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ABSTRACTS

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Research Article

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

Myosin ATPase activity is important in the contraction cycle of the heart. Conventionally prepared solutions of cardiac myosin are inhomogeneous and can be separated into three components by ammonium sulfate fractionation in the presence of 2 M lithium chloride. The major fraction precipitates at 45% ammonium sulfate saturation and has the physicochemical characteristics ascribed to myosin. The two other fractions comprise at a minimum 10 to 15% of the total nitrogen present before fractionation and have characteristics clearly different from native or denatured myosin. The ATPase activity of myosin prepared by lithium chloride-ammonium sulfate fractionation is two to three times that of conventionally prepared cardiac myosin. The increase in enzyme activity results from *a*) removal of enzymatically inactive impurities tightly bound to myosin and *b*) removal of inhibitors of myosin ATPase activity. Canine cardiac myosin ATPase activity is 80% of canine or rabbit skeletal muscle myosin ATPase activity. PCMB titration of canine cardiac myosin fails to reveal stimulation of ATPase activity at either a high or low ionic strength; in contrast canine skeletal myosin ATPase activity is activated by PCMB at high ionic strength. Cardiac myosin ATPase activity is calcium activated. Calcium binding studies indicate that calcium binds to the protein with an n of 6 to 7 and association constant of 6×10^6. Previous work had shown that digoxin was neither bound to cardiac myosin nor influenced its ATPase activity. Calcium binding likewise was uninfluenced by digoxin in either therapeutic or toxic concentrations. Conclusions are: *1*) Although comparison of viscosity, sedimentation coefficient, molecular weight, and SH groups indicates a gross similarity between cardiac and skeletal myosins, there appears to be a subtle although probably important structural difference at or near the enzyme site. *2*) These studies add support to the contention that cardiac glycosides act at a site other than the cardiac myosin filaments.

Hypothesis to Explain Metabolic Defect in Acute Porphyria. GEORGE D. LUDWIG,* Philadelphia, Pa.

Coproporphyrin and uroporphyrin (10^{-6} M) have been shown to inhibit respiration of the succinoxidase system in Keilen-Hartree heart muscle particles and liver mitochondria. Kinetic and spectral studies indicate noncompetitive inhibition at the succinic dehydrogenase (S.D.) level. This led to the hypothesis that this mechanism may explain at least part of the metabolic defect characterizing acute porphyria. Inhibition of S.D. within specific cells (e.g., liver) by excess porphyrins might divert accumulated succinyl-CoA into the Shemin pathway, thus initiating a vicious cycle of overproduction of delta-aminolevulinic acid (ALA), porphobilinogen (PBG), uro- and coproporphyrin that characterizes acute attacks. Moreover triggering or aggravation of acute porphyria by barbiturates might be explained, since they contain malonate, the classical competitive inhibitor of S.D., and recently have been shown to inhibit mitochondrial succinoxidase. The finding of a transient Fanconi syndrome (uricosuria, aminoaciduria, phosphaturia) during acute attacks of human porphyria in six patients studied lends support to the hypothesis that S.D. inhibition occurs, since others have experimentally produced a Fanconi syndrome in rats by injecting maleic acid, a proven S.D. inhibitor. To test this hypothesis attempts were made to induce increased porphyrin and precursor excretion by S.D. inhibition in animals. Administration of sodium malonate (600 to 2,000 mg per kg per day) to rats induced increases of urinary uroporphyrin 8 to 10 times, coproporphyrin 2 to 3 times, precursors 4 to 5 times, with prompt return to control values when the drug was stopped. Maleic acid (3 to 12 mmoles per kg per day) evoked an even more dramatic increase in porphyrin excretion. The experimental production of increased porphyrin excretion by S. D. inhibition in animals coupled with the findings in acute human porphyria suggests that S.D. inhibition may represent an essential part of the metabolic defect, helping to explain the overproduction of porphyrins and precursors that characterizes acute attacks of porphyria, as well as the propensity of barbiturates to induce or aggravate acute attacks.

Interrelation between Jejunal Absorption of Sodium, Glucose, and Water in Man. SIDNEY J. MALAWER, MAYNARD EWTON, JOHN S. FORDTRAN, AND FRANZ J. INGELFINGER,† Boston, Mass., and Dallas, Texas.

The effect of glucose on sodium and water transport in intact human jejunum was studied in 18 normal men (100 studies) using a continuous perfusion technique and 30-cm test segment and correcting for endogenous sodium and water. Perfusion solutions, always isotonic, contained PEG; glucose-sodium ratios were systematically varied. Sodium absorption from isotonic saline without glucose was 3.1 mEq per hour. With 2 to 10 mM glucose, sodium absorption increased by 9.2 mEq per hour to 12.3 mEq per hour ($p < .01$) while only 3.3 mmoles glucose were absorbed per hour. It is essential to account for sodium absorption contributed by nonmetabolic osmotic absorption (solvent drag). This was calculated on the basis of a mean sodium reflection coefficient of 0.5, which we measured in the jejunum of 7 subjects by determining the effective osmotic pressure of hypertonic NaCl compared to hypertonic mannitol. After subtracting the sodium transport due to solvent drag (1.4 mEq per hour when 3.3 mmoles per hour of glucose was absorbed), sodium absorption increased by 7.8 mEq per hour. Thus, when small amounts of glucose were absorbed (sodium concentration in perfusion solution was isotonic to plasma), 2 mEq sodium was transported per millimole glucose, presumably by glucose stimulating the sodium pump. It has previously been asserted that sodium and glucose are transported in a 1:1 molar ratio. We found that the ratio of sodium to glucose absorption depended on the ratio of sodium to glucose perfused; a 1:1 ratio was found only with

30 mM glucose and is an artifact of the experimental conditions. Water absorption was directly proportional to net solute movement and was maximal (303 ml per hour) with 140 mM glucose. This solution elicited a 12-fold increase in water absorption compared to the small water absorption from isotonic saline (26 ml per hour).

Iodide, a Requisite for the Oxidation of Thiourea by Thyroid Tissue. F. MALOOF* AND M. SOODAK, Waltham and Boston, Mass.

We have reported that thiourea, a potent inhibitor of iodination, is oxidized by thyroid tissue, *in vivo* and *in vitro* (mammalian thyroid microsomes). The *in vitro* system, which also iodinates tyrosine, contains a hemoprotein and an essential disulfide bond. Initially, thiocyanate (10^{-3} M), a pseudohalide, and ascorbic acid (a source of H_2O_2) were found to be cofactors for thiourea oxidation, *in vitro*. Recently, iodide was found to replace thiocyanate, providing glucose (10^{-2} M) and glucose oxidase (100 μg) served as the H_2O_2 source. Other halides, chloride, bromide, and fluoride, are ineffective. Unlike thiocyanate, the effect of iodide is catalytic at low concentrations (2×10^{-6} M to 1×10^{-5} M). The requirement for iodide was confirmed by *in vivo* studies. The thyroid of rats on a normal diet (3 μg I^- per g food) oxidizes thiourea at a rate of 50 mμmoles per g per hour, whereas that of animals on an iodide-deficient diet (0.04 μg I^- per g food) for 10 days oxidizes only 20 mμmoles per g per hour. This decrease in thiourea oxidation occurred in spite of increased endogenous TSH stimulation, as is evident by an increase in other parameters of thyroid function. Usually thiourea oxidation and iodination vary similarly; both are inhibited by similar compounds and both are stimulated by the administration of TSH to normal or hypophysectomized rats. These data indicate that the concentration of I^- in thyroid tissue is a limiting factor for thiourea oxidation and suggest that an oxidized form of iodide, equivalent to I^+, an electrophilic species, possibly a sulfenyl iodide, is involved in the oxidation of thiourea as well as in the iodination of tyrosine. An 80-fold excess of tyrosine does not inhibit the oxidation of thiourea and is not iodinated until thiourea is oxidized *in vitro*. Hence, thiourea competes more successfully than tyrosine for the biological iodinating intermediate and thereby inhibits iodination.

Effects of Hypocapnia and Hypercapnia on Intracellular Acid-Base Equilibrium in Man. FELICE MANFREDI, Indianapolis, Ind. (introduced by John B. Hickam†).

Knowledge of the manner in which cells react to extracellular alkalosis and acidosis is essential to the understanding of clinical acid-base derangements. The DMO method recently shown to provide physiologically useful information about the "mean" $[H^+]$ and the "apparent" $[HCO_3^-]$ of cell water was used for studying the patterns of this response. Concentration gradients between extracellular(e) and intracellular(i) fluid compartments for $[H^+]$ and for $[HCO_3^-]$ were measured in five normal young subjects: *1)* at rest, *2)* during steady-state respiratory acidosis where arterial P_{CO_2} was maintained continuously for 3 hours between 15 and 20 mm Hg by voluntary hyperventilation, and *3)* during steady-state respiratory acidosis where arterial P_{CO_2} was maintained continuously for 3 hours between 55 and 60 mm Hg by inhalation of a 7% CO_2 gas mixture. At mean resting arterial P_{CO_2} of 40 ± 3 mm Hg, the $([H^+]i - [H^+]e)$ gradient was $([109 \pm 15] - [37 \pm 2]) = 71 \pm 13$ mμEq per L, and the $([HCO_3^-]e - [HCO_3^-]i)$ gradient was $([25.5 \pm 1.2] - [8.6 \pm 1.4]) = 17.0 \pm 0.5$ mEq per L. With hyperventilation the $[H^+]$ gradient remained unchanged $([91 \pm 19] - [25 \pm 2] = 66 \pm 18$ mμEq per L, $p > 0.5$), whereas the $[HCO_3^-]$ gradient narrowed significantly $([19.4 \pm 1.1] - [5.3 \pm 1.1] = 14.1 \pm 1.3$ mEq per L, $p < 0.001$) due to the $[HCO_3^-]e$ fall greater than the $[HCO_3^-]i$ fall. With CO_2 inhalation, both the $[H^+]$ gradient $([181 \pm 26] - [49 \pm 2] = 132 \pm 25$ mμEq per L, $p < 0.005$) and the $[HCO_3^-]$ gradient $([26.9 \pm 1.4] - [7.5 \pm 1.0] = 19.3 \pm 2.0$ mEq per L, $p < 0.005$) widened; the $[HCO_3^-]$ rise anticipated from the chloride shift mechanism largely accounted for the widening of the $[HCO_3^-]$ gradient. Arterial blood lactate rose with hyperventilation ($p < 0.025$) and fell with CO_2 inhalation ($p < 0.05$); hematocrit level and body water compartments were not statistically different with either of the experimental procedures. These data suggest that during experimentally induced respiratory acid-base disturbances, comparable with those encountered clinically, cells respond to: *1)* extracellular alkalosis with a decrease in bicarbonate concentration less significant than externally, presumably due to a relative cell membrane impermeability, and thus their $[H^+]$ decreases linearly with the external $[H^+]$; *2)* extracellular acidosis with no significant change in bicarbonate concentration, and thus their $[H^+]$ increases more significantly than the external $[H^+]$.

Pregnanediol and Pregnanetriol Excretion in Cushing's Syndrome. MALCOLM M. MARTIN AND BETTE L. HAMMAN, Washington, D. C. (introduced by John J. Canary*).

In the differential diagnosis of 20 patients with Cushing's syndrome a marked increase in urinary neutral 17-KS excretion favored adrenocortical carcinoma, and low values an adenoma, whereas ACTH and SU-4885 hyperresponsiveness and dexamethasone suppressibility suggested nontumorous hyperplasia. None of the above, however, could be relied upon to establish the correct diagnosis unequivocally. Chromatographic separation of neutral 17-KS in 11 patients confirmed abnormal patterns of excretion of A, E, KE, OHE, and OHA, but failed to prove of diagnostic value. DHEA was markedly elevated in 3 of 3 patients with carcinoma as well as in 1 of 3 with adenoma and 2 of 5 patients with adrenocortical hyperplasia. One patient with an adenoma and one with hyperplasia had normal base-line excretion but excessive ACTH responses. Two patients with hyperplasia asso-

ciated with extra-adrenal neoplasia had normal DHEA excretion at all times. Urinary pregnanediol and pregnanetriol were determined in 17 patients. Excretion was elevated in the control collection of 4 of 4 patients with carcinoma tested. Base-line levels were normal in 6 of 6 patients with hyperplasia and 2 of 2 with hyperplasia secondary to extra-adrenal carcinoma, but ACTH responses were elevated in 7 of 8. In the adenoma group in contrast, 5 of 5 had normal control values and normal ACTH responses. These studies suggest that in addition to dexamethasone suppressibility the urinary excretion and response to ACTH of pregnanediol and pregnanetriol may be the most helpful single test in determining the etiology of Cushing's syndrome.

Maturation Defect of Leukemic Blast Cells in Acute Leukemia. ALVIN M. MAUER AND VIRGINIA FISHER, Cincinnati, Ohio (introduced by Richard W. Vilter †).

Only a portion of the leukemic cell population in children with acute leukemia has been found to be actively dividing. The following studies were done to characterize these dividing cells further. A single injection of tritiated thymidine was given intravenously to two children with untreated acute leukemia. Serial blood and bone marrow samples were obtained thereafter and the per cent of labeled leukemic cells and their mean grain count determined by radioautography. In the marrow 5.5 and 6.5% of the leukemic cells were labeled 1 hour after the injection. The per cent of labeled cells then increased, associated with a decreasing mean grain count, doubling the per cent of labeled cells by 8 hours. Twenty hours after the injection a second increase in labeled cells associated with decreasing mean grain count was observed, doubling again the results of the first division. During both of these divisions almost all mitotic figures were found to be labeled. Initially only the larger leukemic cells were labeled, no label being found in small cells. Furthermore, only the larger cells exhibited the characteristics of a dividing cell population with a decreasing mean grain count being associated with the two observed mitotic divisions. Small leukemic cells of the marrow became labeled after 3 hours and subsequently increased at a rate of 10 to 15% per day. The time course for labeled leukemic cells of the blood resembled that of the small cells of the marrow. In these patients, a population of larger leukemic cells, comprising one-fourth of the marrow cells, was dividing with a generation time of about 20 hours. Within one or more divisions after labeling, these cells became smaller and stopped dividing without assuming any other features of maturation. The derivation of these dividing leukemic cells is as yet unknown.

Experimental Studies of Bacterial Antagonism. WILLIAM R. MCCABE, Boston, Mass. (introduced by Chester S. Keefer †).

The protective effect of a normal bacterial flora against superinfection with "opportunistic" pathogens is a recognized clinical phenomenon and has been utilized to prevent colonization and infection of infants with epidemic strains of staphylococci. During studies of experimental staphylococcal infections in embryonated eggs, it was noted that prior infection with avirulent staphylococci afforded significant protection against subsequent challenge with virulent strains. The fatality rate in 10-day-old embryos injected allantoically with avirulent staphylococci and challenged 2 days later with 1 to 100 virulent staphylococci was 32% (153/480) and was 21% (73/348) in eggs infected with avirulent organisms alone. In contrast, the fatality rate in eggs injected with saline and subsequently challenged with 1 to 100 virulent staphylococci was 80% (335/466) ($X^2 = 200$; $p < 0.001$). Eight additional strains of avirulent staphylococci were also shown to protect against subsequent challenge with several strains of virulent coagulase-positive staphylococci and against challenge with egg-virulent *D. pneumoniae, St. pyogenes, E. coli, Pr. mirabilis,* and *S. typhimurium.* Protection became apparent within 2 hours after injection of an avirulent organism, was maximal at 12 hours, and persisted for as long as 7 days. No protection could be demonstrated after injection of heat-killed or zephiran-killed staphylococci. The protective effect could not be overcome with large inocula of the challenge strain ($> 10^8$ bacteria). Millipore-filtered allantoic fluid from infected eggs failed to inhibit growth of the challenge strains *in vitro,* but inhibition of growth of the challenge strain was observed with one strain *in vivo.* With other strains protection was not associated with inhibition of growth of the challenge organism *in vivo.* Interferon production could not be demonstrated nor was there evidence of alterations in virulence or inhibition of toxin production of the challenge strain after infection. Protection was associated with enhanced bactericidal activity of whole blood ($X^2 = 18$; $p < 0.001$).

Extrarenal Regulation of Aldosterone Secretion in Primary Aldosteronism. JAMES C. MELBY,* THOMAS E. WILSON, SIDNEY L. DALE, AND ROGER V. HICKLER, Boston, Mass.

Depressed plasma renin activity in an erect active patient is a distinctive feature of primary aldosteronism (Conn). Plasma renin activity is significantly increased with upright posture in healthy subjects and patients with secondary aldosteronism, and urinary excretion of tetrahydroaldosterone is more than twice the amount excreted when supine. This response is augmented by sodium restriction and is demonstrable in patients with transplanted kidneys. Four of seven patients with primary aldosteronism responded to the erect posture with increased urinary tetrahydroaldosterone excretion, yet effective plasma renin activity was low. Four patients had levels of effective plasma renin ranging from 50 to 165 mμg per 100 ml of angiotensin (normal range = 200 to 680 mμg per 100 ml angiotensin by the Boucher method). One patient who had received spironolactone therapy had 380 mμg per 100 ml. Six-hour infusions of angiotensin

in subpressor amounts resulted in a two- to fourfold increase in aldosterone secretion in patients with primary aldosteronism and a 25 to 40% increase in patients with secondary aldosteronism. Incubation of angiotensin with sectioned aldosteronomas from four patients produced an increase from controls secreting 2.29 to 8.23 μg per g per 3 hours of tumor to 20 to 115 μg per g per 3 hours. Adrenal glomerulosa from contralateral and adjacent glands, normal adrenal glands, and adrenals removed from a patient with secondary aldosteronism when incubated with angiotensin did not increase aldosterone secretion. Subcapsular adrenal tissue (glomerulosa) from contralateral and adjacent adrenals, normal adrenals, and adrenals removed from a patient with secondary aldosteronism did not increase from basal secretion (0.33 to 6.1 μg per g per 3 hours) in response to angiotensin. In conclusion, the upright posture is a potent stimulus to aldosterone production independent of plasma renin activity or secretory sensitivity to angiotensin. Neither corticotropin suppression nor renal denervation alters its effect. An extrarenal humoral stimulus of aldosterone secretion is suggested.

Immune Studies in Vasculitis Associated with 19 S–7 S Type of Cryoglobulinemia. PETER A. MIESCHER,* FIORENZO PARONETTO, AND DAVID KOFFLER, New York, N. Y.

This study was performed on two patients with recurrent vascular purpura and arthralgia of several years duration. The clinical examination was otherwise unremarkable, and routine laboratory tests were normal. Antinuclear antibodies were not detectable. The latex fixation test was positive with aggregated gamma globulin. A 19 S cryoglobulin complexing normal 7 S gamma globulin of the type described by Lo Spalluto and others was found in the serum of both patients. Prednisone and 6-mercaptopurine were of little effect; however, methotrexate medication (50 mg iv once weekly) resulted in complete remission in one and in marked improvement in the other patient. Whole serum complement titers were determined in terms of 50% hemolytic units. C'2 complement component was assayed by the method of Austen and Beer. Both methods revealed markedly decreased complement levels. When the washed cryoglobulin precipitate was incubated with fresh normal human serum, complement was fixed to the cryoprecipitate. Histologic examination of skin biopsies from both patients revealed a similar picture of vasculitis in the superficial dermis associated with neutrophils, some mononuclear cells, granular eosinophilic deposits, and red cell extravasates in the vessel walls and perivascular tissue. Utilizing the fluorescent antibody technique, γ_G- and β_{1C}-globulin and fibrinogen were localized in the vessel walls and surrounding connective tissue. γ_M-Globulin was only detected in one case exhibiting fresh vascular lesions, but not in the second with a 3-day-old lesion. *In vitro* incubation with fresh human serum resulted in increased staining with fluoresceinated anti-β_{1C}-serum. In contrast a case of erythema nodosum vasculitis and of dermatitis factitia did not exhibit positive staining for immunoglobulins and β_{1C}-globulin. In view of these findings it appears that complement fixing cryoprecipitates are formed resulting in a decrease of serum complement and in local vasculitis upon deposition of precipitates on the vascular endothelium.

The Specialized Conduction System and Cardiac Performance. KAY MILLAR, ROBERT H. EICH, HAROLD SMULYAN, AND J. A. ABILDSKOV,* Syracuse, N. Y.

Although abnormal ventricular activation occurs frequently and has established diagnostic utility, its relation to mechanical performance is poorly defined. Evidence ranges from no influence to marked effects on output and pressures. To clarify this relation, hemodynamic effects of simple right bundle branch block and of destruction of the entire canine right ventricular conduction system by intraventricular instillation of Lugol's solution were studied. Ventricular, aortic, and pulmonary pressures were recorded during atrial and ventricular stimulation from multiple sites. With intact conduction system and with right bundle branch block, different activation patterns were associated with slight differences of pressure curves. After conduction system destruction, marked differences of the pressure curves including those from the left ventricle occurred. Premature ventricular stimulation has been shown by others to result in a greater per cent fall in the stimulated compared to the contralateral ventricle. This difference was confirmed. Time of onset of pressure rise in the chambers differed, however, and pressure vs. interval between pressure rise in premature and preceding beat showed little difference with various stimulus sites. This indicated filling time, not activation order, most responsible for differences in the two ventricles. In the absence of the right conduction system, ventricular pressures differed with stimulus sites despite correction for filling time, although specific site differences were not reproducible in different animals. Findings indicate remarkable efficiency of the conduction system in distributing activation in a manner that preserves a normal hemodynamic state. Significant differences of this state occurred after destruction of the right system. This suggests functional consequences of diffuse conduction system disease that may encroach on cardiac reserve. Findings included alteration of left ventricular pressures with destruction of the right conduction system possibly due to abnormal septal activation and reflecting a significant septal role in left ventricular function.

