clearance of circulating ^{131}I after the rate-limiting effect of trapping on thyroidal iodine accumulation had been prevented by acute administration of stable iodide. ¶ Two hours before the cannulation of an inferior thyroid vein, ^{131}I was injected intravenously either carrier free (8 experiments) or with enough stable

iodide to produce a 4- to 25-fold increase in the arterial iodide concentration (8 experiments). In several experiments radiopertechnetate ($^{99m}TcO_4^-$), which is trapped by the thyroid but not organified, was given with the ^{131}I. Net glandular clearances of iodide and pertechnetate were determined at frequent intervals from arterio-thyroid venous concentration differences and thyroid venous flow rates. ¶ After systemic intravenous injection of TSH (10 U), iodine uptake in the normal iodide dogs was unchanged or declined slightly. By contrast, TSH induced a biphasic response in the high iodide group. In the initial 10-minute interval after TSH, mean glandular iodine uptake declined 42%. However, in the next 10-minute interval the mean uptake was 182% of the pre-TSH value, and was 197, 192, and 160% in succeeding 10-minute intervals. ¶ In both groups, a sustained glandular release of ^{99m}Tc began promptly after TSH, resembling the TSH-induced release of trapped iodide observed in methimazole-treated dogs. This effect of TSH upon glandular retention of trapped anion may explain the initial fall in iodide uptake observed in the high iodide dogs. The rapidly ensuing increase in iodide uptake in this group suggests that TSH promptly stimulates organification of circulating iodide and does so within 20 minutes after injection.

Membrane Lipid Depletion during Cation Pumping: A Mechanism for the Genesis of Spheroidal Red Cells in Hereditary Spherocytosis (HS). HARRY S. JACOB, Boston, Mass. (introduced by Frederick Stohlman, Jr.*)

The abnormal spheroidicity of HS red cells (RBC), which increases with incubation, may involve membrane lipid alterations as well as osmotic swelling. The accelerated metabolism of HS membrane phospholipids, associated with increased HS RBC sodium permeability and transport, may render surface lipids vulnerable to catabolism or elution from the cell. The resulting loss of surface material would enhance spheroidicity. Washed, glucose-replete, HS RBC from five splenectomized patients lost phospholipids and cholesterol within 4 hours at 37° C. In 24 hours, as reported by others, total membrane lipids diminished 15 to 20%; normal RBC lost none. Decreasing sodium transport by suspending HS RBC in sodium-poor (choline-potassium) media diminished phospholipid turnover by 34%. Concomitantly no loss of membrane lipid and no increase in osmotic fragility occurred during incubation for 24 hours. Increasing the permeability of normal RBC by transient exposure to butanol doubled their sodium content, and in response phospholipid turnover increased by 35%. With subsequent incubation, they lost 15% of membrane lipid per 24 hours and became smaller and osmotically fragile (hemolysis at 0.80% NaCl). By contrast, butanol-treated RBC of normal sodium content incubated in sodium-poor media were of normal fragility and lipid content and turnover. Analogously, normal RBC made hyperpermeable by osmotic swelling or treatment with nonhemolytic amounts of the sulfhydryl inhibitors, *N*-ethylmaleimide or *P*-mercuribenzoate, became osmotically fragile and spherocytic and lost lipid unless sodium accumulation was prevented. It is concluded that membrane lipids are lost from RBC when metabolizing more rapidly in response to inordinate cation pumping. Therefore, increased permeability to sodium doubly and additively jeopardizes HS RBC: *1*) by directly increasing the tendency for sodium (and water) accumulation, ultimately producing osmotic hemolysis, and *2*) by stimulating turnover and subsequent depletion of membrane lipids, which gradually enhances sphering and diminishes osmotic resistance.

Hereditary Nonspherocytic Hemolytic Disease Associated with Altered Phospholipid Composition of the Erythrocytes. ERNST R. JAFFÉ,* EUGENE L. GOTTFRIED, AND THOMAS B. BRADLEY, JR., New York, N. Y.

Consistent changes in the lipids of erythrocytes in hemolytic disorders have not been reported, except in acanthocytosis, in which mild hemolysis may occur. This report presents results of studies of a 24-year-old Dominican man with long-standing moderate anemia, mild jaundice, splenomegaly, and reticulocytosis (6 to 12%). Analysis of phospholipids of erythrocytes by quantitative thin-layer chromatography revealed a distinct increase in lecithin to 38.4% of the total; normal, $29.7 \pm$ SD 1.6%, $n = 8$. The alteration appeared to constitute an absolute increase in lecithin content, rather than a decrease in other phospholipids. The proportion of lecithin was increased (34.1 to 35.8%) in erythrocytes of three relatives with similar clinical findings and was normal (30.3%) in the cells of an asymptomatic brother. The difference between the lecithin proportion of erythrocytes of affected members of this family and of normal subjects was highly significant, $p < 0.001$. Erythrocytes from other patients with hereditary hemolytic disorders and comparable levels of reticulocytosis had normal phospholipid compositions. Stained smears of the propositus's blood revealed occasional target cells and slight anisocytosis, poikilocytosis, and polychromatophilia. Osmotic fragility was decreased, and the increase in fragility during 24 hours' incubation was less than that observed with normal cells. Autohemolysis after 48 hours was slightly increased and was corrected to nearly normal by addition of glucose. Utilization of glucose and production of lactate by the erythrocytes were increased, the activities of 15 enzymes of the erythrocytes were normal or elevated, and the ATP content was normal. Heinz bodies were not observed, even after prolonged incubation, and an abnormal hemoglobin could not be demonstrated. Erythrocyte life-span was reduced ($t_{\frac{1}{2}}$ $^{51}Cr = 10.6$ days), but compatible normal cells survived normally ($t_{\frac{1}{2}} = 28$ days). A causal relationship between the altered phospholipid composition and the hemolytic disorder has not been established. Both appeared to be inherited as autosomal dominant characteristics.

The Mechanism of Cholestatic Jaundice Induced by Taurolithocholic Acid. NORMAN B. JAVITT AND SIDNEY EMERMAN, New York, N. Y. (introduced by Lewis Thomas †).

Cholestasis and hyperbilirubinemia can be induced in hamsters (*Cricetus auratus*) and Wistar rats by the intravenous administration of sodium taurolithocholate and can be prevented by the simultaneous administration of other bile acids. The mechanism of cholestasis was explored in hamsters by determining the relationship between sodium taurolithocholate excretion and total bile acid excretion. The biliary excretion of sodium taurolithocholate-24-^{14}C was quantitatively estimated by radioisotopic and thin-layer chromatographic techniques. Total bile acid excretion was assayed with 3α-hydroxysteroid dehydrogenase. ¶ After cholecystectomy and cannulation of the bile duct during pentobarbital anesthesia, each hamster was infused (0.05 to 0.12 ml per minute × 20 to 30 minutes) with sodium taurolithocholate-24-^{14}C (2 mM in 7.5% human serum albumin). Although sodium taurolithocholate appeared in bile during the infusion, bile flow and total bile acid excretion fell (cholestasis). The bulk of administered ^{14}C (77%) was excreted after the infusion as total bile acid excretion returned to control levels. Identical infusions of sodium taurolithocholate containing added sodium taurochenodeoxycholate (8 to 16 mM) in the same animal caused an increase in total bile acid excretion together with a threefold or greater increment in sodium taurolithocholate excretion. Sixty-five per cent of administered ^{14}C was excreted during the infusion period. ¶ In nonoperated hamsters the intravenous injection of 13 μmoles of sodium taurolithocholate caused conjugated hyperbilirubinemia within 24 hours. Hyperbilirubinemia did not occur when equimolar amounts of sodium taurocholate or sodium taurochenodeoxycholate were injected with sodium taurolithocholate. ¶ The prevention of cholestasis and jaundice together with the enhanced excretion of sodium taurolithocholate is attributable to the solubilizing properties of micellar solutions of the other bile acids. These findings suggest that cholestatic jaundice might occur spontaneously when absorption of lithocholic acid from the intestine is increased or when hepatic synthesis of bile acids is decreased.

Complement Fixation and Hemolysis by Normal Serum in Sucrose Solutions: The Basis for a Simple Diagnostic Test for Paroxysmal Nocturnal Hemoglobinuria. DAVID E. JENKINS, JR., AND ROBERT C. HARTMANN, † Nashville, Tenn.

Marked hemolysis was observed when paroxysmal nocturnal hemoglobinuria (PNH) erythrocytes were suspended in isotonic sucrose solutions of low ionic strength in the presence of small amounts (e.g., 5%) of either autologous or isologous compatible normal serum. "Sucrose hemolysis" required Mg^{++} and conventional complement components but was not abolished by removal of properdin or thrombin from serum or plasma. Hemolysis was greater than that observed in the standard acid hemolysis reaction, and in contrast the sucrose hemolysis test was equally positive at 24° C and 37° C. Furthermore, serum or plasma containing standard anticoagulant amounts of citrate or oxalate could be used. Both PNH and normal red cells showed striking agglutination in the serum-sucrose system, but the normal cells did not hemolyze. The observed agglutination was maximal below pH 7, whereas PNH hemolysis occurred equally well from pH 6.1 to pH 7.4. Both normal cells and PNH cells were strongly coated with components of complement (β_{1C}- and β_{1E}-globulins) in this system. ¶ To date, sucrose hemolysis has been specific for PNH. Negative reactions were observed in specimens from more than 30 normal subjects, 75 patients with nonhematologic diseases, and 70 patients with various hematologic disorders including other hemolytic anemias and aplastic anemia. Recently we have also employed an even more simplified test. One vol of oxalated, citrated, or defibrinated blood is added to 9 vol of "sugar water" (9 to 10 parts table sugar in 100 parts distilled water) and checked for hemolysis after 30 minutes incubation at room temperature. This test has proved equally sensitive and specific for PNH and has the added advantage of even greater simplicity. The fixation of complement to erythrocytes in the serum-sucrose system provides an important tool for studying PNH and may have additional implications with respect to normal and altered immune mechanisms.

Nerve and Muscle Function in the Myasthenia of Small-Cell Carcinoma of the Lung (Eaton-Lambert Syndrome). RICHARD J. JOHNS * AND MICHAEL P. MCQUILLEN, Baltimore, Md.

From pharmacological evidence, the characteristic weakness in this syndrome has been attributed to a defect in neuromuscular transmission. This study provides direct evidence that this is essentially correct. ¶ Both nerve and muscle action potentials were recorded in response to electrical stimulation of the nerve. The indirectly stimulated muscle exhibited the typical defect, while at the same time no abnormality in nerve conduction was demonstrable. Thus, a nerve abnormality was excluded. However, it remained to be determined whether the defect lay in neuromuscular transmission or in the muscle itself. ¶ Use of the technique of Buchthal permitted the direct electrical stimulation of a few muscle fibers. The directly evoked muscle action potentials showed a progressive increase in conduction velocity with repetitive stimulation. However, the typical defect seen with stimulation via the nerve was absent. Thus, the defect was clearly demonstrated to lie in neuromuscular transmission. ¶ Guanidine hydrochloride, which is known to increase the release of acetylcholine from nerve endings, produced a dramatic clinical improvement in strength. This was paralleled by the improvement in neuromuscular transmission. Guanidine also had a direct effect on muscle in slowing its rate of depolarization and repolarization without affecting its conduction velocity.

Interaction of Penicillin and the Erythrocyte. ALAN S. JOSEPHSON, Brooklyn, N. Y. (introduced by Ludwig W. Eichna †).

Haptens must react covalently with macromolecules to be effective antigens. It has been postulated that penicillin's immunogenic activity may be exerted by a degradation product, penicillinic acid, the antigen being formed by the reaction of this compound with the amine groups of protein. ¶ The transformation of penicillin to penicillinic acid as a necessary step in the binding of this antibiotic to biological materials was studied by the reaction of penicillin with erythrocytes. Penicillin or penicillinic acid when incubated with red cells sensitizes them for agglutination by antipenicillin antisera. Penicillinic acid, unlike penicillin, reacts rapidly with amine groups at pH 7.4. The minimal concentrations of penicillinic acid and penicillin needed to sensitize rabbit erythrocytes suspended in phosphate buffer, pH 7.4, or buffered solutions of 1 M lysine or E-aminocaproate (EACA) were determined. Washed erythrocytes suspended in buffer were sensitized by penicillinic acid in concentrations of 0.12 mg per ml, whereas addition of penicillinic acid to final concentrations of 3.75 mg per ml failed to sensitize erythrocytes suspended in lysine or EACA. Concentrations of penicillin of 0.94 mg per ml sensitized erythrocytes suspended in lysine, EACA, or buffer. ¶ Erythrocytes were treated with 2,4-dinitro-fluorobenzene (DNFB) or 2,4-dinitro-3,5-difluorobenzene (DNDFB) at pH 8.5 in order to block their sulfhydryl and amine groups. These erythrocytes were agglutinable by anti-DNFB antiserum. Sensitization of the DNDFB-treated cells by penicillinic acid was no longer possible. Sensitization of these cells by penicillin was noted. ¶ These results demonstrate that sensitization of erythrocytes by penicillin does not require prior transformation of the penicillin to penicillinic acid and that interaction of penicillin and the erythrocyte may be mediated by groups other than the amine or sulfhydryl groups. Such a direct reaction of penicillin with a protein or formed element may be in part responsible for penicillin's antigenicity.

The Motion of the Normal and Abnormal Mitral Valve. A Study of the Opening Snap. CLAUDE R. JOYNER, JR., AND WAYNE E. DEAR, Philadelphia, Pa. (introduced by Calvin F. Kay †).

The opening snap (OS) of mitral stenosis (MS) is generally accepted as valvular in origin, indicating the time of valve opening. The coincidence of the OS and the moment of maximal descent of the mitral leaflet into the ventricle has been noted by cineangiography. This technique is discontinuous and does not permit accurate assessment of normal valve motion. Continuous graphic registration of position and motion of normal and diseased mitral valves can be obtained by the ultrasound technique. Studies were undertaken to observe motion of the normal and abnormal valve and to define precisely the time and method of production of the OS. A phonocardiogram, electrocardiogram, and UCG were recorded from 45 resting subjects. Fifteen were normal (N); 15 had MS, OS, and sinus rhythm (MS-R); and 15 had MS, OS, and atrial fibrillation (MS-F). Observations were repeated in 7 normals during exercise. The time of beginning mitral opening, velocity of leaflet movement, and the time of completion of opening were determined from the UKG. Aortic closure to complete mitral opening interval (A-O) was determined from the UKG and phonocardiogram. The OS in both MS groups occurred precisely at the completion of mitral leaflet opening. The A-O interval was as follows: MS-R group, 79 ± 5 msec (SEM); resting N, 99 ± 5 msec; N with exercise, 78 msec. Opening velocity was as follows: MS-R, 814 ± 20 mm per second; resting N, 278 ± 29 mm per second; N with exercise, 496 ± 26 mm per second. The interval from the beginning to completion of mitral opening was as follows: MS-R, 25 ± 2 msec; resting N, 55 ± 3 msec, and N after exercise, 34 ± 2 msec. Relative to the normal, the stenotic valve opens more rapidly, completes its excursion quickly, and "snaps" as motion is abruptly checked. In the MS-F group the expected A-O variation was found. Valve velocity was, in general, higher after a short preceding diastolic cycle, but beat-to-beat variation in aortic closure to beginning mitral opening was the main determinant of A-O interval.

Clinical Evaluation of Transtracheal Aspiration: Its Usefulness and Safety. ROBERT W. KALINSKE, RICHARD H. PARKER, DAVID BRANDT, AND PAUL D. HOEPRICH,* Salt Lake City, Utah.