Comparative Potency of the Antidiuretic Hormones Lysine-8- and Arginine-8-Vasopressin in Humans. LOUIS MILLER, LEON FISCH, AND CHARLES R. KLEEMAN,* Los Angeles, Calif.

Previous studies in rats and dogs demonstrated that arginine-8-vasopressin (A.V.) is more potent than lysine-8-vasopressin (L.V.). However, potency incorporates *1*) the hormone's response at the effector site for a given

plasma concentration, *2*) its turnover, and *3*) its affinity for the receptor site. To analyze the above, A.V. and L.V. were given to 10 humans in paired experiments by *a*) acute injection followed by sustained infusions and *b*) sustained infusions per se. Results were expressed in per cent reduction in free water corrected for incomplete bladder emptying by creatinine excretion. Doses of hormone were calculated to give concentrations in plasma between 0.625 μU and 3 μU per ml, assuming that the hormone is distributed in plasma volume. The responses ranged between minimal and maximal antidiuresis. After "*a*" no significant antidiuresis was seen with either hormone in the first 5 minutes, whereas from 5 to 10 minutes the degree of antidiuresis was similar for A.V. and L.V. In the subsequent periods A.V. produced increasingly greater degrees of antidiuresis than L.V. After stopping the constant infusion, the length of time (t) for the effect of the higher, more antidiuretic dose (Q_o) to fall to the level of antidiuresis observed in the same experiment and same subject during the steady state at a lower rate of hormonal infusion (Q_t) was used to calculate the fractional turnover (k) using the standard first order equation $Q_t = Q_o e^{-kt}$. The average k for A.V. and L.V. were 0.031 and 0.050, respectively. These values represent different rates of hormone turnover and/or different affinity for the receptor site in the kidney. The same effect during the 5- to 10-minute period after acute injection of each hormone strongly suggests that affinity, defined as effect per given blood concentration, does not differ. Therefore we conclude that the main cause for the difference in potency between these two hormones is the different rates of turnover (k) in the circulation.

NaCl and $CaCl_2$ Activity Coefficients in Mixed Aqueous Solutions. EDWARD W. MOORE AND JAMES W. ROSS, Boston, Mass. (introduced by Thomas C. Chalmers †).

Ionized calcium is known to play an important role in numerous physiological processes. In many instances, as in studies of membrane transport phenomena and transmembrane ionic activity gradients, it is calcium ion activity rather than stoichiometric concentration that is physiologically meaningful. Other than osmotic data, no thermodynamic data are available, however, which allow estimation of mean ionic $CaCl_2$ activity in mixed electrolytic solutions with composition similar to that of extracellular fluids. Since most of the total ionic strength of plasma and other extracellular fluids is accounted for by NaCl, the activities of NaCl and $CaCl_2$ in these fluids may reasonably be approximated by study of mixed aqueous NaCl–$CaCl_2$ solutions. This study concerns the determination of mean ionic activity coefficients of NaCl in such solutions over the ionic strength range 0.05 M to 0.5 M by potentiometric measurements utilizing a sodium-selective glass electrode-Ag/AgCl electrode system. Log γ_{NaCl} varied linearly, at constant total ionic strength, with the ionic strength of $CaCl_2$ in the mixture, in accordance with Harned's rule. From data thus obtained, γ_{CaCl_2} coefficients in such mixed solutions have been calculated by two different methods, one of which includes published osmotic data. Resulting activity coefficient curves for $CaCl_2$ have been derived for the concentration range encountered in plasma and other extracellular fluids. These data should be useful in the interpretation of *in vitro* studies of membrane ionic transport and also *in vivo* studies if the nonchelated, nonprotein-bound fraction of the total calcium level is known.

Fructose-induced Disruption of Renal Acidification in Patients with Hereditary Fructose Intolerance. R. MORRIS, San Francisco, Calif. (introduced by R. Havel *).

To test whether the metabolic abnormality of hereditary fructose intolerance (HFI) might give rise to an acidification defect like that of renal tubular acidosis (RTA) the effect of fructose on renal acidification was investigated in three patients with HFI. Six studies were done in which urine was collected at 15-minute intervals under oil via indwelling catheter 5 hours after oral administration of ammonium chloride 0.06 to 0.1 g per kg. After three collections, fructose 0.25 to 0.50 g per kg was administered intravenously over approximately 2 hours. Within 45 minutes after initiation of fructose, the urinary pH increased from <5.2 to >6.2, and urinary excretion of titratable acid and ammonium decreased appropriately. After intravenous administration of Na_2HPO_4/NaH_2PO_4 (0.15 M, pH 7.4) sufficient to induce a phosphate diuresis, excretion of titratable acid increased to rates greater than those attained before administration of fructose in spite of continuing urinary pH > 6. Before, during, and after fructose, systemic acidosis was documented. Inulin clearance changed insignificantly or fell slightly after fructose administration. Ninety minutes after discontinuance of fructose urinary pH was 5.2 or less, and excretion of ammonium and titratable acid increased appropriately. In five normal subjects rendered acidotic with NH_4Cl urinary pH remained < 5.2 during intravenous administration of fructose, 0.5 to 0.7 g per kg over 2 hours; the rate of urinary excretion of titratable acid and ammonium did not decrease. In both normals and patients with HFI an abrupt increase in urinary excretion of alpha amino nitrogen occurred with fructose administration. In patients with HFI this was associated with a marked increase in uric acid clearance. Thus, in patients with HFI, fructose induces a reversible renal tubular defect with some of the physiological characteristics of RTA and Fanconi syndrome.

A Clinical Question: Are Vasopressor Amines Beneficial in Treatment of Shock? RUSSELL E. MORRIS, JR., THOMAS D. GRAFF, PATRICIA R. ROBINSON, GEORGE A. SCHEELE, AND ROBERT A. GAERTNER, Baltimore, Md. (introduced by Samuel P. Asper *).

The clinical wisdom of treating shock with vasopressor substances is questioned; such extreme measures to maintain life temporarily through peripheral vasoconstriction

denies some tissues an effective blood supply and may produce irreversible changes that hasten death. Clinical experience shows that few patients survive sustained doses of pressor amines; rather, they decline, progressively failing to maintain an adequate blood pressure despite increasing amounts of drug. Are such deaths hastened by vasopressors, or is death the result of the severity of disease, which makes all efforts futile? To answer this question, 40 normal dogs were infused with norepinephrine, phenylephrine, or angiotensin, and the metabolic effects studied. Intravenous infusion of norepinephrine (1.0 μg per kg per minute) produces metabolic acidosis with blood and lymph pH less than 7.0, a blood volume decrease of 40%, a rising hematocrit, reduction of cardiac output by 60%, and a falling blood pressure. Serum sodium and calcium decrease, potassium is elevated, and phosphorus reaches 10 mg per 100 ml. That this is not respiratory acidosis is shown by a normal Pco_2. These effects of acidosis may be the immediate cause of death but are an incomplete explanation. The twofold rise in lactic acid is inadequate to explain the severe acidosis; however, acetoacetic and β-hydroxybutyric acids increase and equal mole for mole the decrease in bicarbonate. Infusions of sodium bicarbonate with norepinephrine prevent this irreversible acidosis, but administration after development of acidosis is ineffective. Phenylephrine produces a similar change, whereas angiotensin initially produces acidosis, but tachyphylaxis develops with escape and metabolic return towards normal. Four human subjects infused with norepinephrine (0.1 μg per kg per minute) have qualitatively similar acidotic changes; infusions at 2.0 μg per kg per minute are quantitatively identical to results in dogs. It is concluded that prolonged administration of norepinephrine induces fatty acid release resulting in metabolic acidosis, cardiac failure, and irreversible shock despite the apparent initial benefits of blood pressure elevation.

The Relationship of Megakaryocytic and Erythroid Precursor Cells. BERNARD MORSE AND FREDERICK STOHLMAN, JR.,* Boston, Mass.

A common precursor (stem) cell for erythroid, myeloid, and megakaryocytic cell lines is suggested from karyotype studies in chronic myelogenous leukemia and radiation chimeras. The previously reported cessation of erythropoiesis and depopulation of the marrow erythroid compartment in the rat after a single intravenous dose of vincristine (VC) suggests an effect on nucleated erythroid precursors in addition to the stathmokinetic effect of VC. Megakaryocytopoiesis and the levels of circulating platelets in these rats were little affected. The continued presence of immature megakaryocytes (turnover time, 8 to 14 hours) in the marrow of the VC-treated rat suggests that the immediate precursor of the megakaryocyte is little affected. If the erythroid and megakaryocytic precursors are common, then it might be inferred that the erythroid stem cells are unaffected. To investigate this the stem cell response to erythropoietin, (EP) was assessed at intervals before and after VC treatment in hypertransfused rats. The typical sequence of erythroid response was diminished and delayed 4 days in rats receiving EP immediately after VC and delayed 2 days when EP was given 48 hours after VC. When EP was given 6 hours before VC, the erythroid response was abolished. The inability to differentiate erythroid stem cells in the face of megakaryocytic stem cell differentiation points to different immediate precursor cells or a selective block of erythroid differentiation. The delayed response to EP suggests that if there is a block, it does not interfere with the interaction of EP and its intracellular target, but that the information is stored until regeneration permits differentiation. The abolition of the response when erythropoietin is given 6 hours before VC may be interpreted as indicating that resting erythroid stem cells have been triggered into cycle where they are susceptible to VC effects. Regeneration when VC is given after EP, then, would be from resting immediate precursor cells. The data do not permit a final decision, but it appears that the immediate stem cell for erythroid and megakaryocytic cell lines differs with respect to their sensitivity to VC. In view of the karyotype studies, two levels of precursor cells are implicated, a more primitive precursor cell responsible for hemopoietic colonization or repopulation and a more differentiated immediate precursor or stem cell for each cell line.

Association of Fever and Antibody Response in Rabbits Immunized with Human Serum Albumin. PETER D. MOTT AND SHELDON M. WOLFF, Bethesda, Md. (introduced by Vernon Knight †).

Antigen-antibody interactions may be the basis for the development of fever in certain experimental situations, as suggested by others. Using pyrogen-free human serum albumin (HSA) as the antigen, rabbits were immunized intravenously over a 3-week period with 9 injections of 25 mg HSA. Challenge doses of 2 or 5 mg were administered intravenously 10 days later, and 74% of 43 rabbits responded with fever (average rise, 0.83° C). Circulating antibody (bentonite flocculation) was detected in 84% of the febrile rabbits (mean titer, 1:18) on the day before challenge. Although similarly immunized, only 27% of rabbits not reacting with fever had measurable antibody levels (mean titer, 1:1). A state of febrile tolerance to HSA was rapidly induced by daily repetition of the challenge dose. This tolerance could not be passively transferred by serum (20 ml per kg) to immunized recipients. However, the ability to respond to challenge with fever could be passively transferred with 20 ml serum per kg to normal recipients (76% of 25 normal rabbits receiving serum with a mean antibody titer of 1:147). In contrast, when serum of immunized rabbits with no detectable antibody was similarly transferred, only 1 of 7 rabbits became febrile on challenge. The fever after HSA challenge was not altered by prior induction of febrile tolerance to bacterial endotoxin. Sephadex G-200 gel filtration revealed that

almost all of the antibody on the day before challenge was in Fraction II, which contained 7 S gamma globulin. These studies indicate the association between febrile reactivity and the presence of circulating antibody in HSA-immunized rabbits.

Analysis of the Inotropic Effect of Digitalis and *l*-Norepinephrine on Left Ventricular Function. C. B. MULLINS, D. N. GUPTA, AND J. H. MITCHELL,* Dallas, Texas.

Digitalis and *l*-norepinephrine are drugs frequently used for their inotropic action on cardiac muscle. The purpose of this study was to analyze the dynamic effects of these agents on left ventricular function in open chest dogs with controlled aortic pressure, cardiac output, and heart rate. Six lead beads were placed subendocardially to demarcate the major and two minor axes of the assumed elliptical cavity of the left ventricle. These three lengths were determined from biplane cinefluorographic exposures taken at $\frac{1}{30}$ second intervals and then used to calculate ventricular cavity volume and frontal, sagittal, and transverse circumferences. These dimensions and volume measurements were correlated with aortic pressure, left ventricular pressure (intracardiac transducer), and aortic flow (electromagnetic flowmeter). Stroke volumes were compared by simultaneous integration of aortic flow and by the difference between end-diastolic and end-systolic ventricular cavity volumes. The circumferential shortening and lengthening velocities were calculated. Aortic pressure was kept constant by a pressure bottle, cardiac output by an AV fistula, and heart rate by right atrial pacing after excision of the SA node. The administration of both acetyl strophanthidin and norepinephrine caused a decrease in both end-diastolic and end-systolic volumes and a decrease in the ratio of end-systolic to end-diastolic volume. For the same stroke volume the maximal rate of ejection was increased and the duration of ejection decreased. The end-diastolic and end-systolic lengths of all three circumferences were decreased and the per cent shortening of each increased. The circumferential velocities of shortening and lengthening were increased. Also the duration of minimal systolic length was prolonged even though the total time of systole was shortened. The inotropic effect of digitalis and *l*-norepinephrine can be quantitatively measured by the changes in velocity of circumferential shortening and lengthening of the intact left ventricle.

Prolonged Diving in the Seal: A Model for the Study of O_2 Depletion. H. VICTOR MURDAUGH* AND EUGENE D. ROBIN,* Birmingham, Ala., Pittsburgh, Pa., and Salisbury Cove, Maine.

Oxygen depletion is common clinically. Knowledge of physiologic changes and adaptations during O_2 depletion is of obvious importance. Aquatic mammals, capable of prolonged diving, may serve as appropriate models for studying such problems. Measurements of blood flow distribution, cardiac output, heart rate, blood gases, acid-base metabolism, and external gas exchange have been performed in harbor seals (*Phoca vitulina*) before, during, and after diving. Profound regional arterial constriction involving all vascular beds except that of brain occurs during diving. Arterial constriction was documented by dye injection into peripheral veins and monitoring aortic blood dye concentrations. Predive dye injections were followed by inscriptions of typical dye dilution curves. During diving, dye injection resulted in no significant increase in aortic dye concentration. Arterial constriction minimizes O_2 utilization by peripheral tissues, and essentially all O_2 in blood and lungs is available for maintaining cerebral oxygen-dependent metabolism. Accompanying arterial constriction are dramatic decreases in cardiac output and heart rate that presumably permit maintenance of normal pressure-volume relations in cerebral vessels. As regional flow ceases, peripheral tissues utilize anaerobic glycolysis for energy production. Lactate accumulates in tissue, but no hyperlactemia occurs because of perfusion loss. Progressive respiratory acidosis and hypoxemia develop during diving. As the dive terminates, perfusion is restored. Hyperventilation and increased cardiac output develop. Pa_{O_2} normalizes and Pa_{CO_2} falls below normal. In the postdive period, with peripheral tissue reperfusion, blood lactates rise steeply and metabolic acidosis becomes apparent. Normalization of acid-base parameters may take more than 40 minutes after a 10-minute dive. Oxygen "debt" incurred during diving is repaid slowly and apparently incompletely. CO_2 "surplus" is repaid rapidly with an overshoot of CO_2 excretion. Selective perfusion of critical areas, disparity between lactate production and blood lactate concentrations, and complicated changes of acid-base metabolism closely resemble changes occurring in clinical O_2 depletion.

Stimulation of Bone Turnover by Oral Phosphate in Vitamin D-resistant Osteomalacia. CHARLES NAGANT DE DEUXCHAISNES AND STEPHEN M. KRANE,* Boston, Mass.

Healing of vitamin D-resistant osteomalacia has occasionally been achieved by oral administration of inorganic phosphate alone. In the present study, the effects of phosphate therapy on Ca^{47} kinetics, bone matrix turnover (hydroxyproline excretion), calcium and phosphate balances, and the healing of the osteomalacia have been investigated in two female subjects with characteristic blood chemistries, multiple pseudofractures, and wide osteoid seams. After three months on neutral phosphate (2.4 g P daily), A.J. (age 58) showed roentgenological healing; serum phosphate rose from 1.6 to 5.2 mg per 100 ml, and alkaline phosphatase from 11 to 25 B.U., while the serum calcium fell from 9.1 to 6.4 mg per 100 ml. The calcium balance became positive, the bone formation rate (v_{o^+}) increased from 366 to 2,664, and hydroxyproline from 29 to 135 mg per day. After phosphate was discontinued, within 2 days serum calcium normalized and phosphate

fell, and within 2 months alkaline phosphatase fell to 13 B.U., v_{o^+} to 906, and hydroxyproline to 44 mg per day. After two months on neutral phosphate (3.2 g P daily), S.M. (age 37) showed roentgenological healing; serum phosphate increased from 1.2 to 3.2 mg per 100 ml, and alkaline phosphatase from 5 to 8 B.U. Serum calcium remained unchanged, whereas urinary calcium decreased from 63 to 6 mg per day. Calcium balance became positive, v_{o^+} increased from 336 to 1,814, and hydroxyproline from 23 to 103 mg per day. On cessation of phosphate therapy, serum phosphate fell abruptly, urinary calcium rose to over 200 mg per day, and 16 days later, v_{o^+} had fallen to 963 mg per day. Oral phosphate alone induces healing in refractory osteomalacia not only by supplying phosphate for bone salt but also by stimulating initially low normal rates of bone formation and resorption.

Immunosuppression and Altered Graft Rejection Produced by Antilymphocyte Serum. H. NAGAYA, I. L. PEACOCKE, AND H. O. SIEKER,* Durham, N. C.

The lymphocyte has a primary role in delayed hypersensitivity reactions such as tuberculin response and allograft rejection. This study investigated the effects of heterologous antilymphocyte serum and antithymic serum on lymphocytes, immune response, graft rejection, and lymphoid tissue. Antirat lymphocyte serum (ARLS) was produced in rabbits by injection of rat lymph node homogenate and Freund's adjuvant. Antirat thymic serum (ARTS) was prepared using rat thymus homogenate in a similar manner. ARLS agglutinated rat lymphocytes in dilutions up to 1:80. A single intraperitoneal injection of ARLS decreased the absolute lymphocyte count to 30% of the control value, with depression maintained for 48 hours, and suppressed delayed skin reaction to bovine serum albumin in sensitized rats. Daily injection of ARLS produced lymphopenia in rats for periods up to 30 days with periodic fluctuation at 5 and 10 days. Similarly, ARTS produced lymphopenia with fluctuation during the first 10 days of administration, but in contrast to ARLS administration, the lymphopenia was more marked and persistent in the following period. Daily injection of ARLS beginning 1 day before allograft prolonged mean survival time of the graft from 10.8 days to 13 days, whereas mean survival in rats receiving ARLS 7 days before grafting was 17 days. The spleen and lymph nodes of rats receiving ARLS continuously showed complete replacement with plasma cells demonstrated by routine pathologic examination and by electron microscopy. This study demonstrates that antilymphocyte serum will produce lymphopenia with periodic fluctuations, depression of the immune response, and delay of allograft rejection. Antithymic serum chronically administered produces a similar lymphopenia, but in contrast to antilymphocyte serum the effect is more marked and continuous. The replacement of lymphoid tissue with plasma cells provides a model for investigation of the evolution and function of this type of cell.

The Effect of Ouabain Infusion on Cation Binding by Particulate Fractions of Renal Cortex. VICTOR E. NAHMOD AND MACKENZIE WALSER,* Baltimore, Md.

The primary effect of cardiac glycosides may involve changes in the affinity of ion-binding sites on or within the cell. Reversible binding of cations was studied in dog renal cortex homogenized in 0.25 M sucrose at 0° C by measuring concentrations per liter of tissue water in four sediments obtained by differential centrifugation at 4° C and comparing these with concentrations in the final supernatant ($122{,}000 \times g$ for 2 hours). The four sediments contained predominantly nuclear debris, mitochondria, "heavy" microsomes, and "light" microsomes. In normal animals, whole tissue concentrations (millimoles per liter H_2O) were Na 67, K 78, Ca 2.7, and Mg 8.0, and final supernatant concentrations were Na 58, K 65, Ca 0.4, and Mg 4.0. Bound Na was highest in microsomes, whereas the other 3 cations were bound chiefly in the mitochrondrial fraction. Thus bound Na/bound K was 0.3 in mitochondria and 1.4 in heavy microsomes. Ouabain was infused into one renal artery at 5 or 10 μg per kg per minute for either 5 or 20 minutes, and then both kidneys were analyzed as above. The earliest change was a fall in light microsomal binding of K while that of Na increased. At this time (5 minutes) Ca and Mg were unaltered. With increasing time and dosage, tissue Na/K rose from 0.86 to 2.34, and calcium increased markedly in nuclei and mitochondria. Even the uninfused kidneys showed intermediate effects at 20 minutes. Diuresis came later. These results are consistent with a primary action of the drug on binding of alkali cations within the cell.

Muscle- and Nuclear-binding Serum Globulin in Patients with Myasthenia Gravis, Muscular Dystrophy, and Connective Tissue Diseases. T. NAMBA, T. SATO, S. J. MYERS, T. NAKAMURA, AND D. GROB,* Brooklyn, N. Y.

Serum of myasthenic patients, or rabbits immunized with muscle ribonucleoprotein prepared by precipitation with *d*-tubocurarine, contains 7 S globulin that combines with A bands of normal human or animal skeletal or cardiac muscle, with cytoplasm of thymic epithelial cells, and with nuclei of muscle, thymic, and buccal mucosal cells, as demonstrated by immunofluorescent staining. Staining of A bands of skeletal muscle by serum in dilution of 1:90 occurred in 62% of myasthenic patients, 17% of patients with other muscle diseases, including muscular and myotonic dystrophy, 4% of patients with connective tissue diseases, and in no normal subjects. Serum also stained films of *d*-tubocurarine binding ribonucleoprotein strongly and films of muscle extract weakly. Prior incubation of serum of myasthenic patients or immunized rabbits with *d*-tubocurarine binding ribonucleoprotein from normal human or animal muscle, or pretreatment of tissue sections with ribonuclease, markedly reduced both cytoplasmic and nuclear binding of serum globulin, whereas desoxyribonuclease had no effect. Binding of globulin to muscle A bands *in vivo* was demonstrated by intramuscular or intra-

arterial injection of serum of myasthenic patients or immunized rabbits into rat tibialis anterior, followed by immunofluorescent staining of excised muscle. Binding of globulin *in vivo* was also demonstrated, without addition of serum, in muscle removed from myasthenic patients. Despite evidence of binding to muscle, intra-arterial injection into rats of serum of myasthenic patients or immunized rabbits had no effect on action potentials or tension evoked by nerve stimulation. Therefore, while serum of most myasthenic patients, and of some patients with muscular or myotonic dystrophy or connective tissue disease, contains 7 S globulin which binds to cytoplasmic and nuclear ribonucleoprotein in muscle and other tissues, and which may be an autoantibody to this ribonucleoprotein, no effect of such binding on muscle function could be demonstrated in acute experiments.

The Effect of Renal Insufficiency on the Excretion of Tritiated Digoxin. WIL B. NELP AND PHILLIP M. BLOOM, Seattle, Wash. (introduced by Robert A. Bruce †).

The effect of renal insufficiency on the route of excretion and the rate of biological degradation of digoxin was studied in 12 subjects for 1 week after intravenous injection of 1 mg of tritiated digoxin (four normals, four patients with creatinine clearance (C_{Cr}) 50 to 69 ml per minute, and four patients with C_{Cr} 8 ml per minute). During the first 30 minutes, digoxin was rapidly removed from the plasma in all subjects, suggesting that uremia did not influence immediate tissue distribution of digoxin. After 24 hours the $t_{\frac{1}{2}}$ of plasma radioactivity was twice normal in severe renal disease. Normals excreted 70% of the dose in the urine (range, 61 to 80%) and 18% in the stool (range, 15 to 19%). In contrast, patients with severe renal insufficiency excreted only 15% of the dose in the urine and 6 to 36% in the stool. Patients with intermediate reduction of C_{Cr} excreted 41 to 53% of the dose in the urine with a mean total excretion of 70%. The rate of urinary excretion of tritiated digoxin correlated closely with the degree of renal impairment, and in each subject the C_{Cr} and clearance of digoxin was essentially the same. In renal disease the gastrointestinal excretion of digoxin was not significantly increased. An exception to this occurred in one patient where the appearance of diarrhea was associated with excretion of 68% of the dose in the stool. With renal insufficiency, the biological $t_{\frac{1}{2}}$ of digoxin was 4 to 7 times longer than the normal $t_{\frac{1}{2}}$ of 64 hours. The rate of removal of digoxin from plasma and whole blood by the Kiil hemodialyzer was determined under conditions used for clinical hemodialysis. Digoxin was poorly dialyzed with a clearance of only 10 ml per minute. It is concluded that digoxin excretion is reduced in direct relation to renal function and that in the absence of diarrhea the gut does not provide a significant alternative pathway of excretion. Analysis of excretion patterns suggests that in patients with severe renal insufficiency the digitalizing dose of digoxin could be reduced approximately one-third and that maintenance doses might be reduced by two-thirds or more.

***In Vivo* Studies on the Mechanism of Action of ACTH.** R. L. NEY, W. W. DAVIS, AND L. D. GARREN, Bethesda, Md. (introduced by J. E. Rall *).

The following experiments show that protein synthesis in the adrenal is required for the early steroidogenic action of ACTH. Two mU of ACTH was injected intravenously into hypophysectomized rats, and corticosterone was measured in adrenal venous blood obtained 7 to 10 minutes later. Inhibitors of protein synthesis (puromycin or cycloheximide) injected from 2 to 40 minutes before ACTH suppressed the steroidogenic response. The degree of inhibition of adrenal protein synthesis caused by these agents (determined by incorporation of C^{14}-leucine into adrenal protein) correlated with the extent of inhibition of steroidogenesis. The adrenal content of corticosterone did not increase in puromycin- or cycloheximide-treated animals. Therefore, decreased steroid secretion resulted from inhibition of synthesis rather than inhibition of release. When actinomycin D was given 2 hours before ACTH, the steroidogenic response to ACTH remained normal. RNA synthesis, however, was blocked, as reflected in complete inhibition or incorporation of C^{14}-uridine into adrenal RNA. The immediate actions of ACTH, therefore, appeared to be dependent on synthesis of protein regulator(s) of steroidogenesis, utilizing preformed stable messenger RNA. Indirect evidence for rapid turnover of ACTH-stimulated protein regulator(s) of steroidogenesis was obtained from the following experiment. Intravenous ACTH (50 mU) increased corticosterone secretion, reaching a maximum in 10 minutes and maintaining this for over 60 minutes. Inhibition of protein synthesis with cycloheximide during maximal steroidogenesis caused corticosterone synthesis to return to base line with a half-time of approximately 5 minutes. When ACTH was given for longer periods, there was an increase in total RNA and protein synthesis. Thus, while the immediate effect of ACTH seemed to activate protein synthesis at the level of translation of preformed messenger-RNA, the long term effects of ACTH appeared to involve DNA-directed synthesis of RNA and protein.

Heparin Osteoporosis. GEORGE NICHOLS, JR.,* GEORGE C. GRIFFITH, AND JOHN D. ASHER, Boston, Mass., and Los Angeles, Calif.

Treatment of 117 men with up to 100 mg of heparin daily for 2 to 16 years in the prophylaxis of arteriosclerotic and thromboembolic disease was without apparent skeletal complications. When dosage was raised to 2 to 300 mg per day, 5 of 9 men so treated developed marked osteoporosis by X ray and multiple vertebral compression fractures within 2 years. Serum Ca, P, and acid and alkaline phosphatase were normal in all patients affected, as were rates of Ca, P, hydroxyproline excretion, and renal tubular resorption of P when tested.

Parathyroid response to infusions of Ca and EDTA was examined in one patient and found to be normal. Thus disturbance of parathyroid function seemed unlikely as a primary cause of the process and attention focused on bone. Since *in vitro* measurements of bone matrix biosynthesis, O_2 uptake, and lactate production were normal or very slightly elevated in this patient, and no effect on these variables was noted in rat bone after 5 daily injections of heparin, a stimulatory effect of heparin on bone resorptive mechanisms has been postulated. Evidence from rats that supports this view has recently been reported from this laboratory. Increased bone-cell lysosome collagenase activity and decreased lysosome stability after prolonged heparin treatment were demonstrated—effects apparently related to a direct influence of heparin on lysosome membranes. Although final proof that this mechanism is the cause of the osteoporosis seen in these patients remains to be obtained, the experience cited above suggests that heparin is able to cause osteoporosis in man and therefore that this disease must be included among the potential hazards of prolonged anticoagulation therapy with this agent.

Alterations in Erythrocyte Phospholipids Produced by Environmental Change. WILLIAM H. R. NYE AND GUIDO V. MARINETTI, Rochester, N. Y. (introduced by Christine Waterhouse †).

The erythrocyte membrane is known to vary, among mammalian species, in its phospholipid and fatty acid composition, and to differ in permeability in correlation with these two chemical characteristics. Within a species, phospholipid percentage composition remains remarkably constant, whereas fatty acid composition can be varied by dietary manipulation. Moreover, in the human, studies of the red cell phospholipid percentage composition in various hematologic and malabsorptive disorders has revealed no confirmed abnormality, with the sole exception of that found in the rare congenital syndrome of a-β-lipoproteinemia. It appears, on the basis of currently available data, that phospholipid percentage composition is genetically determined. To test this hypothesis, red cell phospholipids were determined by silicic acid chromatography in seven subjects with jaundice and in one subject with hyperlipemia. Plasma phospholipid percentage composition was abnormal (lecithin per cent, high; sphingomyelin and lysolecithin per cent, low) in all eight subjects, although total lipid phosphorus was normal in four of the subjects with jaundice. In each of the eight subjects, red cell phospholipid percentage composition was also abnormal, the lecithin per cent increasing to as high as 48.7% (upper limits of normal, 34%). Normal subjects, subjects with malabsorption, and patients with jaundice or hyperlipemia who had normal plasma phospholipid percentage compositions were used as controls. Fatty acid composition, determined by gas-liquid chromatography, in five subjects with abnormal red cell phospholipids, showed no striking departure from normal. The phospholipid percentage composition of the erythrocyte membrane has been shown to be susceptible to changes in the environment of the erythrocyte that do not necessarily produce changes in its fatty acid composition. Dissociation of the influence of phospholipid percentage composition from that of fatty acid composition on properties of the erythrocyte membrane should now be possible.

Estimation of the Secretion Rate of TSH in Man. WILLIAM D. ODELL, ROBERT D. UTIGER, AND JOHN F. WILBER, Bethesda, Md. (introduced by Mortimer B. Lipsett *).

Relatively little information is available on the secretion rates of pituitary hormones in man. We have used low specific activity human I^{131}-TSH and a radioimmunoassay for human TSH as tools to estimate the secretion rate of TSH in man. Either 0.5 or 5.0 μg of I^{131}-TSH with specific activities of 1 to 50 μc per μg was administered intravenously to six euthyroid subjects. Serial determinations of plasma TCA precipitable I^{131} were made, and from the linear portion of the semilog disappearance curve (10 to 90 minutes), half time of disappearance ($t_{\frac{1}{2}}$) and pool size were estimated. The $t_{\frac{1}{2}}$ averaged 47 minutes (95% limits = 43 to 52) and pool size 5.5% (4.2 to 6.8) of body weight. Similar values for $t_{\frac{1}{2}}$ were found for endogenous TSH in two patients by first elevating levels with methimazole and then suppressing these levels with iv thyroxine. TSH levels were measured in serum extracts from 11 euthyroid patients, and these averaged 1.8 mμg per ml (1.3 to 2.3). The metabolic clearance rate was estimated to be 49 ml per minute (38 to 77) and pool size 6.9 μg (3.8 to 10.9) for a 70-kg man. The secretory rate was 88 mμg per minute (55 to 120) or 126 μg per 24 hours. It is estimated that the entire pituitary TSH content turns over each day.

Submicroscopic Features of the Degranulation of the Human Mast Cell in Localized Mastocytosis. GEORGE F. ODLAND AND FRANK PARKER, Seattle, Wash. (introduced by Cyrus Rubin *).

The localized cutaneous form of mastocytosis (urticaria pigmentosa) provides a unique source of material for the purpose of studying the submicroscopic structure of human mast cell granules and for observing successive stages of degranulation of human mast cells induced by the mechanical stimulus of rubbing the intact lesion. Skin biopsies from three children with urticaria pigmentosa provided samples of intact mast cells (control) and mast cell populations taken arbitrarily at intervals of 30 seconds, 1 minute, and 3 minutes after mechanical stimulus. Two patients showed only local urtication after rubbing of lesions. The third case showed irritability and diffuse flushing of the upper half of the body after rubbing of a large single lesion. High resolution electron microscopic analysis of the mast cell granules in the controls has revealed two components: *1*) a fine granular component of moderate density comprising the major portion of the mast cell granule and *2*) a charac-

teristic rolled lamellar component with a substructure consisting of alternate bands of density with a uniform periodicity of 60 A. Whereas these structures cannot at present be specifically related to known chemical substances, the two component system provides a structural label for identification of extracellular mast cell granules. In the process of degranulation, displacement of granules to the cell periphery is followed by enclosure of peripherally disposed granules either by intracellular membranes or by infoldings of the cell membrane with subsequent release of intact granules into the edematous connective tissue stroma. Degranulated mast cells can be distinguished from other connective tissue cells by distinctive intracellular filaments and surface membrane contours. In the one symptomatic patient, but not in the asymptomatic patients, projections of mast cell cytoplasm as well as released granules can be demonstrated in gaps between adjacent endothelial cells lining vascular channels.

Study of Potassium Absorption at Various Sites of Single Rat Nephrons Using Glass Electrodes *In Situ*. DONALD E. OKEN, Boston, Mass. (introduced by Kendall Emerson, Jr.†).

Although a tendency to progressive lowering of the tubular fluid:plasma potassium concentration ratio (TF/P) was suggested in earlier experiments, wide spread of data obviated any statistical correlation between TF/P potassium and puncture site. In the present experiments, two or more measurements of potassium concentration *in situ* were attempted in individual proximal tubules of rat kidney. Prospective puncture sites were identified by repeatedly injecting small, stained oil droplets and marking their place of reappearance at more distal sites in the proximal tubule. Potassium was measured using Corning KAS 20-5 glass electrodes with a tip diameter of 10 to 15 μ and externally insulated with Q-dope. Micropipettes with 0.5- to 1-μ tip diameter filled with 3 M KCl served as junctions for Ag: AgCl reference electrodes. Puncture was performed first at the most distal of the segments with a reference electrode "downstream" from the glass electrode. Puncture sites were identified by latex injection and microdissection. Forty-eight satisfactory measurements were made in 31 tubules of 15 rats on a standard diet. Single values were obtained in 17 tubules, two values in 11 tubules, and three values in 3 tubules. Plasma potassium concentration ratio of these animals was 4.4 ± 0.1 (SE) mM, proximal tubular fluid potassium concentration of all samples being 3.0 ± 0.1 mM. No TF/P value greater than 0.96 was observed. Mean potassium TF/P was 0.69 $\pm$ 0.02 (SE), a value statistically different from 1.0 (p 0.001). Correlation between puncture site and all values for TF/P potassium did not obtain (p 0.1). When, however, values from more than one segment of each proximal tubule were treated as paired data, potassium concentration was statistically lower at sites more distant from the glomerulus (p 0.001).

Effect of Actinomycin D upon Vitamin K-induced Prothrombin Formation in Dicumarolized Rats. ROBERT E. OLSON,* Pittsburgh, Pa.

It has been shown that actinomycin D inhibits vitamin K-induced prothrombin formation in chicks deficient in vitamin K. Doses of actinomycin that inhibited the normal response to vitamin K inhibited incorporation of adenine-8-C^{14} into hepatic RNA. These results were consistent with the hypothesis that vitamin K acts by stimulating messenger RNA formation for the synthesis of clotting proteins. Since the coumarin drugs antagonize vitamin K, it seemed possible that the site of antagonism was a molecule that regulates this expression of genetic information. To test this hypothesis, rats 200 to 300 g in weight were fed a diet containing 0.05% Dicumarol for 48 hours, at which time they showed prolonged clotting and one-stage prothrombin times. This clotting defect could be corrected in 6 hours by the administration of 1 mg of vitamin K_1 to each rat by mouth. The administration of actinomycin D in doses from 30 to 240 μg per 100 g body weight 4 hours before the vitamin K_1 modified the action of the vitamin. Actinomycin in doses above 120 μg per 100 g body weight completely abolished the procoagulation effect of vitamin K_1. Below this level, the response was dose dependent. If actinomycin was given after the vitamin, or given to normal rats, no anticoagulant effect was noted. These data are consistent with the view that the site of antagonism between vitamin K and the coumarin drugs is a molecule that controls DNA-dependent RNA replication. In view of the recent report of hereditary coumarin drug resistance in human subjects that have a normal sensitivity to vitamin K and a normal drug metabolism, it seems probable that the repressor molecule has multiple sites for anti- and procoagulants.

Effect of Nonthyroidal Disease and Surgical Trauma on the Turnover of I^{131}-labeled Thyroxine-binding Prealbumin (TBPA). JACK H. OPPENHEIMER, GERALD BERNSTEIN, J. CRISPIN SMITH, AND MARTIN I. SURKS, New York, N. Y. (introduced by Louis Leiter †).

Previous studies have indicated that lowered concentrations of serum TBPA in patients with nonthyroidal disease may frequently lead to elevated levels of free thyroxine. Moreover, a rapid fall in circulating TBPA after surgical procedures is accompanied by a rise in the free thyroxine concentration. In order to elucidate the over-all mechanisms responsible for the reduction in serum TBPA, the turnover of I^{131}-TBPA was studied in normal subjects, patients with nonthyroidal illness, and in a patient undergoing exploratory thoracotomy. TBPA was isolated from plasma by column electrophoresis as previously described and iodinated with I^{131}. Data were analyzed on the basis of a two-compartmental model. With the exception of the surgical patient, all individuals had no significant changes of serum TBPA during the study and were assumed to be in a steady state. Mean values determined in four healthy male subjects were as

follows: TBPA serum concentration, 33.7 mg per 100 ml; total body pool, 2.04 g; distribution between vascular and extravascular compartments, 49.5: 50.5; fractional degradation of total body pool, 0.363 per day with a corresponding calculated half-life of 1.90 days; turnover, 9.74 mg per kg per day. Among five patients with nonthyroidal disease studied, diminished synthesis or increased fractional degradation, or both, were found. Reduced synthesis appeared to be the predominant factor in lowering serum TBPA in those patients with the most marked depression in TBPA concentration. In the patient undergoing surgery, estimates of simultaneous synthesis and degradation were made on the basis of the serum specific activity of TBPA and the urinary excretion of I^{131}. A sudden diminution of synthesis of TBPA coupled with the short half-life of this protein appears to account for the rapid postoperative fall in serum TBPA levels.

Nature of the Hepatic Pigment in Schistosomiasis. J. Donald Ostrow and Kenneth S. Warren, Cleveland, Ohio (introduced by Austin S. Weisberger †).

Subjects with schistosomiasis accumulate masses of black pigment in the hepatic Kupffer cells. It is disputed whether this pigment is a hemoglobin derivative or is melanin. To study this problem, pigment was isolated from the livers of mice infected 12 weeks earlier with *S. mansonii cercariae*. The livers were perfused free of blood and homogenized in 0.25 M sucrose using a motor-driven Teflon pestle. The black pigment sedimented completely with the nuclear fraction at $400 \times g$ but remained in suspension when connective tissue was sedimented at $50 \times g$. Further homogenization in water using a ground-glass tissue grinder yielded a more polydisperse brown pigment that still sedimented primarily at 100 to $400 \times g$. This separated pigment was intensely benzidine-positive but Prussian-blue–negative. Treatment with 1.0 N NaOH yielded a turbid yellow solution. The yellow compound, extracted into butanol, exhibited the characteristic absorption spectrum of alkaline hematin, with strong maxima at 402 and 590 mμ. Treatment with 1.0 N HCl produced a reddish suspension. The red compound, extracted into butanol, demonstrated the typical absorption spectrum of acid hematin, with sharp peaks at 399 and 632 mμ. In aqueous or butanol phases, the red and yellow colors were reversibly interconvertible upon addition of strong acid or alkali, respectively. Brown pigment particles reformed in aqueous solution at pH 4 to 5. Pigment flocculated by acetone, ethanol, or methanol dissolved in pyridine to yield a red-purple solution with strong absorption maxima at 417, 521, and 553 mμ, characteristic of pyridine hemochromogen. Black particles, milked from the alimentary tract of female worms flushed from the portal vein of infected mice, behaved identically with hepatic pigment. These preliminary data indicate that the hepatic pigment is a large-particulate, iron-containing derivative of hemoglobin, probably formed from digestion of portal venous erythrocytes in the alimentary tract of the infecting adult worms.

Quantitative Immunochemical Determination of Angiotensin. Lot Page, Edgar Haber, and Saima Lagg, Boston, Mass. (introduced by K. Frank Austen *).

Two-branch chain copolymers of poly-L-lysine and asparagine[1], valine[5], angiotensin II were synthesized. In one compound, the amino terminal end of angiotensin was coupled to poly-L-lysine via *m*-xylylene diisocyanate. In the other compound, the carboxyl terminal end of angiotensin was coupled to epsilon amino groups of poly-L-lysine by a carbodiimide condensation. Both polymers elicited production in rabbits of antibody specific for angiotensin. Binding of isotopically labeled angiotensin to antibody was demonstrated by gel filtration. I^{125}-angiotensin was incubated with serum from immunized rabbits in Tris acetate buffer and chromatographed on columns of Sephadex G-25. Counts in the first peak (void volume) represent I^{125}-angiotensin bound to antibody. Counts in the second peak represent unbound I^{125}-angiotensin. When an excess of antiserum was incubated with I^{125}-angiotensin, virtually complete binding was demonstrated. Specific displacement of I^{125}-angiotensin was demonstrated by adding unlabeled angiotensin to reaction mixtures containing a constant quantity of labeled angiotensin. In sufficient excess, virtually complete displacement was effected. Reproducible displacement produced by adding small increments of unlabeled angiotensin forms the basis of quantitative immunoassay. In buffer, as little as 0.2 mμg was detected. Unlabeled angiotensin added to pooled human serum could be quantitatively determined at a concentration as low as 1 mμg per ml. When sera from nonimmunized rabbits were used, no counts appeared in the void volume, indicating the absence of nonspecific binding of labeled angiotensin. The high specificity of the method is supported by the finding that fiftyfold excesses of vasopressin or bradykinin and a 10^5 excess of peptic digest of lysozyme failed to produce any displacement of I^{125}-angiotensin. The method is being applied to arterial plasma samples obtained from patients with renal vascular disease, hypertension, and edematous states.