Transtracheal aspiration (TTA) was performed 88 times in 81 hospitalized patients with suspected lower respiratory tract infection to assess its usefulness and safety. Sputum was collected simultaneously, and the companion specimens were cultured for bacteria, fungi, and *Mycobacterium*. ¶ Sputum specimens from 40 patients with pneumonia yielded 2 or more potential pathogens without a clear predominance of 1 pathogen on 20 occasions; such confusing cultural information was obtained from but 9 of the companion TTA specimens. Six TTA specimens but only 3 sputa from pneumonia patients were free of pathogens. Forty-eight TTA specimens from patients with chronic bronchitis (13), resolving pneumonia (9), lung abscesses (6), tuberculosis (1), pulmonary infarction (1), and pulmonary disease of undetermined etiology (18) yielded no pathogens or but 1 pathogen in all but 5 instances, whereas 21 of the 48 corresponding sputa contained more than 1 pathogen. ¶ Gram-negative bacilli and tellurite-positive staphylococci were the pathogens most frequently contaminating the 88 sputa. Postmortem studies (4) confirmed the validity of premortem TTA cultures. There were not any serious complications from TTA. ¶ TTA is a safe, practical method of securing precise cultural information. It is particularly indicated in cases of bronchopulmonary infection where sputa yield confusing cultural information.

Tocopherol and the Erythrocyte Membrane. HERBERT E. KANN, JR., AND CHARLES E. MENGEL, Columbus, Ohio (introduced by Charles A. Doan †).

Recent observations in patients with malabsorption, in premature infants, and in humans exposed to hyperbaric oxygen (OHP) have renewed interest in the effects of tocopherol (a lipid antioxidant) on erythrocyte membranes. Erythrocytes of tocopherol-deficient and tocopherol-supplemented mice were studied before and after *in vivo* exposure to 100% oxygen at 4 atmospheres pressure, absolute, for 1.5 hours for *1*) routine hematologic indexes, *2*) glucose-6-phosphate dehydrogenase (G-6-PD), glutathione peroxidase, and catalase activities, *3*) reduced glutathione (GSH), methemoglobin, and Heinz body content, and *4*) erythrocyte and plasma lipid peroxide content. ¶ During OHP no hemolysis occurred in tocopherol-supplemented mice, and all determinations performed on their erythrocytes were unchanged after OHP. Tocopherol-deficient mice developed marked hemolytic anemia during OHP (hemoglobinemia, fall in hematocrit from 50% to 28%). Their erythrocytes contained no Heinz bodies or methemoglobin, had no decrease in GSH content, and no significant change in G-6-PD, glutathione peroxidase, or catalase activities. However, they did contain high quantities of lipid peroxides, undiminished when tocopherol was given after OHP, but 1 hour before exsanguination, a technique proven to prevent *in vitro* lipid peroxidation. Therefore the lipid peroxides in these erythrocytes were formed *in vivo*. ¶ During shorter exposures of tocopherol-deficient mice to OHP, no immediate hemolysis was noted, although large quantities of lipid peroxides were formed in erythrocytes. Hemolysis then began within 10 minutes after OHP (hemoglobinemia, fall in hematocrit from 47% to 42%) and progressed (30 minutes after OHP, hematocrit 30%) while the mice were kept at normal atmospheric conditions. Coincident with the onset of hemolysis, erythrocyte lipid peroxide content decreased, and plasma lipid peroxides appeared. Subsequent rapid disappearance of plasma lipid peroxides reflected urinary excretion and destruction by plasma. ¶ These data are consistent with the hypothesis that lysis of erythrocytes in tocopherol deficiency states results from peroxidation of membrane lipid.

Use of Hapten-Human-Serum-Albumin Conjugates to Study Immunologic Competence in Man. FRED S. KANTOR AND WARD E. BULLOCK, New Haven, Conn. (introduced by Frank D. Gray. Jr.†).

The succession of immune responses to antigenic stimulation is initiated by delayed hypersensitivity and followed by appearance of 19 S and then 7 S antibody production. Patients with sarcoidosis manifest little or no capacity for delayed hypersensitivity, yet antibody production has been reported to be intact. Previous studies have examined the various phases of the immune response with diverse antigens. The present study was designed to investigate all phases of the immune response with a single antigenic determinant. In preliminary experiments guinea pigs sensitized with picryl chloride (PiCl) and dinitrochlorobenzene (DNCB) developed hemagglutinating antibodies directed against the haptenic determinant as well as typical delayed (contact) sensitivity. However, 30 normal and 9 sarcoid patients failed to develop demonstrable antibodies after repeated contact sensitization. ¶ Conjugates of picryl and dinitrophenyl (DNP) human serum albumin (HSA) were prepared *in vitro* and were injected intradermally and subcutaneously into normal and sarcoid patients. Normal patients (8/8) developed delayed hypersensitivity and hemagglutinating antibody titers ranging from 200 to 1,600. Five of 6 patients with sarcoidosis failed to develop delayed sensitivity, none developed antibody titers greater than 100, and 3 developed no demonstrable antibody. Two normals and 1 sarcoid patient developed wheal and flare reactivity, which was hapten specific. ¶ These studies define unique antigens, PiHSA and DNP-HSA, for study of human immunologic deficiency states and suggest that antibody production as well as delayed reactivity is impaired in sarcoidosis.

Studies on the Combining Sites of Human Anti-A Antibodies. M. E. KAPLAN AND E. A. KABAT, St. Louis, Mo., and New York, N. Y. (introduced by Stanford Wessler †).

In man, isohemagglutinin activity has been detected in γM-, γG-, and γA- serum immunoglobulins. To compare combining site characteristics of anti-A antibodies belonging to different immunoglobulin classes, we isolated anti-A from the sera of two hyperimmunized donors by absorption on insoluble polyleucyl-A substance and eluted the anti-A with *N*-acetyl-D-galactosamine (Gal-N-Ac). Each eluate, consisting of mixtures of the three serum immunoglobulins and containing more than 30% of the anti-A present in the original serum, was fractionated by gel filtration or density gradient centrifugation into γM and γG moieties. Only minimal contamination of γG fractions by γA resulted. γM and γG fractions from one serum contained both K and L light chain determinants, the latter predominating. γM fractions from the second serum contained primarily type K molecules, but L determinants were also readily detectable. γG from this serum lacked L determinants, being comprised almost exclusively of type K molecules. Per microgram of antibody N, the hemagglutinating activities of the purified γG equaled or exceeded those of the accompanying γM antibodies. Precipitability of individual antibody fractions by A substance ranged from 43 to 89%. Precipitation of both γG fractions was inhibited most efficiently by a pentasaccharide hapten of A substance; in contrast, γM precipitation was equally well inhibited by equimolar concentrations of monosaccharide (Gal-N-Ac), trisaccharide, and pentasaccharide haptens (the two larger haptens containing Gal-N-Ac as the terminal, nonreducing sugar). These results are interpreted to mean that the isolated γG antibodies have significantly larger combining sites than the γM antibodies. It is not

certain whether the data describe differences in average combining site size of entire anti-A immunoglobulin populations in an individual serum or reflect the properties only of the selected purified antibodies.

The Incidence of Primary Aldosteronism in Patients with "Essential" Hypertension. NORMAN M. KAPLAN, Dallas, Texas (introduced by Roger H. Unger *).