Left Ventricular Contractility in Man Assessed in Terms of Indexes of Force and Velocity. H. W. Paley and Ian G. McDonald, San Francisco, Calif. (introduced by Meyer Friedman †).

Experiments by others have demonstrated the assessment of contractility of afterloaded isolated papillary muscle in terms of its force-velocity relationships. In this study of the human left ventricle (LV), the force-velocity principle was applied by calculation of an index of rate of myocardial shortening which, when related to ventricular load during ejection, more clearly detected the positive inotropic effect of isoproterenol than did other available indexes. Cardiac output (indicator dilution), LV volume (thermodilution), and LV ejection time (LVET, carotid pulse) were measured in supine and 60° head-up tilted positions, under control conditions (C) and during isoproterenol infusion (I) (1.5 to 2.5 μg per minute) in five

normal male volunteers. Assuming a spherical LV, mean rate of circumferential shortening (MRCS, centimeters per second) was derived as the difference between end-diastolic and end-systolic circumferences divided by LVET. This assumption was shown reasonable by analysis of linear surface shortening in LV models with different volumes, shapes, and modes of contraction. Load on the LV circumference during ejection was expressed as circumferential force (tension $\times$ circumference, $Pr/2 \times 2\pi r$). Force at onset of ejection (F_{oe}) exceeded force at end of ejection (F_{ee}) by 10%. Mean force during ejection (F_m) was calculated as $(F_{oe} + F_{ee})/2$. MRCS was markedly increased by isoproterenol (supine: C, 13.0, to I, 21.5, $p < 0.01$; tilted: C, 13.4, to I, 19.4, $p < 0.01$). This effect could not be accounted for by changes in F_m, left ventricular end diastolic volume (LVEDV), or increase in heart rate; atropine-induced tachycardia did not produce significant changes in MRCS. Over the ranges observed, MRCS was only weakly related to F_m and LVEDV, suggesting opposing effects of these factors on MRCS. The force-MRCS relationship is considered analogous to the force-velocity relationship of isolated papillary muscle and has been shown to be a sensitive index of contractility in the human left ventricle.

Lithocholic Acid-induced Choledocholithiasis in Rats. Robert H. Palmer, Chicago, Ill. (introduced by Henry T. Ricketts †).

Lithocholic acid is a potent endogenous pyrogenic and inflammatory steroid in man; it also induces inflammation and bile duct hyperplasia in several species. Young Sprague-Dawley rats fed an 8% protein, 1% lithocholic acid diet for 16 weeks developed a 100% incidence of choledocholithiasis, starting within 2 weeks. Common ducts were dilated, thickened, and contained single or multiple, round or faceted, multicolored stones (dry weights: males, 5.79 ± 0.77 g, females, 4.14 ± 1.75 g), consisting mainly of free or glycine conjugated calcium bile salts. Lithocholic and 6β-hydroxylithocholic acids predominated, with some hyodeoxycholic and chenodeoxycholic acids. Traces of pigments, cholesterol, and other lipids, proteins, and ash were present. The remarkable absence of taurine conjugates prompted study of the effects of higher (27%) protein or 1% taurine-supplemented diets on gallstone formation. At 8 weeks, all low protein, lithocholic acid fed rats had developed large stones; there were rare small stones in the 27% protein group and no stones in the taurine supplemented group. Bile acid taurine and glycine conjugation was investigated with lithocholic acid-24-C^{14}. The per cent of labeled intestinal bile acids conjugated with taurine was: low protein group (with or without added lithocholic acid), 10%; 27% protein group, 50%; taurine-supplemented group, 62%. Urinary excretion of labeled compounds was similar with all diets. In keeping with greater stone formation in males, females excreted significantly more radioactivity in the urine. This study describes another toxic biological effect of the endogenous steroid lithocholic acid and offers a new method for studying experimental gallstone formation. In addition, the important modifying role of specific amino acid conjugation on the biological actions of bile acids, as in the disparate pyrogenicity of tauro- and glycolithocholic acids, is again emphasized.

Does the Bladder Possess an Intrinsic Antibacterial Defense Mechanism? Albert J. Paquin, Jr., Jose Perez, Calvin M. Kunin,* and Eugene A. Foster, Charlottesville, Va.

It is postulated that the bladder is normally sterile by virtue of its ability to empty mechanically and because of antibacterial mechanisms inherent in urine and the bladder wall itself. This report summarizes studies utilizing a model in which mechanical emptying was eliminated so that the contribution of the urine and the bladder wall to the defense mechanism could be assessed separately. In 41 adult male rabbits the bladder was emptied and then isolated by ligating both ureters and urethra. Ten ml of sterile urine obtained from each rabbit was inoculated with 10^1 to 10^8 *E. coli* 018 per ml and divided into two equal portions. One part was reinstilled into each animal's own bladder and one part placed into a sealed sterile glass vial secured in the same animal's peritoneal cavity. Neither bladder nor vial could fill or empty, and both were maintained at the same temperature. The animals were killed at 6 or 24 hours, and the urine removed from bladder and vial was quantitatively cultured. No significant differences were noted between bacterial growth within the bladder and vial in the 41 animals. Bacteria grew equally well in both containers in 25, did not grow in either in 14, and differed slightly in only 2. When there was growth, rates were similar in bladder and vial. Failure of growth was usually associated with urine specific gravity over 1.021, but not with pH. The antibacterial activity of urine of high specific gravity was confirmed *in vitro* in urine from a series of animals studied both under conditions of diuresis and water deprivation. No intrinsic defense mechanism in the bladder wall capable of acting on *E. coli* in its lumen was disclosed by this study. Further study of bladder and urine defense mechanisms against other organisms and in the immunized host is in progress.

Effect of Exogenous Cholesterol on Cholesterol Biosynthesis in Familial Hypercholesteremia. David F. Pawliger and Joseph C. Shipp, Gainesville, Fla. (introduced by H. E. Kaufman *).

Exogenous cholesterol suppresses cholesterol synthesis in liver by an effect on the conversion of β-hydroxy-β-methylgutaryl CoA (HMG CoA) to mevalonic acid, a reaction dependent on HMG CoA reductase (Siperstein). A possible explanation for familial hypercholesteremia (FH) is a defect in this negative feedback mechanism due to absence of or altered activity of HMG CoA reductase. This postulated defect might be shown by a failure of exogenous cholesterol to suppress hepatic synthesis of cholesterol. This study was designed to test the effect of

cholesterol feeding on cholesterol synthesis *in vivo*, and in liver tissue *in vitro*, in patients (no. = 4) with FH (serum cholesterol over 400 mg per 100 ml, vascular disease, 3 kindred affected, nondiabetic). Acetate-1-C^{14} incorporation into cholesterol in liver biopsy specimens and the time course of appearance of label in serum lipids after acetate-1-C^{14} (200 uc iv) were determined before and 5 days after cholesterol feeding (4 g per day for 5 days). Lipids in liver and serum were isolated (Folch) and separated with thin layer silicic acid chromatography, and radioactivity was determined. After cholesterol feeding incorporation of acetate-1-C^{14} into cholesterol by liver from patients with FH was reduced by over two-thirds, an effect similar to that observed in controls. Time course of appearance of label in serum cholesterol, and triglycerides and phospholipids, after acetate-1-C^{14} was similar before and after cholesterol feeding in patients with FH. The results showed that HMG CoA reductase activity in liver of patients with FH was suppressed after exogenous cholesterol feeding. Furthermore, in these patients with FH, as has been shown in normals by Taylor, the failure of exogenous cholesterol to alter the pattern of cholesterol synthesis *in vivo* suggested that nonhepatic tissues may contribute significantly to cholesterol biosynthesis.

Reticuloendothelial Phagocytic Function in Patients with Hypersplenism and Related Disorders. PASQUALE E. PERILLIE, GEORGE S. GROCH, ROBERT S. BRIGGS, AND STUART C. FINCH,* New Haven, Conn.

In this study reticuloendothelial phagocytic function was evaluated in patients with reduced concentrations of the formed elements of the blood. These disorders were believed mostly due to accelerated destruction of the blood elements. The removal rate of I^{131} aggregated human serum albumin from the blood and its differential localization in the liver and spleen was related to the type and severity of the hematologic abnormality. Patients and their controls were injected intravenously with 5 mg per kg of aggregated human serum albumin labeled with about 20 μc of I^{131}. The slope of the blood disappearance curve was determined from radioassay of multiple blood samples obtained during the 15-minute postinjection period. Radioactivity over the spleen and liver was measured for periods up to several hours. Patients with acquired hemolytic anemia showed accelerated blood clearance and increased splenic uptake of the aggregated albumin. Patients with cirrhosis of the liver and associated splenomegaly also demonstrated a reduced liver:spleen uptake ratio, but the clearance of aggregated albumin from the blood usually was very slow. Blood clearance and organ uptake of the aggregated albumin were normal in most patients with idiopathic thrombocytopenic purpura both before and after splenectomy. Normal patterns were observed in patients with pancytopenia due to primary or secondary marrow depression. These data indicate that acquired hemolytic anemia is associated with a nonspecific increase in reticuloendothelial phagocytic activity. Much of this increased phagocytic function occurs in the spleen; advanced chronic liver disease results in reduced liver blood flow with diminution in total reticuloendothelial phagocytic function. The low liver: spleen radioactivity uptake ratio probably is due to reduced hepatic uptake of albumin rather than increased splenic localization. The results suggest that the I^{131} aggregated albumin clearance test may be useful in the clinical evaluation of patients with hemolytic anemias and various forms of hypersplenism.

Evidence for a Human Fetal Myohemoglobin. GERALD T. PERKOFF,* St. Louis, Mo.

Because of conflicting evidence concerning a fetal myoglobin (MbF) in fetal muscle and in some patients with muscle disease, we have *a*) repeated the original experiments by which Singer and others demonstrated human MbF, *b*) studied 30 individual fetuses of known gestation age under several conditions, and *c*) partially characterized fetal muscle heme protein (FMHP) of extracts essentially free of MbA. Our results confirm the existence of FMHP and suggest a molecular size larger than MbA. *1*) FMHP, prepared as the met-heme protein by two different methods, had a faster mobility on starch gels (pH 8.6) than MbA or HbF. As cyan-met proteins MbA, HbF, and FMHP had almost identical electrophoretic mobility. *2*) Neither chromatography nor electrophoresis of the met-heme proteins of individual fetuses revealed significant amounts of MbA until term or after. Immunodiffusion of individual fetal samples vs. anti-MbA showed minute quantities of MbA in some but not all samples. *3*) Most preparations of FMHP contain 5 to 17% HbF. Analytical ultracentrifugation of one such contaminated preparation showed a single peak with an $S_{20,w}$ of 4.22; diffusion constant 6.15; calculated molecular weight 66,250. Spectra were similar to HbF. FMHP had a smaller elution volume on Sephadex G-100 than MbA and was less soluble in ammonium sulfate. Previous confusion concerning FMHP may result from its closer relationship to hemoglobin than Mb and from isolation by methods that rigorously exclude hemoglobins. Also, studies of cyan-met proteins might not show FMHP because of its similar mobility to MbA and HbF in this form. Although FMHP need not be identical to the rapidly migrating heme proteins reported previously in some patients with muscle disease, its properties may help to clarify earlier variable findings in some myoglobinurics and childhood dystrophics. The present data suggest that fetal myohemoglobin may be a preferable term to MbF.

Acute Intermittent Porphyria: The First "Overproduction Disease" Localized to a Specific Enzyme. MARK PERLROTH, DONALD TSCHUDY, HARVEY MARVER, ANNIE COLLINS, GEORGE HUNTER, JR., AND M. RECHCIGL, JR., Bethesda, Md. (introduced by Gordon Zubrod †).

Acute intermittent porphyria (AIP), an inborn error of metabolism, is characterized by excessive urinary ex-

cretion of porphyrin precursors [δ-aminolevulinic acid (ALA) and porphobilinogen (PBG)] and by neurologic dysfunction. The chemical manifestations of AIP can be reproduced in animals by administration of allylisopropylacetamide. This experimental porphyria results from induction in liver of δ-aminolevulinic acid synthetase (ALAS), the rate-controlling enzyme of porphyrin synthesis, and is not associated with any evidence of defective heme production. The induction of ALAS can be inhibited by carbohydrate administration, a phenomenon previously described with certain other inducible enzymes ("the glucose effect"). It accounts for the observation that the ability to provoke experimental porphyria is reciprocally related to the intake of carbohydrate in the diet. The fact that similar dietary changes applied to the human genetically determined disease produced a parallel effect on porphyrin precursor excretion suggested that hepatic ALAS was induced in these patients and that the induction was subject to the "glucose effect." With a new method for the measurement of ALAS, the level of this enzyme in a post-mortem specimen of liver from a porphyric female was compared to levels in post-mortem and surgically obtained hepatic biopsies from nonporphyric individuals. The patient's value exceeded normal sevenfold. This is thought to be the first example of a disease whose biochemical manifestations can be directly attributed to an increase in a specific enzyme. Two explanations are compatible with these facts and the dominant mode of inheritance of AIP: *1*) There might be a constitutive operator mutation for ALAS as suggested by Watson; this requires that neurologic manifestations result from overproduction of ALA and PBG. *2*) Lacking this etiologic relationship, AIP might be explained by a defective structural gene lying outside the porphyrin biosynthetic pathway that leads to induction of ALAS and predisposes the patient to clinical attacks of the disease.

Studies on the Mechanism of Hemolysis in a Sheep Red Blood Cell-Guinea Pig Serum-Cobra Venom System. GERALD B. PHILLIPS,* New York, N. Y.

Cobra venom (*Naja naja*), which did not hemolyze washed sheep red blood cells, did hemolyze these cells when guinea pig serum was included in the system. When the guinea pig serum was heated at 56° for 30 minutes before addition, however, no hemolysis occurred. Lipid analysis indicated that the hemolysis was not on the basis of lysolecithin formation. Furthermore, the venom and serum after incubation together were not hemolytic when heated at 56° for 30 minutes. Venom heated in neutral solution at 100° C for 15 minutes was almost inactive even though it still showed strong phospholipase A activity on lecithin. Moreover, Russell viper venom (*Vipera russelli*), which has potent phospholipase A activity, was ineffective in this system. The cobra venom activity could be washed off the red blood cells and was only slightly dialyzable. Heparin inhibited the hemolysis but appeared to have little, if any, effect on the hydrolysis of lecithin catalyzed by the phospholipase A of the cobra or Russell viper venom. Heparin may have produced this inhibition by inactivating a component(s) of the complement system, as it also inhibited immune hemolysis, apparently in reaction with the guinea pig serum, in the system of sheep red blood cells, antisheep red blood cell antiserum, and guinea pig serum; heparin, however, also reacts with a venom component(s), as it inhibited hemolysis of washed human red blood cells by the cobra venom alone. These results suggest that the cobra venom contains a factor(s) which activates the guinea pig complement system to cause lysis of sheep red blood cells. This factor does not appear to be phospholipase A. The possibility arises that this factor is an enzyme similar to one activated by the antigen-antibody-complement system.

Adipokinetic Action of Amphetamine. E. J. PINTER AND C. J. PATTEE, Montreal, Canada (introduced by J. S. L. Browne †).

Amphetamine and its congeners are being used and abused by several millions of people every day. These sympathomimetic amines bear a structural resemblance to adrenaline. This suggested the possibility that amphetamine had adipokinetic properties similar to those of adrenaline. If so, such nonhemodynamic effects as increased coagulability of the blood, accumulation of triglyceride in the circulation and in the liver, depletion of adipose tissue, and impairment of carbohydrate utilization may be added to the potential hazards of the appetite-suppressant amines. With the exception of a recent account on the *in vitro* adipokinetic action of hydroxyamphetamine on the adipose tissue of hamsters by Rudman and co-workers, no reference to any investigation of amphetamine-induced fat mobilization in man has been found. The effect of amphetamine and some of its analogues on fat mobilization was studied in 12 subjects of both sexes. Amphetamine was administered both intravenously (0.2 mg per kg for 15 minutes) and orally; the other appetite suppressants were given orally at the recommended single oral doses. The process of adipokinesis was assessed by measuring the serial change in the blood levels of FFA, triglyceride, total fat, and also by repeated injections of tracer fatty acid, after the administration of the compounds. These results were contrasted with the effect of adrenaline infusion (2 μg per minute for 15 minutes) in the same subjects. In a limited number of experiments modification of the adipokinetic response was attempted with dibenzyline, dihydroergotamine, reserpine pretreatment, and glucose infusion. It was shown that amphetamine and some of its congeners exhibit an adipokinetic action. In comparison with adrenaline there were differences, however, both in peak response and in the slope of the FFA curves.

Erythropoietic Response to Minor Hemorrhage in Humans. LOUIS F. PLZAK, JR., AND FRANCIS D. MOORE,† Boston, Mass.

Acute, massive hemorrhage has been noted to produce elevated serum and urinary erythropoietin titers in hu-

mans. Erythropoietin has not been satisfactorily demonstrated in normal subjects; consequently its role in minor adjustments of the erythron has been questioned. This study was undertaken to note the effect of a single, small phlebotomy on the serum and urinary erythropoietin levels at subsequent intervals. Twenty-one volunteers including one female were subjected to a 400- to 700-ml (8 to 12% blood volume) hemorrhage. Serum erythropoietin levels were obtained at the time of the bleed and at intervals of 4, 8, 12, 24, 48, and 72 hours thereafter in 13 of the subjects; erythropoietin levels were determined on urine collections beginning 24 hours before the hemorrhage and continuing for 3 days afterward on twelve hospitalized subjects. The urine extracts were concentrated proportional to the volume of the sample in relation to the total urinary output for a given 6- to 18-hour collection period. The erythropoietin assays were performed with the radioactive iron uptake method in either polycythemic rats or polycythemic mice. All the samples from a given individual were assayed simultaneously. Three to five rats and four to seven mice comprised each assay group; results were discarded if the animals were not polycythemic at the time of sampling. An elevated serum erythropoietin titer was noted in 7 of 13 volunteers; the rise occurred as early as 4 hours after the hemorrhage and lasted up to 72 hours. Elevated urinary erythropoietin titers were noted in 4 of 11 subjects 24 hours after the phlebotomy. The average hematocrit dropped from 45% to 41%. It is concluded that erythropoietin mediates relatively minor adjustments of the red cell mass.

Epinephrine Inhibition of Insulin Release. D. PORTE, JR., A. GRABER, T. KUZUYA, AND R. H. WILLIAMS,† Seattle, Wash.

The mechanism of the diabetogenic action of epinephrine (E) was investigated using the double antibody immunoassay technique for serum insulin. In five subjects an iv infusion of E (6 μg per minute for 1 hour) elevated venous plasma glucose to a maximum above basal of 180% ± 18% (±95% confidence interval, mean = 169 mg per 100 ml) and plasma free fatty acid 288% ± 79% (mean = 1.67 μEq per ml), but produced no change in venous serum immunoreactive insulin (IRI) (91% ± 8.6% of a control value of 11 μU per ml). Fifteen minutes after infusion IRI rose significantly to 41 μU per ml ($p < .05$). This rebound in IRI accompanied a decrease in plasma sugar and FFA. In contrast a similar iv infusion of glucose (300 mg per minute in 5 subjects for 1 hour) elevated plasma glucose significantly less (149% ± 11.5%, mean 133 mg per 100 ml), but was accompanied by a significant rise in IRI during the infusion (228% ± 58%, mean 18 μU per ml, control mean 8 μU per ml, $p < .05$). Plasma FFA were reduced to a minimum of 56% ± 16% of the control value of 0.69 μEq per ml. All of these parameters returned to basal levels within 90 minutes of the infusion. In studies to define the mechanism of this inhibition, in each of four subjects the IRI rise caused by *1*) glucose (300 mg per minute) *2*) glucagon (5 μg per minute), or *3*) tolbutamide (1 g as a single dose) was partially or completely abolished by the simultaneous infusion of E (6 μg per minute). Blockade of FFA release from adipose tissue with nicotinic acid (100 mg per 15 minutes iv) failed in four other subjects to reverse the inhibitory effects of E on insulin levels. Prolonged continuous infusion of E for periods up to 6 hours was associated with basal levels of IRI throughout, despite continuous hyperglycemia. These studies show that E inhibits all of the common stimuli to insulin secretion and suggests a direct effect of E on pancreatic release of insulin.

The Effects of Hypertonic Mannitol on Renal Sodium Excretion in Hydropenic Man. JEROME G. PORUSH AND RUTH G. ABRAMSON, Brooklyn, N. Y. (introduced by William Dock †).

During the course of hypertonic mannitol diuresis in hydropenic subjects, fractional sodium excretion increased from 0.7 to 2.0% as solute clearance (C_{osm}) increased from 2 to 10 ml per minute. Thereafter, there was a distinct augmentation in the rate of sodium excretion, so that 11% of the filtered load was excreted at a C_{osm} of 30 ml per minute. Under nondiuretic conditions, the administration of mercaptomerin resulted in a mean increase in fractional sodium excretion of 15.6%. When mercaptomerin was superimposed upon a steady state mannitol diuresis between a C_{osm} of 9 and 15 ml per minute, the mean increase in fractional sodium excretion was 14.6%. Thereafter, the administration of mercaptomerin at increasing levels of C_{osm} resulted in a progressive decrease in the natriuretic response, so that the mean increase in fractional sodium excretion was only 5.6% at a C_{osm} between 25 and 30 ml per minute. However, the fraction of filtered sodium excreted at the peak of the mercurial diuresis was similar at all levels of mannitol diuresis and approximated that obtained in the nondiuretic state. In contrast, when acetazolamide was superimposed upon a mannitol diuresis, the natriuretic response was comparable at all levels of C_{osm} between 9 and 30 ml per minute. The apparent inverse relation between the level of sodium excretion obtained with mannitol and the natriuretic response to mercaptomerin may be explained by a common site of action of these agents in the distal tubule. Thus, a progressive decrease in sodium reabsorption at this site during mannitol loading would leave less sodium available to be acted upon by mercaptomerin. These data also suggest that over the range of C_{osm} examined, the distal tubule receives a relatively fixed percentage of the filtered load of sodium during the course of a mannitol diuresis.

Polarographic Characteristics of Albumin, Gamma Globulin, Ceruloplasmin, and Transferrin. ANANDA S. PRASAD AND M. DAVE POULIK, Detroit, Mich. (introduced by Richard J. Bing †).

Brdička described catalytic reaction at dropping mercury electrode (DME) by proteins dissolved in cobalt

containing buffer at suitable pH. This reaction has gained considerable significance as it measures the dynamic property of a protein molecule. Since studies relating current density (μa per mm^2 surface area of DME) to varying concentrations of pure proteins and effect of alkali digestion have not been reported, these investigations were done in pure albumin, 7 S gamma globulin (γ), ceruloplasmin, and transferrin fractions. Pure proteins were obtained by various fractionation techniques, and their purity was checked by immunoelectrophoresis and analytical ultracentrifugation. Polarograms of proteins in standard buffer were recorded at room temperature between applied voltages of —0.8 and —1.9 v. Current density was plotted against logarithm of molar protein concentrations. Protein digestion (0.2 N KOH) was performed at room temperature, and polarograms were obtained at different time intervals. For each fraction, master curves (for both waves) were different. The current density of each wave increased with increasing protein concentration at first, but later an increase in protein concentration caused a decrease in the wave heights, indicating protein-protein interaction at higher concentrations. After alkali digestion, an initial activation reaction (most marked in albumin) within the first 10 minutes followed by a gradual decrease in wave heights (in all fractions) was noted. When the current density was plotted against time on a semilogarithmic scale, the following $t_{\frac{1}{2}}$ (in minutes) for molecular decay reaction was obtained (albumin, 170; γ, 315; ceruloplasmin, 130; and transferrin, 95). Curve analysis suggested that activation reaction concerned different places on the same molecule, whereas the decay reaction involved the molecule as a whole. In conclusion, the polarographic properties of proteins are specific in nature. These techniques therefore should be used as valuable tools for characterizing normal and abnormal serum proteins.

The Acromegalic Liver: An Excretory Giant. RUDOLF PREISIG, THOMAS Q. MORRIS, JOYCE C. SHAVER, MARTIN S. ROGINSKY, AND NICHOLAS P. CHRISTY,* New York, N. Y.

Visceral enlargement is a prominent feature of acromegaly. The functional capacity of the hypertrophied organs has not been extensively investigated in man. The adrenal cortical hypertrophy of acromegaly was shown in a recent study to be associated with elevated cortisol secretory rate (isotope dilution method) in 9 of 13 patients (21.0 to 45.0 mg per day). The absence of clinical hyperadrenocorticism, despite this cortisol hypersecretion, suggested that the acromegalic liver metabolizes cortisol more rapidly than normal. In a group of six acromegalic patients in whom cortisol secretory rate was found to be elevated in four, and normal in two, circulatory and excretory characteristics of the liver were defined. Measurements were made of splanchnic blood flow (EHBF—dye dilution and extraction) and circulating volume (SBV—regional dilution of I^{131}-labeled human serum albumin), hepatic storage capacity (S), and hepatic transport maximum (Tm) for Bromsulphalein (BSP). Scanning with Au^{198} provided an estimate of liver size. Standard biochemical tests (SGOT, SGPT, serum bilirubin, and alkaline phosphatase) were normal, as were the hepatic scintigrams relative to body size. Whereas EHBF, SBV, splanchnic oxygen uptake, and wedged hepatic venous pressure were within normal limits, the ratio Tm/S was nearly doubled. The mean values of 15.6 mg per minute (normal 8.9 $\pm$ S.D. 2.3) for BSP Tm and of 66 mg per mg per 100 ml (normal 62 $\pm$ S.D. 18) for BSP S imply a large increase in excretory capacity per unit functional hepatocellular mass. The magnitude of Tm/S and of cortisol secretory rate was not related to treatment status (one untreated subject, 5 post pituitary irradiation) or to serum growth hormone level (radioimmunoassay). It is concluded that large increments in hepatic excretory capacity are characteristic of acromegaly, whether or not the disease is "active." Although cortisol and BSP may well be handled by different hepatic mechanisms, the demonstrated unique abnormality in BSP excretion may be paralleled by a similar abnormality of cortisol metabolism, thus explaining the absence of clinically apparent Cushing's syndrome in acromegaly.

Mixed-System Agglutination. GIORGIO PUGLISI AND ANGELO TARANTA,* New York, N. Y.

Rabbit antisera versus sheep red cells (SRC) mixed with rabbit antisera versus pigeon red cells (PRC) produced mixed clumps of SRC and PRC, although at dilutions lower than the end-point for homogeneous clumps of SRC or PRC. Similar mixed clumps occurred with some, but not with all, the pairs of red cell agglutinating systems tested, which comprised, in addition to SRC and PRC, human, alligator, and camel red cells. This phenomenon persisted after cross-absorption of the antisera, and thus differs from the "mixed-agglutination reaction" of Coombs. To differentiate it from the latter, it is better called "mixed-system agglutination" (MSA). Heat-inactivation did not decrease MSA; incubation of the sera with antigen-antibody complexes actually increased the titer of both mixed and homogeneous clumps. Therefore, MSA differs from the "complement-dependent mixed aggregation" of Nelson. Mechanical trapping of erythrocytes within clumps of heterologous cells was excluded as the cause of MSA because *1*) mixed clumps could comprise as few as two cells, and *2*) mixed clumps of SRC and PRC did not occur with either antiserum alone. Electrophoresis of the sera on starch blocks separated fast gamma fractions with little or no MSA activity from slow gamma fractions with high MSA activity. This activity was concentrated in the gamma globulin breakthrough on DEAE chromatography and was absent in the second peak of agglutinating antibody eluted at the end. It was present in the second peak on Sephadex G-200 gel filtration and was absent in the first peak of agglutinating antibody activity. Digestion of the antisera's gamma globulin preparations with pepsin abolished the capacity

to produce MSA. It is concluded that MSA is a feature of 7 S and not of 19 S antibodies and that within the 7 S antibody molecule it is dependent on Fragment III of Porter.

Blood Hypercoagulability Induced by Intravascular Hemolysis. S. FREDERICK RABINER, LILA H. FRIEDMAN, AND STEWART T. ROSENFELD, Chicago, Ill. (introduced by Eric Reiss *).

Intravascular coagulation is a common feature in a group of syndromes including septicemia due to gram negative organisms and incompatible blood transfusion reaction. The present studies suggest that intravascular hemolysis may be an important factor in pathogenesis of these syndromes. Infusion of autologous hemolyzed erythrocytes (hemolysate) into mongrel dogs produced transient hypercoagulability as demonstrated by shortening of plastic tube clotting times and improved prothrombin consumption in plastic. With depressed activity of the reticuloendothelial (R.E.) system (carbon infusion or splenectomy), the intravenous administration of hemolysate resulted in intravascular coagulation with fall in Factors V and VIII, fibrinogen, and commonly, pulmonary arterial thrombi. Controls including saline infusion into carbon-treated dogs gave no evidence of hypercoagulability. *In vitro* hemolysate was found to activate the intrinsic rather than extrinsic system of thromboplastin production. The *in vitro* and *in vivo* coagulant activity was found to reside in stroma fraction rather than hemoglobin. Other syndromes, possibly related to the phenomenon of hypercoagulability secondary to intravascular hemolysis, include thrombotic thrombocytopenic purpura (T.T.P.), the hemolytic uremic syndrome (Gasser), and paroxysmal nocturnal hemoglobinuria. Their common feature is chronic intravascular hemolysis that could result in hypercoagulability. Coagulation would not occur until there is saturation of the R.E. system by erythrocyte stroma. Thrombocytopenia and thrombotic episodes could, therefore, be a result of intravascular coagulation activated by erythrocyte stroma. On this basis, two patients with the clinical syndrome of T.T.P. were treated with heparin, one for as long as 6 months. There was a decrease in thrombotic and thrombocytopenic episodes.

Relation of Ventricular Arrhythmias during Coronary Thrombosis to Myocardial K^+ Transfer. TIMOTHY J. REGAN,* MAUREEN HARMAN, ANGEL MARKOV, HENRY OLDEWURTEL, AND HARPER K. HELLEMS,* Jersey City, N. J.

Ventricular arrhythmias due to ischemia are allegedly more difficult to control than those from other causes, presumably related to inadequate delivery of antiarrhythmic agents to the poorly perfused ischemic area or due to the ischemic process itself. To assess this problem and the relationship to myocardial K^+ movement, an electrode catheter was placed in the left anterior descending coronary artery for thrombus production in a group of intact anesthetized dogs. Kr^{85} injections distal to the thrombus site permitted serial coronary flow measurements. Sequential paired sampling of arterial and anterior descending venous blood for K^+ was performed to assess the relation of ion loss to *1*) the development of ventricular tachycardia and *2*) the efficacy of attempted rhythm correction with procaine amide or insulin. Coronary blood flow declined from 75 ± 8 ml per 100 g per minute to a mean of 22 ± 4 ml by 60 to 80 minutes. In animals in whom flow and arterial ion concentration remained constant, the onset of K^+ egress into coronary venous blood usually corresponded with the appearance of an injury potential on the epicardial EKG. The largest cumulative ion loss appeared just before the onset of the arrhythmia ($p < .001$). After the onset of ventricular tachycardia, the use of procaine amide 10 mg per kg iv in 9 animals restored sinus rhythm within 2 to 5 minutes, without recurrence or further ion loss during 3 hours of observation. Six untreated controls progressed to fibrillation. Although insulin (0.1 U per kg) was found to produce significant K^+ uptake in normals when injected into the coronary artery, during ischemia it failed to modify ion loss or the arrhythmia. Thus, it is concluded that myocardial K^+ loss is closely related to the genesis of ventricular arrhythmias due to ischemia and that pharmacologic regulation of this ion movement is associated with correction of potentially fatal arrhythmias despite the persistence of low flow to the ischemic tissue.

Exaltation and Abatement of Urinary Ammonia Excretion. EDWARD L. REID, Miami, Fla. (introduced by A. Gorman Hills †).

The rate of urinary ammonia excretion by healthy men was studied during imposition of rapid alteration of urine flow rate, using relatively short urine collection periods (1 to 4 minutes). Values associated with rapidly increasing flow rate exceed the values characteristic of urine of the same flow rate and pH when urine flow rate is stable; values associated with rapidly falling flow are exceeded by the stable values. These phenomena are analogous respectively to the "exaltation" and "abatement" characterizing urea excretion under similar circumstances. Synoptic evidence suggests that under physiological conditions renal ammonia excretion is the reflection of a physiological system maintaining renal ammonia balance by passive means (nonionic diffusion to equilibrium), and that the kinetic behavior of the system can be formulated adequately in terms of disposition of the ammonia being produced within the kidneys at a constant rate via two first-order outflow channels—the urine and the renal venous blood. Under stable conditions the sum of the two outflows must equal the production rate of ammonia, but this cannot be true immediately after abrupt variation of the value of one outflow velocity constant. If its value increases, then a transitional period ensues during which outflow must exceed inflow, due to progressive decrease in the size of the intrarenal ammonia pool. After decrease of the values of the velocity constant, inflow must temporarily exceed outflow. Exaltation and abatement of urinary

ammonia excretion are interpreted as respective expressions of transitional periods of these types intercalated between two stable states of the system. The evidence indicates that such transitional states, following an abrupt change in the urine flow rate, are (practically speaking) of relatively brief duration (less than 15 minutes).

The Effect of Vitamin Deficiencies on the Intestinal Transport of Amino Acids. S. REISER, R. IMAMI, AND P. A. CHRISTIANSEN, Indianapolis, Ind. (introduced by William P. Deiss *).

Vitamin deficiencies were nutritionally induced in rats in order to correlate the well-established coenzymatic roles of the water soluble vitamins and possible role of the lipid soluble vitamins in membranes with the requirements for intestinal transport. A representative amino acid, valine, was studied *in vitro* with everted intestinal sacs, and results were described as tissue uptake (valine taken up by 500 mg tissue), net transport (valine transported into the serosal medium per 500 mg tissue), and 1/0 ratio (the ratio of valine in the serosal to that in the mucosal medium). All values given have a probability <0.05 or better. A thiamine deficiency, confirmed by defective pyruvate oxidation, decreased net transport, tissue uptake, and 1/0 ratio 25, 35, and 75%, respectively, compared to controls. A 32% decrease in the tissue wet weight in the deficient animals explains the variability of these values. A vitamin B_2 deficiency reduced the tissue wet weight 60% causing an apparent increase in the net transport of valine. This was not reflected by a significant increase in tissue uptake or 1/0 ratio. In agreement with findings on other amino acids, a vitamin B_6 deficiency decreased the tissue uptake (50%) and net transport (75%) of valine. A vitamin E deficiency, verified by direct tissue analysis, decreased the tissue uptake 30% and net transport 40%. A deficiency in the essential fatty acids, corroborated by gas chromatograms, reduced net transport 54%, but did not significantly affect tissue uptake, indicating a specific effect on the slow as opposed to the fast step of valine transport. These results confirm the complexity of the metabolic reactions involved in intestinal transport with the apparent requirement for vitamins B_1, B_6, and E and essential fatty acids for optimal activity.

Metabolic Studies in Young Men with Coronary Heart Disease. PENTTI K. REISSELL, LILLIAN M. HAGOPIAN, AND FREDERICK T. HATCH, Boston, Mass. (introduced by John B. Stanbury †).

To study metabolic factors in the genesis of coronary heart disease in previously healthy young adults, 24 men (aged 23 to 49 years), who had recovered from proven myocardial infarction were compared with an age-matched series of 20 healthy subjects—10 business and professional men and 10 prisoners. Both groups averaged 10% above ideal body weight. The patients were of shorter stature, were heavier smokers, and had a high familial incidence (70%) of coronary heart disease; however, the controls had more familial diabetes. Oral glucose tolerance tests without and with cortisone showed higher peak blood sugar levels and delayed return in the patients. Four patients were found to have fasting serum triglycerides above 300 mg per 100 ml and were placed in a separate category of essential hyperlipidemia. In the remaining 20 patients, serum cholesterol and triglycerides in combination were higher than in the controls, together with elevation of β- and pre-β-lipoproteins. α_1-Liproprotein was decreased. Mean postheparin lipolytic activity was significantly less in the patients—in the lower part of the normal range. In the combined series, the 1-hour blood sugar and fasting triglyceride levels were directly correlated with relative body weight, and the α_1-lipoprotein was inversely correlated with weight. The total incidence of abnormal findings was very high among the patients (and low among the controls), but the positive items were grouped in many different ways in individuals. The analysis suggests that overnutrition and heavy smoking may combine with hereditary or diet-mediated hypercholesterolemia to accelerate the onset of coronary heart disease. This becomes manifest particularly in individuals of short stature from afflicted families. Since individuals fitting many of these criteria are not uncommon in our population, their identification as candidates for coronary heart disease is a significant public health problem.

Correlation of Airway Resistance and Maximal Ventilatory Rates in Normal Subjects and Patients with Chronic Obstructive Pulmonary Disease. A. D. RENZETTI, JR., AND S. WATANABE, Salt Lake City, Utah (introduced by M. M. Wintrobe †).

It is generally accepted that respiratory muscle force and airway resistance are the principal determinants of the maximal air flow rates that can be achieved by the human ventilatory system. This study was performed with the purpose of assessing the relative importance of these two factors in health and in patients with chronic obstructive pulmonary disease (C.O.P.D.). The subjects studied consisted of 25 normals and 39 patients with C.O.P.D. whose mean ages were, respectively, 38.6 and 58.6 years. Airway resistance (Ra) and thoracic gas volume (TGV) were measured by the methods of DuBois, Botelho, and Comroe, utilizing a body plethysmograph. Conventional methods with a 13.5-L spirometer were used to measure maximal voluntary ventilation (MVV), maximal expiratory flow rate (MEFR), maximal mid-expiratory flow rate (MMEF), maximal inspiratory flow rate (MIFR), and forced expiratory volume in one second (FEV_1). Conductance (C) was calculated as $C = 1/Ra$, and its ratio to TGV (C/TGV) was correlated with the flow rates. The mean Ra (cm H_2O per L per second) was 1.11 (± 0.326) for the normals and 3.40 (± 1.34) for those with C.O.P.D. No significant difference was found in the normal group between the 17 below and the 8 above 40 years of age. Correlation coefficients between 0.71 and 0.85 ($p < 0.001$) were found in the C.O.P.D. group between C/TGV and the following: MVV, MEFR, MMEF, and FEV_1. For C/TGV vs. MIFR, $r = 0.49$ ($0.02 > p > 0.01$) in the C.O.P.D.

group. In the normal subjects r was less than 0.33 for all correlations (p ranged from <0.10 to <0.30). The results indicate that in C.O.P.D., airway resistance is the major determinant of all flow rates studied with the exception of the MIFR. By contrast, the ventilatory flow rates studied are probably determined in normal subjects at least as much by respiratory muscle force and other factors as they are by airway resistance. In addition, this study confirms the observation that aging is not associated with increasing airway resistance.

A Mechanism of Bacterial Interference. JOHN C. RIBBLE, New York, N. Y. (introduced by Edward W. Hook *).

Coagulase-positive staphylococci, phage type 7, injected into the allantoic cavity of 10-day-old chick embryos grow to high titer (10^9 per ml) and kill 80 to 90% of embryos in 48 hours. Growth of the coagulase-positive strain can be suppressed to 10^5 per ml and the mortality from infection reduced to 15% by prior establishment of intra-allantoic infection with avirulent coagulase-negative staphylococci. Interference with growth of the coagulase-positive strain by the coagulase-negative strain also occurs *in vitro*. Coagulase-positive staphylococci in trypticase soy broth or normal allantoic fluid grow to a concentration of 10^9 ml in 24 hours. However, the same strain inoculated into a multiplying culture of coagulase-negative staphylococci reaches a concentration of less than 10^5 per ml in 24 hours. Interference can also be demonstrated when the coagulase-positive strain is placed in broth inside a collodion bag that is immersed in a growing culture of coagulase-negative staphylococci. The growth of the coagulase-positive strain is also suppressed to concentrations of less than 10^5 per ml in bacteria-free filtrates of allantoic fluid or broth in which coagulase-negative staphylococci have grown. pH and Eh of inhibitory filtrates do not differ significantly from those of normal allantoic fluid or broth. Glucose concentrations in the inhibitory filtrates are significantly decreased, but addition of glucose does not restore ability to support growth. The inhibitory effect of bacteria-free filtrates of broth cultures of coagulase-negative staphylococci can be reversed by addition of Eagle's basal medium, or a mixture of the vitamins which it contains. The only vitamin in the mixture which reverses the inhibitory effect is nicotinamide, active in a concentration of 0.001 mg per ml. These findings indicate that one of the mechanisms responsible for interference with growth of one staphylococcal strain by another is competition for nicotinamide or production of an antibacterial substance that can be inhibited by nicotinamide.

Fundamental Determinant of CO_2-HCO_3^- Activities in Biological Systems. EUGENE D. ROBIN,* H. VICTOR MURDAUGH,* AND CARROLL E. CROSS, Pittsburgh, Pa., Birmingham, Ala., and Salisbury Cove, Maine.

A bimodal distribution of CO_2 tensions and $[HCO_3^-]$ concentrations exists in animals. Low P_{CO_2} (< 5 mm Hg) and low $[HCO_3^-]$ are found in aquatic forms. High P_{CO_2} (> 10 mm Hg) and high $[HCO_3^-]$ are found in terrestrial forms. Steady-state relationships between P_{O_2} and P_{CO_2} may be expressed as follows:

$$Pa_{O_2} = PI_{O_2} - K_1 - \left(\frac{1}{R}\right)\left(\frac{\alpha CO_2}{\alpha O_2}\right)(Pa_{CO_2} - PI_{CO_2} - K_2).$$

Pa = arterial tension; PI = ambient tension; R = respiratory exchange ratio; α = solubility coefficient; K_1 = concentration gradient arterial blood—mean expired water/air O_2 tension; K_2 = concentration gradient arterial blood—mean expired water/air CO_2 tension. In air, $\alpha CO_2 = \alpha O_2$, and P_{O_2} of approximately 100 mm Hg result in $P_{CO_2} > 10$ mm Hg. In water, at biological temperatures, $\alpha CO_2/\alpha O_2 > 20$ and $P_{O_2} > 50$ mm Hg require $P_{CO_2} <$ 5 mm Hg. $[H^+]$ and $[HCO_3^-]$ vary as functions of P_{CO_2} so that animals with low P_{CO_2} have low $[HCO_3^-]$ and those with high P_{CO_2} have high $[HCO_3^-]$. This problem was studied in an aquatic gas exchanger, the dogfish shark, by determining individual parameters of gas exchange. Control P_{CO_2} are <3 mm Hg and $[HCO_3^-]$ <7 mEq per L. However, quantitatively P_{CO_2} is disproportionately great for the level of P_{O_2}. Three possibilities may explain this finding: *1)* negative K_1 (active secretion of O_2 across the gill), *2)* negative K_1 and K_2 (countercurrent exchange across the gill), or *3)* positive K_2 (steady-state CO_2 concentration gradient across the gill). The third possibility has been demonstrated experimentally. As oxygen-dependent metabolism developed, an obligatory requirement for P_{O_2} was established. The primary mode of gas transport is passive diffusion so that P_{O_2}, in turn, establishes the level of P_{CO_2} and HCO_3^-. These levels can, in special situations, be modified by specific transport mechanisms. However, the values found in biological systems closely reflect passive diffusion as the transport mechanism. The bimodal distribution of CO_2/HCO_3^- activities results from an obligatory requirement for relatively high P_{O_2} to subserve oxygen-dependent metabolism.