The evidence used by Conn in suggesting that at least 20% of "essential" hypertensives have primary aldosteronism was examined. ¶ First, adrenal adenomas from 7 patients with primary aldosteronism and 11 essential hypertensives and nonadenomatous tissue from 7 normotensives were assayed by a double isotope derivative technique. The aldosterone content of the aldosterone adenomas was markedly higher (mean = 10.3 μg per g tissue) than the hypertensive adenomas (0.4) and normal tissue (0.3). The corticosterone content was also much greater in the aldosterone adenomas (20.2) than in the other tissues (4.4 and 3.1). Cortisol content was similar in all (10.9, 8.4, and 10.2). ¶ Second, the excretion (UA) and secretion (ASR) of aldosterone were in the normal range in all of 43 essential hypertensives. The mean values (in μg per day) were higher in the hypertensives (UA = 10.9, ASR = 112) than in 39 normotensives (UA = 10.1, ASR = 90), but the differences were not statistically significant ($p < 0.2$). ¶ Third, only 2 of 58 hypertensives who developed hypokalemia (below 3.5 mEq per L) while on thiazides had increased aldosterone excretion after supplemental potassium was discontinued and when the serum potassium was normal. ¶ Fourth, angiotensin infusion tests were performed as an indirect measure of plasma renin activity in 20 unselected essential hypertensives. All were initially sensitive, and all became resistant after 4 days on a low-salt diet. Three patients with primary aldosteronism remained sensitive. ¶ In conclusion: *1*) Adenomas from essential hypertensives contain much less aldosterone than adenomas causing primary aldosteronism. *2*) Essential hypertensives do not excrete significantly increased amounts of aldosterone. *3*) The overwhelming majority of hypertensives who develop hypokalemia on thiazides do not have aldosteronism. *4*) Unlike patients with primary aldosteronism, none of 20 unselected hypertensives failed to reflect increased plasma renin after salt depletion. Primary aldosteronism appears to be rarely the cause of "essential" hypertension.

Parotid Fluid Corticosteroids: A Good Measure of Cortisol Available to the Cell. FRED H. KATZ AND IRA L. SHANNON, Chicago, Ill., and Brooks AFB, Texas (introduced by H. H. Hecht †).

Our previous studies have demonstrated that the concentration of 17-hydroxycorticosteroids (17-OHCS) in human parotid fluid, measured by the Porter-Silber reaction, is independent of salivary flow rate and increases substantially after ACTH administration. Moreover, despite significant elevations of plasma 17-OHCS in pregnancy, parotid fluid 17-OHCS are only slightly increased. Since the increased concentration of plasma 17-OHCS during gestation represents almost exclusively protein-bound, and therefore physiologically inactive, hormone, the likelihood that parotid fluid 17-OHCS concentration reflects only the level of dialyzable plasma cortisol was investigated. ¶ Parotid fluid contains cortisone as well as the cortisol found in plasma, and parotid enzymes can effect the cortisol → cortisone transformation *in vitro*. Therefore, both cortisol and cortisone concentrations in parotid fluid and plasma cortisol were measured by highly specific isotopic techniques. The free plasma cortisol was estimated by dialyzing plasma containing a cortisol tracer at 37° C. ¶ The data indicate that *1*) morning parotid fluid cortisone concentration (0.22 to 0.68 μg per 100 ml, mean 0.42 ± 0.17 SD) regularly exceeds that of parotid fluid cortisol (0 to 0.46 μg per 100 ml, mean 0.16 ± 0.14); *2*) after ACTH administration the ratio of cortisone to cortisol reverses (cortisol, 1.10 to 2.37 μg per 100 ml, mean 1.63 ± 0.39; cortisone, 0.97 to 1.77 μg per 100 ml, mean 1.17 ± 0.26); *3*) absolute levels of cortisone plus cortisol in parotid fluid are far lower than Porter-Silber chromogens, more so after ACTH, suggesting that other steroids are then present; *4*) estrogen treatment, which increases dialyzable plasma cortisol to a far smaller extent than ACTH treatment, similarly affects parotid fluid cortisol and cortisone concentrations; *5*) the sum of parotid fluid cortisol and cortisone is directly correlated with plasma free cortisol ($r = +0.95$) in control, estrogen, and ACTH periods. Thus parotid fluid cortisol plus cortisone represents an accurate index of the amount of circulating biologically active hormone available to the tissues.

Demonstration That 2-Monoglycerides Are Incorporated Intact into Human Lymph Triglycerides. HERBERT J. KAYDEN, JOHN R. SENIOR, AND FRED H. MATTSON, New York, N. Y. (introduced by Charles E. Kossmann †).

Until recently, it had been thought that dietary fat was completely hydrolyzed in glycerol and fatty acids, and that the latter after absorption were combined with glycerophosphate to triglycerides. This study is designed to show the quantitative importance in man of the additional pathway of triglyceride synthesis from 2-monoglycerides, which are absorbed intact and directly esterified; the magnitude of this pathway is similar to that observed in animal studies. Thoracic duct cannulation was carried out in three male patients who did not have disease of the gastrointestinal tract. Doubly labeled monoglycerides were synthesized from glycerol-2-^{3}H and stereate or oleate-1-^{14}C, solubilized into micellar form, and introduced via stomach or duodenal tube. Lymph was collected for 18 to 24 hours and total radioactivity counted for each isotope, and the fatty acids present in the triglycerides were counted and identified by sequential hydrolysis. When a mixture of the 1- and 2-monoglyceride 2-^{3}H glyceryl monostearate-1-^{14}C in the propor-

tion of 80:20 was used, the total recovery for ^{3}H was 35% and for ^{14}C 49%. When the proportion of 1- and 2-monoglyceride was 30:70, the recovery was ^{3}H 53% and ^{14}C 57%. A study with 2-^{3}H glyceryl 2 mono-olein-1-^{14}C gave recoveries of 35% ^{3}H and 36% ^{14}C, but the collection of intestinal lymph was incomplete. Analyses of the ratio of ^{3}H to ^{14}C and identification of the fatty acids in the first 4- to 8-hour lymph samples indicated that the 2-monoglyceride was absorbed into the intestinal cell and esterified to triglyceride. Subsequently, the remainder of the fed monoglyceride appeared to have been completely hydrolyzed either within the lumen or within the intestinal cells as evidenced by a marked fall in the ratio of ^{3}H to ^{14}C. These data indicate that in man dietary triglyceride that is hydrolyzed to 2-monoglyceride intraluminally is absorbed as the intact 2-monoglyceride; this latter pathway of fat absorption is quantitatively important.

Evidence for a Neural Origin of Cushing's Syndrome Associated with Bilateral Adrenal Hyperplasia. JOHN W. KENDALL, MONTE A. GREER,* AND GEORGE AUSTIN, Portland, Ore.

The etiology of Cushing's syndrome (CS) is obscure. Recent evidence strongly suggests that CS associated with adrenal hyperplasia is due to a higher "setting" of the negative feedback mechanism regulating ACTH secretion. Although opinion favors a location for this deranged feedback setting in the hypothalamus rather than in the adenohypophysis, evidence supporting this hypothesis is lacking. ¶ We have studied a woman who developed CS at age 33 and had both hyperplastic adrenals removed at age 34. The stigmata of CS cleared, but at age 39, while receiving 30 mg cortisol daily, she had developed a deeply pigmented, Negroid skin without evidence of pituitary tumor. Melanotropic activity was readily demonstrated in the plasma. The plasma ACTH content averaged 0.5 mU per ml and was depressed by 2 mg dexamethasone every 6 hours for 2 days to <0.06 mU per ml. We reasoned that if the increased ACTH secretion was caused by some neural drive, sectioning the pituitary stalk and inserting an impermeable plate between the hypothalamus and pituitary should reduce this drive. Since MSH secretion is known to be increased in some species after stalk section, we were concerned that she might become more deeply pigmented, but considered this a risk worth taking. At operation no pituitary enlargement or tumor was found. The patient's skin lightened within a few days after the stalk section, and the pigmentation had regressed markedly within 1 week and had almost completely disappeared within 1 month. Plasma ACTH and melanotropic activity were undetectable 2 weeks postoperatively after the patient had been without steroids for 36 hours and showed evidence of impending adrenal crisis. The data suggest that *1*) the deranged feedback mechanism causing CS is located in the brain; *2*) hyperpigmentation after bilateral adrenalectomy for CS is a result of a stimulatory rather than inhibitory influence from the brain.

Sodium Excretion as a Function of Rate of Expansion of the Extracellular Volume. EDWARD KESSLER, RONALD C. HUGHES, AND CHARLOTTE ORLANDO, Youngstown, Ohio (introduced by T. S. Danowski †).

The present experiments were undertaken to determine the role of rate of expansion of the extracellular volume as a stimulus to the excretion of sodium. Paired studies were performed in hydropenic dogs loaded with vasopressin and DOCA. Nine-tenths per cent NaCl was infused at two different constant rates in each animal for periods up to 4 hours. Both infusions produced a rise in urine flow, $U_{Na}V$, $U_{K}V$, and C_{osm}, reaching a new steady state despite continuously increasing cumulative fluid balances. $U_{Na}V$ and C_{osm} often could not be related to GFR or ERPF, and the onset of a rise in C_{Na}/GFR and C_{osm}/GFR was more prompt with rapid infusions, suggesting that changes in filtered load only partially explain the increased sodium excretion. ¶ Additional paired experiments were performed with gradually increasing rates of infusion and, after an initial rapid infusion, with gradually decreasing rates. The increases or decreases in urine flow, C_{Na}/GFR, and C_{osm}/GFR, again appeared, followed by stabilization until the infusion rate was changed, despite continuously increasing or decreasing cumulative balances. ¶ At equal cumulative balances or volumes infused more rapid infusions produced significantly higher rates of urine flow, C_{Na}/GFR, C_{osm}/GFR, and $U_{K}V$, and lower U_{osm} than slower infusions. $T^{c}_{H_2O}$ was not significantly different. ¶ It is concluded that the rate of extracellular volume expansion, not volume per se, is a stimulus for sodium excretion. The changes suggest that a disruption of the steady state in the extracellular space may trigger an alteration in renal sodium excretion.

The Effect of Independent Changes in Glomerular Filtration (GFR) and Sodium (Na^{+}) Excretion on the Renal Excretion of Calcium (Ca^{++}) and Magnesium (Mg^{++}) in Acutely Hypercalcemic Dogs. CHARLES R. KLEEMAN,* SHUN LING, DONALD BERNSTEIN, MORTON H. MAXWELL,* AND LLOYD CHAPMAN, Los Angeles, Calif.

In five normal dogs under Nembutal anesthesia, GFR (inulin clearance) was acutely altered by clamping the aorta above the renal arteries for periods of 1 to 3 hours. A sustained hypercalcemia (12 to 15 mg per 100 ml) was maintained in each animal by the continuous infusion of $CaCl_2$ throughout the experiment. Thus, it was possible to maintain the filtered load of Ca^{++} (FL Ca^{++}) at normal levels in many periods despite decreases in GFR of 30 to 60%. Na^{+} excretion was independently altered by infusing at varying rates of 0.45 to 1.2% NaCl containing 25 to 35 mEq per L of HCO_3. Urine collection periods averaged 20 minutes in duration. Ca^{++}, Mg^{++}, and Na^{+} in plasma and urine were measured by flame spectrophotometry. Concentrations of Ca^{++} and Mg^{++} were measured in ultrafiltrates of the plasma after anaerobic ultrafiltration. The latter were utilized to calculate Ca^{++}

and Mg^{++} clearances. ¶ Results: Despite hypercalcemia, if the FL Ca^{++} and the excretion of Na^{+} decreased, the excretion and clearance of Ca^{++} decreased. The greater the fall in Na^{+} excretion, the greater the decrease in Ca^{++} clearance. If the FL Ca^{++} was sustained despite 30 to 60% reductions in GFR, Ca^{++} excretion and clearance were directly related to the simultaneous changes in Na^{+} excretion. If the GFR and FL Ca^{++} decreased, but Na^{+} excretion increased, the excretion and clearance of Ca^{++} also increased. Throughout all experiments plasma total and diffusible Mg^{++} remained constant or fell slightly. Therefore reductions in GFR caused comparable reductions in the FL Mg^{++}. Despite this, Mg^{++} excretion and clearance were directly related to the simultaneous changes in calcium excretion and not to the FL Mg^{++}. ¶ Conclusion: The change in Ca^{++} clearance secondary to acute reductions in GFR in the hypercalcemic dog is primarily the consequence of the simultaneous changes in Na^{+} excretion. Mg^{++} clearance is directly related to Ca^{++} excretion rather than the FL Mg^{++}.

The Reticuloendothelial Uptake of *Staphylococcus aureus*. M. Glenn Koenig, M. Ann Melly, and David E. Rogers,* Nashville, Tenn.

It has been clearly established that serum opsonins are necessary for phagocytosis of staphylococci by polymorphonuclear leukocytes in fluid systems. Whether similar opsonins are required for phagocytosis by other cell populations is not known, and few studies have dealt with the serum factors necessary for the uptake of staphylococci by the reticuloendothelial system. ¶ The isolated perfused rabbit liver was utilized to study humoral factors governing the hepatic removal of viable staphylococci. Microbial uptake was determined by cultural or by radioisotope-labeling techniques. Hepatic trapping of staphylococci was reduced but not abolished by the metabolic inhibitor sodium arsenite or by temperatures below 20° C. Both wide-type and encapsulated staphylococci required serum for efficient hepatic removal. A heat labile serum component increased hepatic removal of wild-type strains, but its effect on the uptake of encapsulated staphylococci was less apparent. Hepatic trapping of encapsulated strains was enhanced by immune serum. Differential absorption of immune sera suggested that immunization with either wild-type or encapsulated strains elicited a common opsonic antibody. However, immunization with encapsulated strains elicited an additional opsonin that promoted hepatic uptake. ¶ These studies indicate that serum factors are necessary for the hepatic removal of staphylococci. It would thus appear that phagocytosis of staphylococci by polymorphonuclear leukocytes and the removal of staphylococci by the reticuloendothelial system are both serum-dependent host defense mechanisms.

The Inhibition of Bone Resorption by Thyrocalcitonin. Heinz F. Kohler and Maurice M. Pechet,† Boston, Mass.

The action of freshly prepared porcine thyrocalcitonin on Ca, P, Mg, ^{90}Sr, and hydroxyproline metabolism was investigated with an unanesthetized rat preparation specially designed for a prolonged metabolic study on a single rat. Dextrose, NaCl, and KCl were constantly infused through indwelling gastric and venous catheters. Urine was collected at 30-minute intervals over a 4-day period through an indwelling bladder catheter. Each 30-minute specimen was analyzed for Ca, P, Mg, hydroxyproline, and radioactivity. Normal and thyroparathyroidectomized rats were used as well as rats whose bones had been labeled 60 to 90 days previously with ^{90}Sr. Constant infusions over periods of 6 to 18 hours of thyrocalcitonin (TCT) caused in each instance a decreased excretion of Ca, P, Mg, ^{90}Sr, and hydroxyproline. Constant infusions over periods of 6 to 18 hours of parathyroid hormone (PTH) caused an increased excretion of Ca, P, Mg, ^{90}Sr, and hydroxyproline. The parathyroid-induced excretions of Ca, P, Mg, ^{90}Sr, and hydroxyproline were negated by the concomitant infusion of thyrocalcitonin with parathyroid hormone. The consistently reproducible changes in urinary excretion patterns were pronounced; for example, infusions of 5 μg PTH per hour caused an increased excretion over control of 84.3 μg Ca per hour, 1,052 μg P per hour, 38.5% increase in ^{90}Sr, and 120% increase in hydroxyproline excretions. The concomitant administration of 50 μg TCT per hour with 5 μg PTH per hour in the same rat preparation caused a decreased excretion over control of 77.9 μg Ca per hour, an increase of only 98.4 μg P per hour, 58.3% decrease in ^{90}Sr, and 88% decrease in hydroxyproline excretions. These studies indicate *1*) that thyrocalcitonin inhibits the action of parathyroid hormone on bone resorption and *2*) that thyrocalcitonin inhibits bone resorption in the absence of parathyroid hormone.