Micropuncture Study of Renal Ammonia Excretion by the Dog. ROSCOE R. ROBINSON, EDWARD E. OWEN, AND JAMES R. CLAPP, Durham, N. C. (introduced by Eugene A. Stead †).

Contrary to traditional opinion, recent micropuncture experiments in the rat have shown that measurable concentrations of ammonia are present in proximal tubular fluid during normal acid-base balance, thereby demonstrating that the proximal tubule provides an important potential source of urine ammonia. To evaluate the proximal tubular contribution to urine ammonia excretion in the dog, the ammonia concentration and pH (quinhydrone electrode) were measured in samples of proximal fluid from antidiuretic dogs during normal acid-base balance and acute or chronic ammonium chloride acidosis. In normal acid-base balance (average blood pH 7.35 ± 0.04; urine pH 7.09 ± 0.32) when the pH of proximal fluid resembled that of blood, measurable concentrations (> 0.3 mM) of ammonia were not observed in 19

of 20 samples. Similar results were obtained during acute ammonium chloride acidosis (average blood pH 7.26 ± 0.07; urine pH 6.13 ± 0.38) despite an average 0.43 ± 0.20 U reduction of tubular fluid pH and a two-fold rise in urine ammonia excretion. In contrast, the average ammonia concentration in midproximal fluid increased markedly to 0.8 ± 0.3 mM during chronic acidosis (average blood pH 7.21 ± 0.06; urine pH 5.91 ± 0.39), when the average reduction of midproximal fluid pH (-0.44 ± 0.25 U) was no different than that observed during acute acidosis. Nevertheless, even if ammonia had been present in proximal fluid in the highest concentration, which might have gone undetected (0.3 mM), the proximal tubule could still have provided 90% of the urine ammonia during normal acid-base balance and 50% during acute acidosis. On the other hand, during chronic acidosis, the data clearly demonstrate that the proximal tubule can provide as much as 70% of the urine ammonia and that this segment of the dog nephron definitely contributes to the adaptive rise of renal ammonia excretion that is known to occur during chronic metabolic acidosis.

Effect of Hydrocortisone in Lethal Pneumococcal Infection in Rabbits. HUGH G. ROBSON AND LEIGHTON E. CLUFF,* Baltimore, Md.

These studies evaluate the effect of pharmacologic doses of hydrocortisone on mortality, bacteremia, and cardiovascular function of rabbits with lethal pneumococcal infection. Intraperitoneal inoculation of 2.5×10^9 type I pneumococci caused death of 89% of rabbits within 120 hours. Fifty-three per cent of the animals died in the first 24 hours. Penicillin treatment initiated 6 to 12 hours after infection failed to prevent deaths destined to occur within 24 hours, but prevented most deaths between 24 and 120 hours. Treatment with hydrocortisone, 40 mg per kg, given concurrently with penicillin at 6 or 8 hours after infection reduced 24-hour mortality to 7 and 17%, respectively. Hydrocortisone treatment given at 10 or 12 hours did not lower mortality below that associated with penicillin therapy alone. The protective action of hydrocortisone could be demonstrated with doses of 20 to 40 mg per kg; smaller doses were ineffective. Serial quantitative blood cultures in rabbits treated at 4 or 6 hours after infection with penicillin or penicillin and hydrocortisone, 40 mg per kg, showed no differences in decline of bacteremia between steroid and nonsteroid treatment groups. Pneumococcal bacteremia in rabbits is associated with declining cardiac output (CO), increasing total peripheral resistance (TPR), and mean arterial blood pressure (MABP) maintained near normal until the terminal period. Administration of penicillin plus hydrocortisone, 40 mg per kg, to rabbits 6 to 9 hours after infection resulted in increase of CO and fall of TPR over the course of 6 hours, with no change in MABP. Treatment with penicillin produced no change in hemodynamic parameters. It is suggested that hydrocortisone exerts its protective action by decreasing peripheral vasoconstriction, thereby allowing better tissue perfusion with blood.

Intrarenal Distribution of Blood Flow in Normal and Autotransplanted Dog Kidneys. Effect of Hemorrhagic Hypotension and Mannitol. S. M. ROSEN, B. TRUNIGER, H. R. KRIEK, D. E. OKEN, J. E. MURRAY, AND J. P. MERRILL,† Boston, Mass.

Intrarenal distribution of blood flow (IDBF) has been studied using the Xe^{133} washout technique of Barger and associates. With this method renal blood flow (RBF) has been shown to be separable into 4 components corresponding to cortex (CP I), corticomedullary zone (CP II), inner medulla (CP III), and perirenal fat (CP IV). Ten determinations of IDBF were performed on 8 nontransplanted dogs 7 days to 3 months after surgical implantation of an indwelling renal artery catheter. Distribution of RBF was: CP I 85 ± 4 (SE)%; CP II 10 ± 1%; CP III 2 ± 1%; CP IV 2 ± 1%. For the first week after this operation, however, marked redistribution of RBF was observed in 10 determinations on the same dogs, the greatest changes occurring in CP I and CP II ($p < 0.001$): CP I 55 ± 6%, CP II 32 ± 5%, CP III 9 ± 2%, CP IV 5 ± 1%. By contrast 15 determinations of IDBF on 11 dogs during the first week after autotransplantation were indistinguishable from results obtained from 1 to 25 weeks after operation (CP I 79 ± 1%, CP II 15 ± 2%, CP III 3 ± 1%, CP IV 3 ± 1% of total). Response to hemorrhagic hypotension (mean BP 50 mm Hg) produced the same qualitative changes in IDBF in 4 autotransplanted as in 4 nontransplanted kidneys. CP I was reduced to as little as 10%, whereas CP II was increased to as much as 72%. Mannitol did not prevent or reverse these effects. Since autotransplantation requires denervation of the kidney the results suggest that *a*) redistribution of RBF seen in the intact kidney after surgical manipulation does not occur in the denervated, autotransplanted kidney, and *b*) renal denervation does not appreciably change basal IDBF and does not prevent redistribution of RBF during hemorrhagic hypotension.

Cystinuria: Biochemical Evidence for Two Genetically Distinct Diseases. LEON E. ROSENBERG AND STANTON SEGAL,* Bethesda, Md.

A previous study of urinary amino acid excretion in families of patients with cystinuria identified two groups: families in which parents of affected subjects excreted normal quantities of cystine and dibasic amino acids and those in which parents excreted moderate excesses of cystine and lysine. The genetic implications of these findings have been examined in the present study using *in vitro* and *in vivo* methods. Investigation of amino acid transport by jejunal mucosa and kidney cortex, response to oral amino acid loading, and quantitation of urinary cystine in normal subjects and in five cystinuric patients and their families indicate that two forms of cystinuria are distinguishable. Each propositus had formed numerous cystine renal calculi, excreted gross excesses of

cystine, lysine, arginine, and ornithine, and showed defective dibasic amino acid transport in kidney tissue. Type 1 cystinuria, found in three patients, was characterized by absence of active transport of cystine and lysine by intestinal mucosa, by failure to elevate plasma cystine after an oral cystine load, and by normal urinary cystine values in all parents, children, and siblings of affected subjects. In contrast, type 2 cystinuric subjects were found to retain the mechanism for mediated, active transport of cystine and lysine by gut mucosa and to respond to oral cystine loading with elevations of plasma cystine equal to those seen in controls. Furthermore, several parents, children, and relatives of type 2 patients excreted marked excesses of cystine. These differences cannot be explained by postulating that the two groups of patients represent homozygotes and heterozygotes, respectively, for a single disease. It is, therefore, concluded that the two types of cystinuria result from two different, heritable, autosomal defects separable by investigation of intestinal transport or absorption of the involved amino acids.

Platelet Glycolysis, Platelet Phosphorus Turnover, and Platelet Aggregation. E. C. Rossi, Milwaukee, Wis. (introduced by William W. Stead*).

Although numerous investigators have demonstrated deleterious effects of saline upon platelets, the mechanism of platelet damage remains unclear. This work began as an attempt to study this problem. Rat platelets incubated at 37° C in balanced salt solution (BSS) containing EDTA and 90 mg per 100 ml glucose exhibit clumping after 10 to 12 hours. Platelets incubated in plasma-EDTA (PL) exhibit clumping after 26 to 30 hours. Decreasing glucose concentration in BSS accelerates the appearance of platelet clumping. This suggests that platelet clumping may be related to platelet glycolytic activity. It further suggests that differences in the time at which clumping occurs in different media (glucose concentration remaining constant) may be related to differences in rate of platelet glycolysis. Glucose consumption, lactate production, and $C^{14}O_2$ evolution from glucose-1-C^{14} were measured. 10^9 platelets in BSS consumed 1.40 μmoles glucose per hour and produced 3.6 μmoles lactate per hour. 10^9 platelets in PL consumed 0.69 μmole glucose per hour and produced 1.5 μmoles lactate per hour. $C^{14}O_2$ evolution of platelets in BSS was also markedly increased. The association of earliest clumping with greatest glycolytic activity, and the known clumping action of ADP, suggested that phosphate utilization by platelets might also be altered by changes in medium. This was studied by measuring platelet uptake of $P^{32}O_4$ and its rate of incorporation into "10-minute phosphorus" (phosphonucleotides). Platelets incubated in BSS showed the most rapid uptake of $P^{32}O_4$, the most rapid incorporation of $P^{32}O_4$ into phosphonucleotides, and subsequently, the most rapid depletion of phosphonucleotide $P^{32}O_4$. Repetition of all studies using a medium of BSS and PL in 1:1 proportion produced results that were intermediate in all parameters investigated. These data suggest the following hypothesis: Platelets in BSS demonstrate increased glycolytic activity. Earlier substrate exhaustion arrests glycolysis. As glycolysis ceases, ADP accumulates. Accumulation of ADP within the platelet may then lead to platelet clumping.

Cleavage of Insulin by Mammalian Adipose Tissue: Release of a Biologically Active Peptide from the Hormone Molecule. D. Rudman,* L. A. Garcia, M. Di Girolamo, and P. W. Shank, New York, N. Y.

Previous work in this laboratory showed that rat adipose tissue, which is highly responsive to insulin, contains an insoluble enzyme system that cleaves this hormone. The mode of cleavage and nature of the enzyme system have now been defined by *a*) measuring concentrations of –SH, NH_3, free amino acids, and free-COOH during the cleavage process; *b*) isolating 26 cleavage products from the incubation mixture by phosphocellulose column chromatography and determining the amino acid composition of each product; and *c*) testing the capacity of adipose tissue to hydrolyze synthetic substrates of known peptidases. These data show that the disulfide bridges and acid amide groups of insulin remain intact. The initial event is hydrolysis of internal peptide bonds A13–14, A18–19, B11–12, B15–16, and B25–26 by an adipose tissue endopeptidase. The five peptides thus formed then undergo stepwise loss of amino acids from both termini under the influence of an adipose tissue aminopeptidase and carboxypeptidase. The endopeptidase activity does not cleave synthetic substrates and therefore may be specific for insulin; the two exopeptidase activities hydrolyze appropriate synthetic peptides. One fraction from the phosphocellulose column, which contained peptides B12–19–21 and B17–19–21 linked by disulfide bridges to A20–21, was equipotent with insulin (as revealed by minimal effective dose and maximal response) in stimulating rat adipose tissue *in vitro* to convert C^{14}-labeled glucose in CO_2, triglyceride-glycerol, and triglyceride-fatty acids. Hamster and mouse adipose tissues cleaved insulin in a chemically similar but less rapid manner. It is concluded that insulin is hydrolyzed by a system of three peptidases in mammalian adipose tissue, with release of one or more peptides that are as active *in vitro* as the parent molecule.

Dynamics of Plasma Triglyceride Turnover. Will G. Ryan and Theodore B. Schwartz,* Chicago, Ill.

Although the plasma triglyceride (TG) level has received increasing attention as a factor in the pathogenesis of atherosclerosis in man, the mechanisms regulating these levels are unknown. It is generally agreed that, in the fasting state, the liver is the major source of plasma TG in the form of low density lipoproteins, but quantitation of this influx in health or disease has posed problems. We have devised a quantitative measure of TG influx in the fasting state. The procedure is advantageous in that it is independent of the plasma TG pool size. Because influx equals efflux in the steady state, clearance of plasma

TG can be calculated from the efflux and plasma TG pool size. The technique involves the determination of free fatty acid (FFA) turnover rate during a constant infusion of palmitic acid-1-C^{14} and the rate of appearance of C^{14}-labeled TG in the plasma measured concurrently. By using the reasonable assumption that labeled FFA mix freely with the pool from which plasma TG are derived, the total TG influx can be calculated. Twenty-six studies were performed on seventeen patients. Mean TG influx rate in nine normoglyceridemic individuals was 8.4 ± 3.1 μmoles per minute ($\pm$ SE), a value not significantly different from that of three hyperlipemic subjects (9.6 ± 6.9 μmoles per minute). Individual TG influx rates do not correlate with plasma TG levels, suggesting that TG influx is not the major determinant of the plasma TG level. On the other hand, calculated plasma TG clearance, which ranged from 1.0 to 24.3 ml per minute was, of necessity, better correlated. Four patients treated with chlorophenoxyisobutyl ethyl ester showed a significant lowering of plasma TG levels without change from control values of plasma TG influx, indicating that this drug acts by enhancing TG clearance. We conclude that defective clearance is the primary cause of hyperlipemic states and that further investigation of this mechanism may elucidate its control.

Identification of Intrinsic Factor and Intrinsic Factor Autoantibody in Man by Radioimmunodiffusion and Radioimmunoelectrophoresis. I. MICHAEL SAMLOFF AND EUGENE V. BARNETT, Rochester, N. Y. (introduced by Ralph F. Jacox †).

This report describes a micromethod for the detection of both intrinsic factor (IF) and autoantibody against intrinsic factor (IFAb), which combines the techniques of immunodiffusion and immunoelectrophoresis with radioautography. A radioactive precipitin arc developed when a mixture containing normal human gastric juice (NHGJ), $Co^{60}B_{12}$, and rabbit antihuman gamma globulin (RAHgG) diffused in agar gel against the sera from 8 of 32 patients with pernicious anemia (PA). IFAb was not demonstrable in the serum of 20 patients without PA. With positive sera the test was negative if RAHgG was omitted from the system, if nonintrinsic factor B_{12} binders were substituted for NHGJ, and if $Co^{60}B_{12}$ uptake by IF was blocked by either unlabeled vitamin B_{12} or by antibody-containing serum. As detected by IFAb, IF was demonstrated in the gastric juice of 12 normal patients and in none of 3 patients with PA. Radioimmunoelectrophoretic experiments revealed that in PA sera IFAb was present in γ_2-globulin (IgG) of both type I and type II L-chain types. IFAb was not found in either γ_{1A} (IgA)- or γ_{1M} (IgM)-globulins. The finding of IFAb in IgG, but not IgA or IgM globulin may be a function of the sensitivity of the test system. Alternatively, the presence of IFAb only in the IgG globulin class may indicate immunization of long duration since antibody of this immunoglobulin class occurs late after immunization with certain foreign antigens.

Transcortin: A Candidate for a Progesterone-specific Binding Protein. AVERY A. SANDBERG,* HANNAH ROSENTHAL, AND W. R. SLAUNWHITE, JR., Buffalo, N. Y.

A fuller understanding of factors influencing cortisol metabolism and the transport of this steroid in the blood was gained by the description of transcortin, a plasma protein with high affinity for cortisol. For a number of years we have been impressed with the observation that there may exist in human plasma a protein, besides albumin, with very high affinity for progesterone. Scatchard plots of binding data obtained with native and mildly heated (60° for 20 minutes) plasma have afforded a more direct approach to the binding of progesterone to plasma proteins. Progesterone, like cortisol, appears to bind to a specific plasma protein of high affinity but low capacity. Since the albumin-progesterone interaction is rather strong, definitive demonstration of this protein requires a somewhat high dilution of plasma, i.e., 1:20 in binding experiments. This protein is present in the plasma at about the same concentration as transcortin (0.7 to 1 μmole per L) and exhibits the same heat lability as transcortin. Because of this marked similarity and the fact that progesterone can easily displace cortisol from transcortin, the specific progesterone-binding protein may be transcortin itself. It has been established that transcortin-bound cortisol is biologically and metabolically inactive and that the physiologically effective level of cortisol is related, not to the total plasma level, but to that not bound to transcortin. In view of the much lower concentration of progesterone in plasma and the very high affinity of the protein for this steroid, it is probable that progesterone is almost quantitatively bound to transcortin and, hence, physiologically inert. Transcortin levels are greatly increased during pregnancy and after estrogen administration, resulting in an increased capacity to bind cortisol and progesterone. Thus, it appears that transcortin (and albumin) plays a major role in the metabolism not only of cortisol but also of progesterone.

Calculation of Left Ventricular Volume from Single Plane (A-P) Angiocardiograms. HAROLD SANDLER, RICHARD R. HAWLEY, HAROLD T. DODGE,* AND WILLIAM A. BAXLEY, Seattle, Wash.

A method has been developed and evaluated for determining left ventricular (LV) volume (V) from angiocardiograms taken in a single anteroposterior (A-P) projection and compared with methods previously described for determining LVV from biplane films. In 15 subjects with heart disease 365 paired observations of A-P and lateral LV dimensions were determined from biplane angiocardiograms. A-P and lateral transverse diameters (D_A and D_L) were derived, assuming that each LV image represented an ellipse so that $D = 4A/\pi L$, where A is the respective planimetered area of the image and L its longest measured length. All measurements were corrected for X-ray distortion. Calculated D_A and D_L varied from 2.8 to 8.2 cm and were nearly equal, the

relationship being $D_L = 0.973\ D_A + 0.19$ (SEE 0.45 cm). LV spatial apex to aortic valve length (P) was related to L in the A-P projection (LAP) by $L_{AP} = 0.73\ P + 2.6$ (SEE 0.9 cm). LVV varied from 36 to 410 ml and was calculated from D_A, D_L, and L by using an ellipsoid reference figure. V was corrected to known volumes (V′) by $V' = 0.93\ V - 4$ (SEE 8.2 ml) derived from observations in post-mortem hearts. Volumes derived from the A-P films (V^{AP}) as the product of $(D_A)^2$ and L_{AP} were related to V′ by $V' = V^{AP} - 12$ (SEE 13 ml). These volumes when calculated from directly measured axes using a modification of Arvidsson's technique (V″) were related to V′ by $V' = 0.73\ V'' - 15$ (SEE 25 ml). These studies demonstrate that LVV can be calculated from films taken in a single (A-P) projection and that a method based on direct measurement of chamber axes overestimates V with a larger SEE than a method based on film area measurements. These methods are applicable to calculate LVV from cineangiocardiograms.

The Antigenic Determinants of Bovine, Human, and Porcine Chondromucoprotein. JOHN SANDSON, New York, N. Y. (introduced by David Hamerman*).

Chondromucoprotein (CMP), the only proteinpolysaccharide present in hyaline cartilage, is composed primarily of protein, chondroitin sulfate, and keratan sulfate. Most reports suggest that both chondroitin sulfate and keratan sulfate are present as side chains bound to the same protein that serves as the backbone of the macromolecule. Antisera were prepared in rabbits to CMP isolated from bovine, human, and porcine cartilage. Tanned sheep cell agglutination and inhibition studies clearly showed that CMP from each of these species contained at least two antigenic determinants. One was a common determinant (CD) present in all three species; the other was a species specific determinant (SSD). Undegraded CMP is too large to diffuse well in agar and usually formed no precipitin lines with the antisera. After digestion with testicular hyaluronidase (pH 4.9, 48 hours, 37° C), immunoelectrophoresis (pH 8.6) of CMP from each species with its homologous antiserum revealed two distinct and separate precipitin arcs, one of which migrated faster than the other. Immunoelectrophoresis with the heterologous antiserum revealed only the fast precipitin arc, proving that the fast component contained CD. When antiserum absorbed with heterologous CMP was used, only the slow precipitin arc was obtained, indicating that the slow component contained SSD. The intensity of these precipitin arcs is not reduced by absorbing the antisera with chondroitin sulfate, keratan sulfate, hyaluronidase, or serum. If the hyaluronidase-digested CMP was treated with trypsin (pH 7.4, 24 hours, 37° C), no precipitin arcs were obtained. These results indicate that both CD and SSD are closely associated with the protein of CMP and that after hyaluronidase digestion CD and SSD can be separated. These findings suggest either that *1*) CD and SSD are present in different molecules or that *2*) CD and SSD are present in the same molecule but are linked together by bond(s) susceptible to hyaluronidase. Studies are in progress to determine which of these two possibilities is correct.

The Role of Adenosine Triphosphate (ATP) Depletion *In Vivo* on Sulfobromophthalein (BSP) Excretion into Bile. STEVEN SCHENKER AND BURTON COMBES,* Dallas, Texas.