Histochemical and Biochemical Study of the 3β-Hydroxysteroid Oxidoreductase Activity of Human Term Placenta. Samuel S. Koide, New York, N. Y. (introduced by Martin Sonenberg *).

Formazan deposition was used as indicator for the 3β-hydroxysteroid oxidoreductase activity in the histochemical study. Positive staining reactions were observed in the trophoblasts, amnion, chorion, and connective tissue of the cord obtained from term and 10-week fetal placenta. ¶ The 3β-hydroxysteroid oxidoreductase and Δ^5-3-ketosteroid isomerase activities were determined in the subcellular fractions of placental homogenate. The activities were demonstrated in the soluble, mitochondrial, and microsomal fractions. The greatest activity resided in the particulate fractions. ¶ The placenta was subjected to acetone and deoxycholate treatment and ammonium sulfate fractionation. The resulting fractions possessed isomerizing activity and were devoid of dehydrogenating activity. Variations in the ratios of isomerizing activity

with androstan-5-ene-3,17-dione and pregn-5-ene-3,20-dione indicate that the isomerase for these two substrates might be different. ¶ The 3β-hydroxysteroid oxidoreductase activity was determined with various 3β-hydroxysteroids as substrates in the histochemical and biochemical study. The greatest activity occurred with 5β-androstan-3β-ol-17-one.

The Effect of Erythropoietin on Human Bone Marrow Cells *In Vitro* and Lack of Effect on Polycythemia Rubra Vera Marrow. SANFORD B. KRANTZ, Chicago, Ill. (introduced by Clifford W. Gurney *).

Recent work has shown that human bone marrow in cell culture responds to erythropoietin with a marked increase in hemoglobin synthesis. The dose response curve for human marrow and sheep erythropoietin shows a 500% increase in heme synthesis above controls after 70 hours' *in vitro* contact with the hormone. Since overproduction of red cells is a feature of polycythemia vera, it has been suggested that this disease results from excessive stimulation of red cell precursors by erythropoietin. ¶ When marrow from polycythemia vera patients was incubated *in vitro* with erythropoietin and either normal human plasma or the patient's plasma, heme synthesis was increased by only 50% after 70 hours. This is only one-tenth of the normal response. Furthermore, the rate of heme synthesis of normal marrow without added hormone was lower when incubated in polycythemia vera plasma than in normal plasma, suggesting that erythropoietin plasma levels are not increased in this disease. Normal human marrow incubated in polycythemia vera plasma responded normally to addition of the hormone. ¶ These experiments demonstrate that polycythemia vera is characterized by a relative lack of marrow response to erythropoietin and that this characteristic is not an environmental or plasma defect but an intrinsic marrow cell defect. Since it is believed that erythropoietin acts on primitive stem cells to promote the synthesis of messenger RNA, which effects differentiation into erythroblasts, the cell defect might be the absence of an inhibitor that normally prevents this messenger RNA formation unless the hormone is present. ¶ In terms of the Jacob and Monod hypothesis, the hormone acts as a derepressor that allows a specific capacity of the stem cells to become manifest. Polycythemia vera is thus best seen as a disease with an intrinsic defect in the specific stem cell repressor acted on by erythropoietin.

$^{47}Ca/^{85}Sr$ Studies of the Mechanism of the Hypercalcemia of Cancer. W. P. LAIRD MYERS, ERNEST J. GREENBERG, EDMUND O. ROTHSCHILD, MARIO MERLINO, DAVID A. WEBER, AND KARIN R. COREY, New York, N. Y. (introduced by Rulon W. Rawson †).

The occurrence of hypercalcemia in cancer is a well-recognized phenomenon, but the mechanisms involved are poorly understood. The occurrence of hypophosphatemia and absence of demonstrable bone metastases in some patients have suggested that parathyroid hormone excess (tumor-elaborated) is the underlying reason for the hypercalcemia. However, there are other patients with the hypercalcemia of cancer whose clinical and biochemical features do not mimic hyperparathyroidism, suggesting, therefore, a different mechanism. As one means of investigating this problem, $^{47}Ca/^{85}Sr$ kinetic studies of calcium metabolism have been undertaken in nine patients with cancer and hypercalcemia and in six patients with cancer and normocalcemia. The hypercalcemia group included five patients with breast cancer, two with lung cancer, one with renal adenocarcinoma, and one with rhabdomyosarcoma. The normocalcemic group all had breast cancer. The patients were hospitalized on a metabolic ward and studied under balance conditions. Kinetic analyses were carried out according to the method of Bauer and his colleagues, and measurements of total body retention were made with a high energy gamma (HEG) scanner. Compared to normocalcemic patients, the hypercalcemic group showed approximately a twofold increase in skeletal accretion rate of calcium and a three- to fourfold increase in the resorption rate. The exchangeable calcium pool was increased by approximately one-third. Successful treatment of the hypercalcemia in three patients was associated with a return to normal of these parameters, indicating a proportionately larger reduction in resorption rate than in accretion rate. These data were similar to those obtained in three other patients with primary hyperparathyroidism. They suggest that the primary defect in the hypercalcemia of cancer is an enhanced resorption of bone that is of greater magnitude than the increased accretion rate.

Clinical and Experimental Observations on Pre-Eclampsia. H. G. LANGFORD, B. B. DOUGLAS, R. ARHELGER, J. BONAR, J. J. BROWN, D. L. DAVIES, A. F. LEVER, AND J. I. S. ROBERTSON, Jackson, Miss., and London, England (introduced by Harper K. Hellems *).

Although pre-eclampsia, because of its self-limiting nature, seems an excellent model to study mechanisms of human hypertension, to date there is no explanation for the blood pressure elevation or the pathogenesis of the syndrome. One hypothesis gives renin a role in the BP elevation. Renin activity was measured (method of Brown and co-workers) on patients at term. In seven normal patients it was 58.7 U per L, and in nine pre-eclamptic patients it was 28.4 U per L, significantly lower, $p < 0.02$. ¶ If pre-eclampsia were associated with excess sodium ingestion or difficulty in sodium excretion, such a lowering of renin might occur. However, we showed previously that pregnancy occurring in rats during salt hypertension, DCA-salt hypertension, or post-DCA hypertension was associated with no further rise in BP. Increased proteinuria and edema did occur with a 10% increase in body weight in 24 hours in 6 of 30 rats. Two other proposed models also failed in rats—uterine constriction, where BP was significantly elevated after expected term, but no proteinuria or edema developed, and vitamin E deficiency, which produced histologic changes suggestive of pre-eclampsia, but no hypertension, proteinuria, or

edema. Because immunization of rats with rat placenta in Freund's adjuvant had produced mild hypertension and proteinuria, pregnant and nonpregnant rats were injected iv with rabbit antirat placenta serum. In the pregnant rats significant blood pressure elevation had developed in 5 days, associated with edema and proteinuria. Changes were less striking in the nonpregnant rats. We suggest that *a*) renin is not the prime mover in the hypertension of pre-eclampsia; *b*) an immune mechanism affecting the kidney seems the best lead to the pathogenesis of pre-eclampsia. Such a kidney lesion would have to interfere with Na excretion without stimulating renin production.

Diagnostic Criteria for Clinical Diagnosis of Primary Aldosteronism. DAVID P. LAULER, ROGER B. HICKLER, AND GEORGE W. THORN,* Boston, Mass.