Observations that BSP is delivered into bile against a high concentration gradient suggest that biliary excretion of BSP is an energy-requiring transport process. The importance of ATP, the prime source of hepatic energy, for BSP transport was appraised in these studies by examining the effect of ethionine-induced hepatic ATP depletion on BSP excretion in adult female rats. The maximal BSP concentration gradient between liver cells and bile remained unaffected 2 hours after intraperitoneal administration of ethionine (75 mg per 100 g), despite a 51% decrease of hepatic ATP (luciferin-luciferase assay). Maximal rate of BSP delivery into bile decreased 15%, however, because of a fall in bile flow. By contrast, total BSP excretion in 30 minutes fell from 2.34 to 0.18 mg per 100 g rat (6.67 mg per 100 g administered), and maximal concentration gradients between liver cells and bile for both free and conjugated BSP were markedly depressed 24 hours after ethionine, when hepatic ATP was decreased to 40% of control, approximately to the same extent as at 2 hours. The profound difference in BSP excretion 2 and 24 hours after ethionine, despite a comparable decrease in hepatic ATP levels, suggests that ATP per se does not limit BSP transport into bile at 24 hours. Although impaired BSP conjugation depresses maximal BSP excretion, no significant decrease in activity of the enzyme catalyzing BSP conjugation, and of glutathione, the substrate for conjugation, was noted in liver at these times, indicating that conjugation of BSP was probably not decreased. These findings suggest that ethionine impaired BSP transport by *a*) decreasing the synthesis of one of the essential components of the carrier mechanism, *b*) altering the permeability of cell membranes permitting backdiffusion of BSP compounds from liver cells and bile, or *c*) inducing fatty metamorphosis and interfering with intracellular movement of BSP to the transport mechanism.

Radiation Damage of Erythrocytes: A Limiting Factor in Therapeutic Extracorporeal Irradiation of the Blood. LEWIS M. SCHIFFER, ARJUN D. CHANANA, EUGENE P. CRONKITE,† MICHAEL L. GREENBERG, HORTON A. JOHNSON, AND JAMES S. ROBERTSON, Upton, N. Y.

Extracorporeal irradiation of circulating blood (ECI) has favorably influenced the peripheral blood picture in human and bovine leukemia and prolonged the acceptance of bovine skin homografts. The duration of ECI

in treatment of leukemia and autoimmune disease and in preparation of patients for organ homotransplantation is limited by radiation damage to erythrocytes. In calves hemolysis appeared when the radiation dose to some erythrocytes exceeded 100,000 rads. This study is one phase of a program to evaluate the behavior of blood during therapeutic ECI in man. Blood collected from normal and leukemic individuals was irradiated by Co^{60} gamma rays before and after chromation and reinjected into the donors. The loss of radioactivity from the blood was then compared to the loss following infusion of nonirradiated, chromated blood. A two component loss of radioactivity from the peripheral blood was observed. The rapid and variable first component has a half-time between 12 and 24 hours. The 24-hour loss increases linearly from 15% at 35,000 rads to 70% at 200,000 rads. The half-time of the second component decreases with the radiation dose from 17 days at 35,000 rads to 5.8 days at 200,000 rads. The phase and electron microscopic appearance of irradiated erythrocytes, osmotic fragility, organ localization of Cr^{51}, and effect on transfusion requirement will be presented. A computer program has been devised to calculate the accumulated dose distribution of radiation to the erythrocytes during prolonged ECI. From these *in vivo* and *in vitro* studies on radiation sensitivity and the estimation of the dose distribution of radiation to the erythrocytes, one can design a course of ECI to get the best therapeutic effect without inducing severe hemolysis.

Isolation and Characterization of a Lactic Dehydrogenase (LDH) Inhibitor of Human Urine. GUIDO SCHOENENBERGER AND WARREN WACKER,* Boston, Mass.

The measurement of urinary LDH and alkaline phosphatase activities is useful in the diagnosis of diseases of the urinary tract. Dialysis is a prerequisite to eliminate marked variations in successive samples from the same person and extreme differences among individuals of control groups. As a result of this procedure a reliable base line for diagnosis has been established. The unexplained effect of dialysis on these enzyme activities suggested that the removal of enzyme inhibitors accounts for the normalization of the assays. The inhibition by urine of purified *E. coli* alkaline phosphatase and rabbit muscle lactic dehydrogenase lent credence to this interpretation. To define the role of such inhibitors in biological control mechanisms and to assess their physiological and pathological importance requires their isolation and chemical characterization. We have separated substances that inhibit either alkaline phosphatase or LDH but not both. During the isolation of an LDH inhibitor, several polypeptides were obtained from urine by solvent extraction, paper and ion exchange, chromatography, and electrophoresis. Only one of these peptides, now completely purified, inhibits both urinary and crystalline rabbit muscle LDH. This establishes a basis for LDH inhibition of undialyzed urine. The polypeptide inhibitor contains aspartic and glutamic acids, glycine, leucine, isoleucine, alanine, lysine, threonine, and serine. Digestion with the proteolytic enzyme Nagarse destroys its inhibitory properties. The molar ratios of the constituent amino acids and their sequence are under study. The biological and pathological implications of the discovery of urinary polypeptide inhibitors will be discussed.

Erythrocyte Membrane Function in Phosphate Transport and ATP Production. STANLEY L. SCHRIER, LYDIE DOAK, AND BARBARA CARR, Palo Alto, Calif. (introduced by David A. Rytand †).

Important biologic reactions take place at cell surfaces; however, the rapid, complex interchanges that occur in intact metabolizing cells make these reactions difficult to follow. Human erythrocyte membranes can be prepared so that they contain only membrane-bound enzymes and carriers and known amounts of specifically introduced substrates. This circumscribed system was used to test two current hypotheses of inorganic phosphate (Pi) transport, which are: *1*) Pi traverses RBC membranes unchanged, perhaps via an equilibrating carrier mechanism, and *2*) Pi transport requires energy; Pi is converted to ATP at the cell surface by glyceraldehyde phosphate dehydrogenase (GAPD) and phosphoglycerate kinase (PGK). Membranes (containing bound GAPD and PGK) were prepared either without intramembrane substrates (< 0.01 μmole per ml) or with 0.5 μmole per ml of the substrates for the GAPD-PGK reaction, which are: DPN, ADP, and glyceraldehyde-3-phosphate (GA3P). After incubation with added Pi^{32} in the suspending medium, washed membranes and medium were extracted and chromatographed. Fractions were analyzed for radioactivity, ATP, ADP, DPN, GA3P, and Pi. Whether or not substrates were present, Pi was taken up maximally in the first 5 minutes and reached 2 to 3 μmoles Pi per ml in 30 minutes at 37° C. In membranes containing substrates, labeled ATP appeared after 10 to 30 minutes and reached levels of 0.1 μmole per ml. Therefore, Pi traversed RBC membranes unchanged and without mediation by GAPD and PGK since *1*) substrates were not required, *2*) the rate of Pi entry was more rapid than ATP appearance, and *3*) the concentration of Pi achieved exceeded both the amount of ATP formed and the limiting substrate concentrations. A further conclusion is that enzymes, structurally bound to RBC membranes, can convert intramembrane substrates into intramembrane ATP. It remains to be determined whether this membrane-synthesized ATP can be used by pump ATPase in mediating Na^+ and K^+ transport.

Differing Effects of Immunity Induced by Infection and Inactivated Influenza Virus Vaccine on Transmission of Infection. JEROME L. SCHULMAN, New York, N. Y. (introduced by Edwin D. Kilbourne *).

Mice infected with influenza A2 virus are completely refractory to reinfection with homotypic (A2) virus 4

weeks later. In contrast, partial immunity results from either prior parenteral immunization with inactivated A2 virus or from infection with an antigenically less related influenza A virus. This partial immunity is reflected by lower titers of pulmonary virus and less extensive lung lesions after A2 virus challenge. Infection with heterologous influenza B virus affords no protection to A2 virus challenge. Unimmunized mice infected with influenza A2 virus transmit infection to 50% of uninfected contacts. The effects on transmission related to the immunity induced by infection with homotypic (A2) virus or heterotypic (A) virus and the immunity following homotypic (A2) vaccine were compared. Because mice immunized by prior homotypic (A2) virus infection could not be reinfected, they were unable to transmit infection to normal contacts. On the other hand, mice immunized with inactivated homotypic (A2) virus transmitted infection as readily as nonimmune infectors, whereas mice immunized by influenza A virus infection, even though this virus was antigenically dissimilar, transmitted subsequent A2 challenge infection to fewer contacts. Contact mice partially immunized either by homotypic (A2) inactivated virus or heterotypic (A) virus infection acquired transmitted infection less frequently than unimmunized contacts. The absolute resistance to reinfection in mice immunized by homotypic infection compared to the partial immunity induced in mice by homotypic vaccine is probably not related to humoral antibody since the titers in both groups are similar. The data suggest that local immunological mechanisms are responsible. Similarly local factors are suggested by the reduced transmission observed in infectors immunized by heterotypic (A) virus pulmonary infection but not in infectors immunized with inactivated homotypic (A2) vaccine at a peripheral site. These findings may have relevance to human immunization procedures.

Effect of Heme on Hemoglobin Synthesis. HERBERT C. SCHWARTZ * AND T. JOHN GRIBBLE, Palo Alto, Calif.

The mechanism for the final assembly of hemoglobin is poorly understood. Since the heme groups and polypeptide chains are intimately related structurally, the relationship of heme to globin synthesis was studied in a "cell free" system. Reticulocytes, obtained from rabbits pretreated with phenylhydrazine, were hemolyzed with one volume of a 1.5 M sucrose solution and centrifuged at $12{,}000 \times g$ for 10 minutes. The supernatant solution was separated into ribosomes, pH 5 enzymes, and soluble supernatant cell fractions. These cellular fractions were incubated at 37° in reaction mixtures that also contained a complete amino acid mixture, an ATP generating system, 2.6 μmoles Mg^{++}, 50 μmoles Tris buffer pH 7.5, and 1 μc L-leucine-C^{14} in a final volume of 1.5 ml. The uptake of leucine-C^{14} into TCA precipitable material was used as a measure of hemoglobin synthesis and was expressed as both specific activity (cpm per mg protein) and total counts incorporated. Ribosomal systems, prepared from rabbit reticulocytes, can synthesize heme from protoporphyrin and iron. Since heme is less soluble than its precursor, protoporphyrin IX, this porphyrin was used in these studies. In eight separate experiments, when 1.2×10^{-4} M protoporphyrin was added to reaction mixtures, 20 to 30% of the total counts incorporated into protein was released as soluble protein. In control reaction mixtures without added protoporphyrin, 8 to 13% of the total counts appeared as soluble protein. The same effect was observed whether the protoporphyrin was added initially or after the reaction mixture had been incubated for 10 minutes. This increase in the radioactivity of the soluble protein was maximal when the concentration of protoporphyrin IX was 3.2×10^{-5} M. Despite this increase in labeled soluble protein, the ribosomal protein was unchanged or slightly decreased. Since the two- to threefold increase in soluble protein occurred in the absence of marked increase in total protein synthesis, heme may be involved in the regulation of hemoglobin synthesis by augmenting the release of the completed molecule from the ribosomes.

Malignant Lymphomas in Runt Disease: Escalation from an Immunological to a Neoplastic Disease. ROBERT S. SCHWARTZ,* Boston, Mass.

It has been proposed that continuous antigenic stimulation of lymphoid cells might induce mutations or other transformations in them which result in malignant lymphomas. This hypothesis was tested by the prolonged exposure of immunologically competent cells (immunocytes) to an environment consisting entirely of antigens. These circumstances were obtained in mice by inoculating parental (C57B1/6) spleen cells into normal F_1 hybrid (C57B1/6 × DBA/2) recipients. The hybrid cannot reject parental grafts, and all of its tissues are antigenic to C57B1/6 immunocytes. The mortality due to runt disease was 70%. This was reduced to 30% by treatment of the hybrid with amethopterin. Mice surviving acute runt disease were observed for 8 to 15 months. They were then examined for *a*) presence of C57B1/6 immunocytes and *b*) lymphoid tumors. One-half of these mice had neoplasms of the lymphoid system. The tumors were comprised of lymphocytic and reticulum cell elements and in some respects resembled Hodgkin's disease. C57B1/6 immunologically competent cells were detected in every tumor-bearing mouse by the discriminant spleen cell assay method. In each case the parental cells were specifically tolerant of hybrid antigens. Control hybrids, including normal, aged mice, those given only amethopterin, and recipients of disrupted, cell-free parental spleen preparations did not develop lymphoid tumors. The development of immunological tolerance by the C57B1/6 immunocytes is a reflection of their continuous exposure to hybrid antigens. It is conceivable that this same exposure resulted in the malignant transformation of some of the parental cells. These experiments support the above hypothesis and strengthen previous analogies between runt disease and malignant lymphomas.

The Possible Association of Erythrocyte Glucose-6-Phosphate Dehydrogenase Deficiency and Regional Enteritis. RICHARD G. SHEEHAN, ROBERT J. LINDEMAN, HEIDI J. MEYER, JAMES F. PATTERSON, AND THOMAS F. NECHELES, Boston, Mass. (introduced by William Dameshek †).

Three cases of erythrocyte glucose-6-phosphate dehydrogenase deficiency were discovered among 17 consecutive, unrelated Caucasians with a diagnosis of regional enteritis. Two further siblings having both conditions were found in the immediate family of one of the probands. All five individuals were of Ashkenazic Jewish background in which the incidence of G6PD deficiency is 1% or less. A survey of 41 Caucasians with a similar ethnic distribution suffering from ulcerative colitis revealed no cases of erythrocyte G6PD deficiency ($p < 0.01$). Starch gel electrophoresis of hemolysates from males with G6PD deficiency and regional enteritis showed no bands of enzyme activity; affected females showed a single band, and normal family members showed two distinct bands with a mobility of the common type B enzyme complex. In order to further elucidate the relationship of these two diseases, studies were carried out on rat intestinal mucosa. Electrophoretic studies of the G6PD enzyme derived from intestinal mucosa showed that each portion of the intestine—duodenum, jejunem, terminal ileum, and colon—had a typical unique enzyme pattern consisting of one or more bands. The terminal ileum shared some, but not all, bands in common with those found in the red cell hemolysates from the same animal. It is therefore suggested that a genetically determined deficiency of one of the G6PD isozymes, occurring in both the erythrocytes and terminal ileum, may predispose to the development of inflammatory disease of that portion of the bowel.

Synthesis and Degradation of RNA by Normal and Leukemic Leukocytes. ROBERT SILBER, KENNETH W. UNGER, AND JOAN KELLER, New York, N. Y. (introduced by Jonathan W. Uhr *).

Although human leukocytes are known to synthesize RNA, this pathway has not been fully characterized. In view of the profound alterations reported in RNA synthesis in the course of cell differentiation and during viral infection, a study was undertaken to compare the types and turnover of RNA in normal and leukemic cells. Leukocytes were labeled *in vitro* or *in vivo* with P^{32} or C^{14} uridine. RNA was purified by a phenol-dodecyl sulfate procedure and subjected to sucrose gradient ultracentrifugation. Material with sedimentation velocities (S) corresponding to ribosomal RNA (28 S and 16 S) and transfer RNA (4 S) was found in all cells. Pulse label experiments to date have revealed a 45 S precursor of ribosomal RNA in cells from patients with chronic lymphocytic leukemia. Pulse experiments show the label in nuclear RNA with subsequent incorporation into the cytoplasm. The highest specific activity of the nuclear material was noted in the uracil-rich 10 to 14 S region that presumably represents messenger RNA. Minimal labeling of ribosomal RNA was noted after 3 hours of incubation of lymphocytes or granulocytes from normal subjects, whereas six of ten patients with chronic lymphocytic leukemia showed up to a fivefold increase in specific activity above normal lymphocytes, thereby suggesting accelerated synthesis. Similarly, one-third of patients with chronic myelocytic or acute leukemia had increased incorporation of label. Whereas the synthesis of RNA appears to be greater in certain immature cells, the degradation of RNA *in vitro* increases in the course of granulocyte maturation. The mean activity of ribonuclease was ten times higher in normal granulocytes (16 pts) than in myeloblasts (6 pts), whereas intermediate values were found in chronic myelocytic leukemia. The changes of RNA metabolism reported above may reflect alterations in a control mechanism in the course of cellular differentiation and maturation.

The Occurrence of Lysosomal Enzymes in Blister Fluid. J. GRAHAM SMITH, JR., C. FRANKLIN CHURCH, AND PETER G. BURK, Durham, N. C. (introduced by William M. Nicholson †).

Morphologic studies have provided conflicting opinions concerning the presence of lysosomes in human epidermis. However, blistering of the skin may be produced by a number of agents known to labilize lysosomes, such as heat, cold, ultraviolet light, papain, etc., and is prevented by glucocorticoids that stabilize lysosomes. Assays for lysosomal enzymes such as β glucuronidase, acid phosphatase, and cathepsins on blister fluid from various bullous diseases and in bullae produced experimentally with cantharidin demonstrate an increase in these enzymes as compared with serum levels in the same individuals. Epidermis that is homogenized in a tissue grinder after being stretch-separated from the dermis has much higher levels of β glucuronidase after repeated freeze-thawing or treatment with Triton X-100 than control homogenized epidermis. Differential centrifugation localizes the enzymatic activity in the cytoplasmic rather than the nuclear elements. These preliminary observations support a lysosomal mechanism for certain types of blistering in human skin.

The Effect of Hematoporphyrin and Light on Human Fibrinogen. H. M. SOLOMON AND P. D. ZIEVE, Baltimore, Md. (introduced by A. I. Mendeloff †).

Many organic dyes sensitize biologic systems to injury by light. In the current studies hematoporphyrin was added to human fibrinogen, pH 7.4. The samples were exposed to light for 10 minutes at 37° C; control specimens were incubated in the dark. Samples were subjected to ultrafiltration according to the technique of Rehberg. Hematoporphyrin was quantitated by fluorometric assay. Both in the light and dark, binding of hematoporphyrin to fibrinogen was linear on a Line-

weaver-Burk plot; a maximal number of 5 binding sites was thereby determined. The radiated specimens demonstrated enhanced binding of hematoporphyrin although the total number of receptor sites was not increased. The thrombin time of irradiated fibrinogen was prolonged but became independent of hematoporphyrin concentration above 0.33×10^{-4} M. The clotting of untreated fibrinogen by thrombin was inhibited competitively by fibrinogen treated with hematoporphyrin and exposed to light. Paper electrophoresis of treated and untreated samples of fibrinogen showed identical migration. These results demonstrate that hematoporphyrin and light alter fibrinogen and that this altered molecule is able to compete with normal fibrinogen for thrombin. These experiments provide an opportunity to characterize the binding of hematoporphyrin to fibrinogen and demonstrate the effect of this photosensitizing dye on the functional property of the protein.

The Metabolic Effects in Man of Bovine Growth Hormone Digested with Trypsin. MARTIN SONENBERG,* CHARLES A. FREE, JUAN M. DELLACHA, GIOVANNI BONADONNA, ASHER HAYMOWITZ, AND ALLEN C. NADLER, New York, N. Y.

Purified bovine growth hormone was subjected to controlled tryptic proteolysis, the reaction being terminated with soybean trypsin inhibitor. The extent of hydrolysis, as determined by alkali uptake on a pH-stat and change in zone electrophoretic pattern, has been correlated with growth-promoting activity in the rat. Nearly complete disappearance of the electrophoretic components present in untreated bovine growth hormone occurred after 10 minutes of tryptic digestion, accompanied by an alkali uptake of 5 to 6 moles per mole of protein. The 10-minute digests, although containing a series of intermediate, faster migrating components, retained growth promoting activities in the rat as high as 1 U.S.P. U per mg or more. Digestion for 60 minutes increased the alkali uptake to over 13 moles per mole of protein and produced a mixture of yet faster moving components containing activity of 0.1 U per mg. Under conditions of complete metabolic balance, seventeen studies were performed in ten hypophysectomized and one hypopituitary patient injected with tryptic digests of bovine growth hormone, undigested bovine growth hormone, or human growth hormone. In studies employing tryptic digests of bovine growth hormone, responses were noted among diabetic patients of aggravated hyperglycemia and glycosuria, decreased carbohydrate tolerance, and production of ketonuria. In other studies, the digests promoted retention of nitrogen, phosphorus, sodium, and potassium; increased urinary calcium; and caused depression of blood urea nitrogen and elevation of serum inorganic phosphorus. Instances were also noted of increased urinary hydroxyproline and decreased urinary creatine. In two of three studies, typical metabolic responses to human growth hormone occurred but not to undigested bovine growth hormone administered to one patient.

Velocity of Contraction: A Major Determinant of Myocardial Oxygen Consumption. EDMUND H. SONNENBLICK, JOHN ROSS, JR., JAMES W. COVELL, GERARD KAISER, AND EUGENE BRAUNWALD,* Bethesda, Md.