Because of renewed interest in primary aldosteronism, definite preoperative diagnostic criteria are needed. Such procedures have been evaluated by us in normal subjects, in patients with sustained hypertension, and in patients with primary aldosteronism. As a result of these studies, four major diagnostic criteria have been established: first, a continued high aldosterone secretion on high sodium intake (200 mEq sodium per day); second, a low level of plasma renin activity despite a low sodium intake (10 mEq sodium per day); third, increased urinary potassium excretion during high sodium intake; fourth, presence of hypertension without significant edema. ¶ The key feature of these criteria is the demonstration that hypersecretion of aldosterone fails to decrease appropriately on a standard suppression procedure, i.e., salt loading, along with a diminished response of the tropic hormone (renin) to a standard stimulation procedure (salt restriction). Failure of aldosterone secretion to decrease reflects the autonomous nature of the hypersecretion, whereas impaired plasma renin response is due to prolonged suppression by aldosterone, presumably acting through expanded extracellular fluid volume. These conclusions are strongly supported by our patient studies.

The Adverse Effects of High Oxygen Breathing and Hypoxia on Tracheobronchial Mucus Flow. GUSTAVE A. LAURENZI, BRIAN J. COLLINS, SAM YIN, AND JOSEPH J. GUARNERI, Jersey City, N. J. (introduced by Harold Jeghers †).

The inhalation of oxygen-enriched gas mixtures is generally employed and of prime importance in a wide variety of situations extending from the treatment of patients with cardiorespiratory failure to the exploration of space and sea. This study demonstrates that oxygen inhalation has a deleterious effect on tracheobronchial mucus flow, thereby interfering with the clearance of secretions. Mucus flow was measured by determining the time for heterogeneous carbon particles to move cephalad a distance of 5 mm in the intact tracheae of barbiturate-anesthetized kittens. Particles were sprayed into the distal trachea, and observations were made under magnification through the transparent, anterior tracheal wall. The mean basal flow rate at room air breathing was 0.412 ± 0.026 mm per second. Marked slowing of mucus flow (66%) was consistently observed during 100% oxygen (moist) breathing. Inhibition was also observed during hypoxia, and graded interference occurred during inhalations of 25% and 40% oxygen mixtures. These events started within 3 minutes and peaked at 12 minutes. The clinical circumstance of administering oxygen to hypoxic patients was simulated by exposing animals for sequential periods to 10% and 100% oxygen. This combination reduced mucus flow 91%. Basal mucus flow was significantly improved; and the adverse oxygen and hypoxia effects were prevented and rapidly reversed, in separate studies, by aerosolized racemic epinephrine, intramuscular epinephrine, and intravenous G-strophanthin in therapeutic doses. These studies indicate that the mucociliary system functions optimally at ambient oxygen tension. The beneficial effects of epinephrine make it advisable that it, or related agents, be nebulized during high oxygen breathing. Epinephrine, through oxidative metabolism, and digitalis, by a more direct effect on contractile proteins, have the capacity to increase contractile activity. It follows that enhancement of mucus flow by these agents may be by a direct effect on ciliary activity.

Marked Elevation in Plasma Free Fatty Acids Due to Exercise in Obese Subjects. A. M. LAWRENCE, Chicago, Ill. (introduced by Richard L. Landau †).

Remarkable elevations in plasma free fatty acids (FFA) have been observed in obese individuals during and immediately after moderate standardized exercise. This rise in FFA contrasts sharply to the small rise seen in exercising normal subjects and to the picture of apparent impaired lipolysis in overweight subjects seen after, e.g., growth hormone or epinephrine administration. Conversely, exercise-induced elevation of serum growth hormone was conspicuously lower in obese than in normal subjects under identical experimental conditions. ¶ Normal and obese, nondiabetic, subjects on constant maintenance calories containing 250 to 300 g of carbohydrate were studied. After a 12-hour fast each subject exercised on a bicycle ergometer for exactly 30 minutes at a constant rate of 500 kg-m per minute. Blood samples for blood sugar, plasma FFA, and serum growth hormone were obtained at 0, 10, 30, 45, 60, 120, 150, and 180 minutes after beginning exercise. ¶ After an initial early drop in FFA, normal subjects showed an approximate 25% rise with peak elevation 45 minutes after start of exercise and gradual return to base-line values by 90 minutes. Average growth hormone rise in this group was 33 mμg per ml with peak increase appearing between 20 and 40 minutes. ¶ Obese subjects averaged greater than 70% increase in plasma FFA with peak rise at 45 minutes and delayed return with values elevated above base-line values at 90 minutes. Maximal growth hormone response in exercising obese subjects was less than half that observed in normal, nonobese individuals. ¶ It is concluded that exercise may result in more rapid than

normal mobilization of fat stores or delayed plasma clearing of FFA or both in exercising obese subjects. Since the apparent growth response to exercise was less than 50% of normal, it is suggested that the rise in plasma FFA seen with short term moderate exercise in obese subjects is not directly growth hormone mediated.

Antigenic Deficiency of Hybrid Myeloma Proteins: The Importance of Symmetry in the Region of Fd Fragment. THOMAS G. LAWRENCE, JR., AND RALPH C. WILLIAMS, JR.,* Minneapolis, Minn.

Twenty per cent of normal human sera contain anti-γ-globulin factors reacting with buried sites on γG not obvious in native γ-globulin but revealed by digestion with pepsin at pH 4.1. Myeloma proteins of the four major H-chain subgroups (We, Vi, Ne, and Ge) have been studied with respect to their relative antigenic potency at the sites revealed by proteolytic digestion of γG with pepsin. The test system employed inhibition of agglutination of Rh positive cells coated with pepsin-digested human incomplete antibody. Normal sera as well as sera from patients with rheumatoid arthritis or subacute bacterial endocarditis supplied agglutinating factors for the test system. ¶ All myeloma proteins of Ge and Ne H-chain subgroups showed antigenic deficiency at the pepsin site; reduction of antigenically complete 5 S pepsin fragments with 0.1 M mercaptoethanol abolished antigenic potency. Restoration of antigenicity could be effected by reoxidation in an atmosphere of 100% oxygen. An attempt was made to determine whether symmetry of the 5 S fragments was essential to antigenic activity. 5 S pepsin fragments of antigenically deficient Ge or Ne myeloma proteins were mixed in varying ratios with pepsin fragments from antigenically complete We or Vi myeloma proteins. The mixtures were then reduced with 0.1 M mercaptoethanol, the reducing agent removed by Sephadex G-25 gel filtration, and reoxidation carried out to produce 5 S hybrids. In all instances no antigenic activity for the site revealed by pepsin digestion could be ascribed to hybrids combining antigenically deficient (Ge or Ne) and antigenically complete (We or Vi) 5 S pepsin fragments. Molecular symmetry across the intact disulfide bond joining the heavy chain portions of the pepsin fragment seems essential for full antigenic expression of the pepsin site. Human sera containing anti-γ-globulin factors can by their fine specificity serve as useful probes in the study of human immunoglobulin structure.

Vasodepressor and Antihypertensive Prostaglandins of PGE Type with Emphasis on the Identification of Medullin as PGE_2-217. J. B. LEE, J. Z. GOUGOUTAS, B. H. TAKMAN, E. G. DANIELS, M. F. GROSTIC, J. E. PIKE, J. W. HINMAN, AND E. E. MUIRHEAD,† Worcester and Cambridge, Mass., Kalamazoo, Mich., and Memphis, Tenn.

The discovery of antihypertensive and vasodepressor lipids in extracts of renal medulla (Muirhead and associates, Lee and associates, Hickler and associates) has enhanced interest in the concept of a humorally mediated antihypertensive renal function. Recently, medullin was isolated by Lee and co-workers from crude extracts of medulla and found to be a more unsaturated, less hydroxylated derivative of prostaglandin-E_1 (PGE_1), possessing weak intestinal smooth muscle stimulation but marked vasodepressor activity. Medullin produced vasodepression by direct peripheral arteriolar dilatation. ¶ Daniels and co-workers isolated crystalline 15-hydroxy-9-oxoprost-10, 13-dienoic acid (PGE_1-217) from the enzymatic conversion of homo-γ-linolenic acid. PGE_1-217 differs from PGE_1 (Bergstrom and associates) by having a double bond at position 10-11 and is also prepared from PGE_1 by dehydration in 90% HOAc at 60° C. PGE_2-217 has now been prepared by dehydration of PGE_2. These compounds were identified by UV and IR spectra, NMR, and mass spectrometry. In contrast to PGE_1 and PGE_2, the Δ^{10-11} analogs have markedly reduced stimulating action on intestinal muscle, do not antagonize epinephrine-induced mobilization of FFA from adipose tissue, but are extremely potent vasodepressor agents. In addition, PGE_1-217 protects against the development of accelerated canine renoprival hypertension at 75 μg per kg per day (nine test animals, ten controls, $p < 0.001$). ¶ A UV spectrum of medullin isolated from rabbit renal medulla showed absorption at 215 mμ. The mass spectrum of free acid gave fragmentation patterns consistent with a formulation of 15-hydroxy-9-oxoprost-5,10,13-trienoic acid (PGE_2-217). Direct comparison of medullin and authentic PGE_2-217 revealed identical TLC mobility on $AgNO_3$-impregnated plates. ¶ These studies show that medullin appears to be PGE_2-217 and that it is a major vasodepressor lipid of renal medulla. Another dehydrated PGE (PGE_1-217) has antihypertensive properties. Existence of dehydrated PGE in renal medulla and the vasoactive nature of such compounds add interest to the concept of a renomedullary antihypertensive function.

The Metabolic Effects Caused by the Administration of Bovine Pancreatic Ribonuclease to Patients with Cancer. ROBERT D. LEEPER AND ASCHER HAYMOVITZ, New York, N. Y. (introduced by Joseph Burchenal †).

Previous work on the antitumor effects of bovine pancreatic ribonuclease in lower animals suggested that the enzyme might exert metabolic and antitumor effects in man. Daily intramuscular injections of ribonuclease (50 to 600 mg) were administered to patients with metastatic cancer for short term studies of up to 30 days. Complete metabolic balance techniques were used in five studies. ¶ Increases in serum ribonuclease (up to tenfold) were measured during injection periods and were dose dependent. Serum uric acids increased by 1 to 3 mg per 100 ml of serum, and urine urate excretion increased by 100 to 200 mg per day in four of the five patients studied. ¶ Urinary nitrogen excretion increased 1 to 3 g a day in four of five patients, and a negative

nitrogen balance was demonstrated in three patients. Blood urea nitrogen increased 5 to 15 mg per 100 ml in all patients. Negative calcium balances (100 to 500 mg per day) were induced by the enzyme in four of five instances. Phosphate excretion was not increased, and theoretical phosphorus excretion was 0.5 to 1.0 g above the observed. Elevated serum alkaline phosphatase levels decreased in two instances, in one case from 36 Bodansky U to 18, with rebound to over 40 U in the post-treatment period. Daily 17-hydroxysteroid excretion decreased to subnormal levels in two of three studies. Positive indirect Coombs' tests became negative in two studies and reverted to positive on enzyme cessation. Serum isozymes of ribonuclease and the kinetics of intravenous ribonuclease-^{131}I will be described. ¶ The studies indicate that the enzyme is absorbed and active and that its administration is accompanied by deranged protein metabolism. The effect is presumably mediated through hydrolysis of ribonucleic acid. It is suggested that this and other serum enzymes, either endogenously secreted or exogenous, may have effects in tissues other than the tissue of enzyme origin.

Regional Coronary Blood Flow Measurements by Simultaneous Use of Two Inert Gases. P. H. LEHAN, W. M. BURKE, A. MARKOV, H. A. OLDEWURTEL, AND T. J. REGAN,* Jersey City, N. J.

Measurement of coronary blood flow by inert gas methods is readily accomplished in the intact subject with homogeneous distribution of blood in the left ventricle. However, the presence of physiologically significant coronary artery obstruction is not usually reflected in such flow measurements due to the predominant distribution of gas to normal flow areas. ¶ The present study was undertaken to determine if regional rather than generalized alterations of coronary flow might be accurately localized in the intact dog by selective retrograde delivery of two inert gases into separate areas of left ventricle tissue via a coronary venous catheter. The transient reversal of the coronary venous pressure gradients created by rapid injection of hydrogen at the level of the great cardiac vein was found to selectively localize this gas in the region of myocardium subserved by that vein and the left anterior descending artery, as detected by tissue electrodes. Similarly, injection of ^{85}Kr gas into the area subserved by the coronary sinus and circumflex branch of the left coronary artery demonstrated localization of isotopic activity in tissue. Further, characteristic clearance curves for ^{85}Kr were obtained by precordial counting of isotope and for hydrogen by use of a platinum electrode in the coronary vein. Sequential coronary flow values so derived closely corresponded to those obtained by coronary arterial injection of gas during steady state hemodynamic conditions ($r = 0.89$). ¶ Simultaneous rather than sequential flow determinations should obviate the problem of an altered hemodynamic state between measurements. To determine if simultaneous use of the two gases discriminates normal from low flow areas, coronary thrombosis was induced via a catheter electrode in either of the major left coronary branches and was reflected in substantial flow reductions (93 ± 14 to 24 ± 8 ml per 100 g per minute) when gas was delivered via the artery distal to the thrombus. A similar flow decrement was observed when the gas was delivered to the corresponding venous site ($r = 0.89$), whereas normal blood flow values were obtained simultaneously in the nonischemic area. Thus, selective retrograde injection of two inert gases into myocardial tissue clearly differentiates low flow from normal flow areas when major branch obstruction in the coronary arteries exists and suggests a feasible method for detection of regional reduction of coronary flow in man.

Evidence for Renal Distal Tubular Impermeability to Urea in Man. PAUL R. LENZ, MARVIN H. GOLDSTEIN, AND MARVIN F. LEVITT,* New York, N. Y.

Urea excretion ($U_{Ur}V$) and the fraction of the filtered urea excreted (C_{Ur}/C_{In}) were determined in normal man during hydropenia and sustained water hydration. In hydropenia $U_{Ur}V$ averaged 17.6 mg per minute and C_{Ur}/C_{In} 0.42. During the transition from hydropenia to hydration $U_{Ur}V$ and C_{Ur}/C_{In} increased rapidly ("urea exaltation"), declining and stabilizing thereafter as hydration was sustained. The magnitude of the urea exaltation was directly proportional to hydropenic U_{Ur} values. This process probably results from the diffusion of medullary urea along its concentration gradients into tubular fluid. In the steady state of hydration, after exaltation, $U_{Ur}V$ averaged 24.7 mg per minute and C_{Ur}/C_{In} 0.65. ¶ Experiments were performed to determine tubular permeability to urea in postexalted states of sustained high urine flow. Agents inhibiting proximal tubular sodium and water reabsorption (acetazolamide, mannitol) were administered to hydrated subjects with steady urine flow rates. A prompt rise in C_{Ur}/C_{In} from 0.65 to 0.74 was observed with an average rise in solute clearance (C_{osm}) of 5.7 ml per minute. In contrast, administration of agents inhibiting distal salt and water reabsorption (organomercurials) in the same subjects failed to increase C_{Ur}/C_{In} despite a larger rise in C_{osm} averaging 8.6 ml per minute. A similar disparity in C_{Ur}/C_{In} was noted when proximal and distal agents were administered to hydropenic subjects during a steady state of mannitol diuresis. The failure of the distal agents to increase C_{Ur}/C_{In} under these conditions suggests that, at high rates of urine flow, there is virtually no back diffusion of urea in the distal tubule. The increase in C_{Ur}/C_{In} produced by hydration must therefore represent the maximal fraction of the filtered urea reabsorbed in the distal tubule during hydropenia. Reabsorption of less than one-fourth of the filtered urea in hydropenia at low urine flow rates and favorable urea concentration gradients reveals the limited permeability of the human cortical distal convolution to urea.