It is widely held that myocardial tension is the only important determinant of myocardial O_2 consumption ($M\dot{V}O_2$). Since a proportionality between velocity of shortening and rate of heat liberation exists in skeletal muscle, this study was designed to determine whether or not velocity of myocardial contraction influences $M\dot{V}O_2$. In 12 dogs on right-heart bypass, with heart rate, stroke volume, and aortic pressure constant, the velocity of contraction was augmented to comparable levels by three fundamentally distinct interventions in the same heart: *1*) sustained post-extrasystolic potentiation produced by paired, electrical stimulation, *2*) norepinephrine infusion, and *3*) Ca^{++} infusion. In each instance the correlation between velocity of contraction and $M\dot{V}O_2$ was striking. During paired stimulation, maximal rate of left ventricular ejection, measured with an electromagnetic flow meter on the ascending aorta, increased by an average of $50.2 \pm 3.8\%$ (SEM) above control, and $M\dot{V}O_2$ increased by 1.96 ± 0.10 ml per 100 g per minute ($39.8 \pm 1.6\%$ above control), while the tension time index (TTI) fell $12.4 \pm 1.1\%$. With norepinephrine, and with Ca^{++}, left ventricular ejection rates were increased $52.9 \pm 3.2\%$ and $55.1 \pm 3.2\%$, respectively, and $M\dot{V}O_2$ was augmented 2.03 ± 0.08 ml per 100 g per minute and 1.87 ± 0.04 ml per 100 g per minute, while the TTI decreased $15.9 \pm 0.6\%$ and $12.4 \pm 2.4\%$. When paired stimulation and norepinephrine were combined, left ventricular ejection rate increased $106.5 \pm 7.7\%$ above control, while $M\dot{V}O_2$ increased 5.53 ± 0.28 ml per 100 g per minute and TTI decreased $28.9 \pm 3.3\%$. It is evident that since $M\dot{V}O_2$ always increased substantially while TTI fell, alterations in the TTI were not responsible for the observed increases in $M\dot{V}O_2$, and tension therefore cannot be considered to be the sole determinant of $M\dot{V}O_2$. Since comparable increases in the velocity of contraction produced by different interventions were associated with similar, large augmentations of $M\dot{V}O_2$, it appears that velocity of contraction is an important determinant of $M\dot{V}O_2$. Furthermore, it is likely that the so-called O_2 wasting effect exerted by norepinephrine on myocardial metabolism may be explained largely by an increased velocity of contraction.

Biochemistry of Human Platelet Clumping by Adenosine Diphosphate. THEODORE H. SPAET,* New York, N. Y.

Clumping of platelets by adenosine diphosphate (ADP) is probably an important factor in growth of the hemostatic plug. The present studies concern the mechanism of this clumping reaction; the data are derived from experiments with ADP-8-C^{14}. Radioactive ADP added to heparinized platelet rich plasma (PRP), in concentrations just sufficient to clump platelets, gave

less than 2% of the nucleotide remaining with the platelets after repeated washing, confirming the results of Salzman and associates. This was not due to nonspecific trapping, since "cold" ADP prevented the "binding" reaction. Radioactive ADP was incubated with various types of plasma and platelet preparations; the nucleotides were extracted, chromatographed, and radiographically scanned. Spots were also subjected to liquid scintillation counting. Radioactivity sedimented with the platelets was identified as a mixture of approximately equal ADP, adenosine monophosphate (AMP), and adenosine triphosphate (ATP). Platelet poor plasma (PPP) was found to convert ADP into AMP, but this property was destroyed at 56° C. Heated EDTA PPP used as washing fluid for platelets preserved their ability to clump in the presence of ADP. Platelets so washed converted ADP into AMP. Clumping and ADP conversion required divalent cation, were inhibited by concentrated AMP, and were absent when platelets were washed in buffered saline. Benadryl inhibited clumping without preventing ADP conversion. Red cells or cephalin failed to produce ADP conversion in the presence of heated plasma and divalent cation. The hypothesis is favored that ADP is a high energy source in the platelet clumping reaction, rather than participating in a bridge. The small amount of ADP-platelet binding is not specific for ADP, for Salzman has shown that this is a property of nonclumping nucleotides as well.

Dissociation of Immunological and Biological Activities of ACTH. LOWELL L. SPARKS, HIROO IMURA, SYLVIA D. GRANT, AND PETER H. FORSHAM,† San Francisco, Calif.

The unexpected finding of a dissociation between the immunologically and biologically determined plasma levels of ACTH in a patient with pigmentation after adrenalectomy led us to investigate the molecular requirements for antibody binding of ACTH. The displacement radioimmunoassay was used to measure binding of ACTH to antibody and the hypophysectomized rat adrenal vein cannulation method to measure steroidogenic activity of ACTH. Studies using synthetic 1-17, 1-19, 1-24, 1-26, and 1-39 amino acid ACTH corresponding to the N-terminal sequence showed that the immunologic activity resides in the C-terminal and the steroidogenic activity in the N-terminal portion of the molecule. Treatment of sheep ACTH with periodate, which oxidizes the N-terminal serine, destroyed biologic activity but did not influence immunologic activity. Fibrinolysin treatment, which splits the molecule into three fragments, destroyed biologic activity but enhanced immunologic activity slightly. A highly purified subfraction of ACTH, corresponding to the 22nd to 39th amino acids in the C-terminal portion of the molecule, had no biologic activity but was immunologically three times as active as native ACTH on a molar basis. It is concluded that the biologic and immunologic activities of ACTH can be completely dissociated. Examples of such dissociations observed in commercial lots of ACTH and in the plasma of patients with postadrenalectomy pituitary tumors and pigmentation (Nelson's syndrome) will be presented.

The Relationship of Renal Excretion of Sodium and Water to Atrial Pressures and Cardiac Output. WILLIAM M. STAHL, Burlington, Vt. (introduced by Thomas B. Tomasi, Jr.*).

Diuresis and natriuresis have been related to stretching of the walls of the cardiac atria due to increased pressure or amplitude of pulsation. Explanation of the antidiuresis and antinatriuresis of cardiac failure has been difficult to reconcile with these findings. Thirty-seven studies were performed in 12 dogs loaded with DOCA, 9α-fluorohydrocortisone, and vasopressin. Atrial pressures and renal excretory parameters were altered by the following: isotonic saline infusion, thoracic inferior vena cava constriction, pulmonary artery constriction, and aortic constriction. By these maneuvers, right atrial pressures varied from 0 to + 9 mm Hg, and left atrial pressures varied from 0 to + 19 mm Hg. Cardiac output varied from a low of 40% to a high of 200% of control values. Urine volumes, sodium excretion, and osmolar clearance were nearly proportional to changes in cardiac output, whereas no correlation was shown with changes in atrial pressure, except in those experiments where cardiac output and atrial pressure changes were parallel (saline infusion and thoracic inferior vena cava constriction). In 76% of these experiments glomerular filtration rate as measured by inulin clearance varied less than 15% from control levels. Changes in tubular resorption of sodium and water accounted for most of the observed changes in excretion. Correlation between changes in tubular resorption of sodium and changes in urinary osmolality and potassium excretion suggest that alterations in excretion were produced by changes in countercurrent flow. Under the conditions of these experiments, renal excretion varied inversely with levels of total peripheral resistance as calculated from cardiac output and mean arterial blood pressure and were not related to atrial stretch phenomena.

Demonstration of a Maximal Free Water Clearance in Hydrated Dog. RICHARD M. STEIN, RUTH G. ABRAMSON, AND MARVIN F. LEVITT,* New York, N. Y.

The present studies reveal that maximal free water clearance (C_{H_2O}) could be produced in anesthetized hydrated dogs when solute clearance (C_{osm}) was increased by the infusion of hypotonic saline (0.45%) or mannitol (2.5%). In the saline studies, C_{H_2O} per 100 ml GFR (C_{H_2O}/GFR) rose sharply as C_{osm} per 100 ml GFR (C_{osm}/GFR) increased to 6 ml per minute. Thereafter, C_{H_2O}/GFR failed to increase and remained stable as C_{osm}/GFR increased to 25 ml per minute. In the mannitol studies, C_{H_2O}/GFR increased more slowly and continued to rise until C_{osm}/GFR reached 18 ml per minute. Thereafter, as in the saline studies, C_{H_2O}/GFR remained at maximal levels as C_{osm}/GFR increased to 40 ml per

minute. Maximal C_{H_2O}/GFR averaged 13.5 ml per minute in the saline studies compared to 17 ml per minute in the mannitol studies. In several experiments, unilateral reductions in GFR were produced before the administration of saline or mannitol. These studies demonstrated that C_{H_2O}/GFR attained maximal levels at different times in the two kidneys (C_{H_2O}/GFR continued to rise in the kidney with the reduced GFR after maximal levels had been attained in the control kidney). These findings exclude the possibility that C_{H_2O}/GFR stabilized as a result of the secretion of ADH. The failure for C_{H_2O}/GFR to progressively increase might be explained by a limit on net sodium transport at the water clearing site (primarily the ascending limb). However, the alleged limit would have to be independent of tubular fluid sodium concentrations or flow rates. Alternatively, an inhibition of sodium transport at a nonwater clearing site, distal to the ascending limb, might also explain an increase in C_{osm}/GFR without a change in C_{H_2O}/GFR.

An Evaluation of the Interrelationships between the Distribution of Rubidium^{86} and Myocardial Blood Flow. SHELDON H. STEINER AND ROBERT D. KING, Indianapolis, Ind. (introduced by Stuart O. Bondurant *).

The early uptake phase following a bolus intravenous injection of tracers of potassium has been suggested as a measure of the nutrient myocardial blood flow fraction of the cardiac output. In 32 dogs both coronary arteries were perfused with left atrial blood at flows calibrated from 45 to 230 ml per minute. Three hundred μc of $Rb^{86}Cl$ and 2.5 mg of indocyanine green were injected simultaneously into the vena cava. Cardiac output was determined by indicator dilution using a photodensitometer. Since myocardial tracer uptake shows negligible change from the maximal value obtained during initial delivery to at least 2 minutes postinjection, the heart was excised after 1 minute, and the Rb^{86} uptake fraction was determined. This fraction multiplied by cardiac output was defined as nutrient myocardial blood flow. At pump flows of 227 ± 0.9, 147 ± 2.7, 96 ± 11.8, 51 ± 7.0, and 0 ± 0 ml per minute calculated flows were, respectively, 230 ± 17.8, 125 ± 8.0, 86 ± 10.3, 60 ± 21.3, and 21 ± 7.0 ml per minute. The correlation coefficient is $+0.96$, and the regression equation is $y = 1.10x - 11.1$, where y = pumped flow, and x = calculated nutrient myocardial flow. Pulmonary tracer loss has been demonstrated previously to be negligible. However, the noncoronary endomyocardial contribution may be significant and was assessed by comparing flows calculated both immediately after bilateral coronary occlusion and after perfusion at 144 to 312 ml per minute from a delay reservoir containing tracer free blood. Under these conditions calculated flows were, respectively, 17 ± 4.5 and 19.5 ± 10 ml per minute, which agree reasonably with that obtained from the y intercept of the equation. These data suggest that under certain circumstances the uptake of Rb^{86} immediately following an intravenous injection may reflect the nutrient myocardial blood flow fraction of cardiac output. Control measurements from indicator injections into 16 trained, awake dogs averaged 134 ± 35 ml per minute, where myocardial tracer content was determined in the extirpated heart subsequent to sacrifice by electronarcosis and ventricular fibrillation.

Isolation of Lipoproteins from Thyroxine-labeled Human Serum. KENNETH STERLING,* MILTON A. BRENNER, AND HERBERT G. ROSE, Bronx, N. Y.

Immunoelectrophoretic studies elsewhere have suggested that the thyroxine-binding α-globulin (TBG) may be a lipoprotein or closely associated with a lipoprotein, based on lipid stains of the protein arcs. The question was investigated by isolation of lipoproteins from sera or from Fraction IV after the addition of I^{131}-labeled thyroxine. The lipoproteins were separated by ultracentrifugation in high density media and also by precipitation with specific antisera. No detectable radioactivity was found in the washed precipitates from three antisera (against α_1-, α_2-, and β-lipoprotein) with either fresh serum or Fraction IV enriched with labeled thyroxine. Ultracentrifugation in high density media (1.21) yielded a top layer of yellowish lipid-rich material under which was a clear area above the rest of the serum proteins. The tubes were divided through the clear area with a tube cutter, and the radioactivity was determined in the $D < 1.21$ ("top") and $D > 1.21$ ("bottom") fractions. The bottom fractions had more than 89% of the radioactivity in all instances; total recovery of radioactivity ranged from 86% to 98%. The studies do not exclude the possibility that TBG could contain a small amount of lipid; however, TBG is not a lipoprotein of the major lipoprotein classes.

The Effect of Sulfonylureas and Phenformin on Lipolysis in Adipose Tissue. DANIEL B. STONE AND JOSEPH D. BROWN, Iowa City, Iowa (introduced by Elmer L. DeGowin †).

Recent studies suggest a reciprocal relationship between glucose and fatty acid metabolism, one feature of which is restriction of glucose uptake by release of fatty acids from glyceride stores. Our purpose was to study some effects of oral hypoglycemic drugs on lipolysis in adipose tissue. Epididymal fat pads from fasted rats, each pad divided into three portions, were incubated in Krebs-Ringer bicarbonate buffer containing albumin but neither glucose nor lipolytic hormones. Tolbutamide or phenformin was added to the medium in pharmacologic concentrations. Tolbutamide, in contrast to phenformin, induced significant decreases in the net release of free fatty acids (FFA) and glycerol. In experiments with 15 rats, tolbutamide decreased the net release of FFA by 22% ($p < 0.01$) and of glycerol by 13% ($p < 0.02$). Decreased net release of FFA into the medium was not caused by accumulation in the tissue, for the difference between tissue concentrations of FFA, control versus tolbutamide (0.037 uEq per g), was not significant (p

> 0.9). Phenformin (15 rats) had no effect on net release of FFA ($p > 0.5$) or glycerol ($p > 0.1$). *In vivo* studies were performed by giving one dose (3 g) of oral acetohexamide to 26, maturity-onset, fasting, diabetic patients. After acetohexamide administration, the decrease in concentration of plasma FFA preceded that of blood glucose. At 1 and at 2 hours after the ingestion of acetohexamide, the concentration of plasma FFA had decreased ($p < 0.001$) despite no significant change in blood glucose concentration until the third hour. These *in vivo* data are consistent with the direct action of sulfonylureas observed *in vitro*. In terms of the glucose fatty-acid cycle, one factor in the hypoglycemic effect of the sulfonylureas may be enhanced glucose uptake following inhibition of lipolysis and decreased release of FFA from glyceride stores.

Effects of Physiological Concentrations of Cortisol on Sodium Transport in Erythrocytes. DAVID H. P. STREETEN, ARNOLD M. MOSES, AND MARY KEARNEY, Syracuse, N. Y. (introduced by Eugene L. Lozner †).

Previous studies from this laboratory have demonstrated that cortisol raises the level of plasma osmolality required for vasopressin release in human subjects infused with hypertonic saline. Assuming that the *in vitro* behavior of erythrocytes might provide a clue to the mechanism of the cortisol effect on osmoreceptor cell stimulation, the following observations were made: Cortisol (120 μg per 100 ml) significantly reduced the shrinkage of human erythrocytes produced by adding increasing amounts of hypertonic sodium chloride but not of hypertonic sucrose. This suggested enhancement of sodium influx by cortisol. Na^{22} influx into the erythrocytes of adrenalectomized dogs was accelerated by cortisol to rates 36 to 115% above control values, with log dose-response relationships between 1 and 300 μg per 100 ml ($p < 0.01$). Cortisol had no effect on the efflux of Na^{22} previously incorporated into such erythrocytes. The effect of cortisol on sodium influx was abolished when the plasma glucose concentration was allowed to fall below 10 mg per 100 ml. However, the enhancement of sodium influx by cortisol did not result from stimulation of glycolysis, since cortisol failed to influence the changes in blood hexose, lactate, or ATP concentrations during the incubations. Preliminary studies have shown that cortisol produces dose-related increases in adenosine triphosphatase activity in erythrocyte membranes. Since we have previously demonstrated that aldosterone retards sodium influx without affecting sodium efflux, it is concluded that the relative plasma concentrations of aldosterone and cortisol may control cellular sodium content by their effects on an inwardly directed sodium carrier that requires energy from glycolysis and may involve adenosine triphosphatase. These observations on sodium influx into erythrocytes suggest that the above described *in vivo* effects of cortisol on vasopressin release may result from similar action of cortisol on the osmoreceptor cells.

Folic Acid Deficiency as a Cause of Uterine Hemorrhage in Pregnancy. RICHARD R. STREIFF AND A. BRIAN LITTLE, Boston, Mass. (introduced by W. B. Castle †).

Evidence has accumulated to suggest that folic acid deficiency, or inhibitors of folic acid metabolism in animals, greatly decrease the response of the female reproductive organs to estrogenic stimulation. Clinically, a correlation between megaloblastic hematopoiesis and uterine bleeding late in pregnancy has been reported. Increased urinary formiminoglutamic acid excretion, although not specific for folic acid deficiency, has been found in practically all pregnant women with abruptio placentae. Folic acid antagonists are known to be effective abortifacients. We have studied serum folic acid and vitamin B_{12} levels in prenatal patients and in patients at delivery in the Boston City Hospital. Routine peripheral blood studies were also made. In 66 patients with early spontaneous abortions, the incidence of folic acid deficiency was no greater than in patients with uncomplicated pregnancy (10% ±). In 20 patients with uterine bleeding in the third trimester, or placenta previa, folic acid deficiency was about 3½ times as frequent as in patients with uncomplicated pregnancy. In 10 of 11 patients with clinical separation of the placenta, serum folic acid levels were low (< 3 mμg per ml by the *L. casei* method). All patients with low serum folic acid levels showed hypersegmented polymorphonuclear leukocytes, indicating that the deficiency had been present for some time before the hemorrhage. Serum vitamin B_{12} levels showed little or no correlation with hemorrhage during pregnancy. Only three pregnant patients had vitamin B_{12} levels in the deficient range (< 100 $\mu\mu$g per ml by the *E. gracilis* method). These results suggest that folic acid deficiency not only leads to megaloblastic erythropoiesis in pregnancy, but is also associated with placental hemorrhage in the third trimester.

Relationship of Histamine to Gastrin-stimulated Acid Secretion Elucidated by Enzymatic Pathways in the Dog. MARCELO I. STUBRIN, BARBARA J. DYCE, AND B. J. HAVERBACK,* Los Angeles, Calif.

Studies in our laboratory have shown that the polypeptide, gastrin, releases histamine from the glandular mucosa of the rat's stomach. If the release of histamine is an important factor in the stimulation of acid by gastrin, enzymatic alteration of the rate of formation or degradation of the amine during gastrin stimulation should profoundly affect acid secretion. Accordingly, the degradation of histamine, which proceeds mainly by diamine-oxidase and by a combination of *N*-methyl transferase and monamine-oxidase, was increased or decreased by the administration of *1*) a highly purified diamine-oxidase, *2*) heat inactivated diamine-oxidase, *3*) a selective inhibitor of diamine-oxidase, aminoguanidine, *4*) simultaneous administration of diamine-oxidase and aminoguanidine, and *5*) serotonin, an inhibitor of *N*-methyl transferase, in dogs with total innervated gastric fistulae.

In addition, the effect of alteration in the rate of formation of histamine by administration of the enzyme histidine decarboxylase on gastrin-stimulated secretion was accomplished in the same animals. The activity of all the enzymes was checked by *in vitro* studies. The following results were obtained on gastrin-stimulated-acid secretion: *1*) 75 to 100% inhibition by diamine-oxidase, *2*) no inhibition by heat inactivated diamine-oxidase, *3*) 100% increase by aminoguanidine, *4*) complete blocking of the inhibitory effect of diamine-oxidase by aminoguanidine, *5*) 100% increase by serotonin, and *6*) 50% increase by histidine decarboxylase. The results of these studies suggest an interrelationship between gastrin and endogenous histamine in acid secretion.

A Study of Expiratory Airway Collapse in Excised Human Lungs. SUNG SUH PARK, OK HI YOO, AND M. HENRY WILLIAMS, JR.,* New York, N. Y.

In order to elucidate the nature of expiratory collapse in different lung diseases, a study of pressure-volume-flow relationships was carried out on 18 excised human lungs, including 7 normal lungs and 11 lungs from patients with asthma, bronchitis, and/or emphysema. In each case, the diagnosis was confirmed by pathological study. The lung was deflated under atmospheric pressure after being inflated with air under positive pressure ranging from 10 to 20 cm H_2O. Under these conditions, the airway resistance (R) remained fairly constant throughout deflation and varied markedly among the cases (from 2 cm H_2O per L per second to 123 cm H_2O per L per second). However, when the lung was deflated under positive pressure, R increased progressively as the applied pressure was increased, and the following general rules were observed regardless of the pulmonary pathology: *1*) for a given applied pressure, the increase of R was curvilinearly related to the decrease of lung recoil force (P_L), the R increasing markedly with lower P_L; *2*) for a given P_L, the increase of R was proportional to the applied pressure; *3*) for a given applied pressure and P_L, the increase of R was directly proportional to the R during natural deflation. It is concluded that expiratory airway collapse is related to *1*) the recoil force of the lung, *2*) the airway resistance during natural deflation, and *3*) the applied pressure around the lung. Severe abnormalities of any of these factors, singly or in combination, may result in expiratory airway collapse. Identical flow-volume curves were obtained on lungs taken from patients with severe asthma, bronchitis, and emphysema.

The Serological Epidemiology of *M. pneumoniae* Infections. RICHARD H. SUHS AND HARRY A. FELDMAN,† Syracuse, N. Y.

The recent development of a direct hemagglutination-inhibition (HI) test allows antibodies for *M. pneumoniae* to be measured with facility, thus permitting population studies to determine the extent of general experience with this respiratory disease-producing agent. The HI titers of eight current human gamma globulin lots varied from 1:40 to 1:160, whereas four outdated ones ranged from 1:10 to 1:80, suggesting that these antibodies are either infrequent or of low titer in the normal population. The sera of 124 persons, mean age 80 years (range, 44 to 99), have been examined similarly. Titers were distributed as follows: 59 (48%), $<1:2$; 33 (27%), 1:2; 15 (12%), 1:4; 11 (9%), 1:8; 5 (4%), 1:16; and 1 (0.8%), 1:64. Males were more often positive than females. Of ten pairs of maternal and cord bloods, eight were negative. In the others, maternal and infant titers were identical. Similar studies of other populations are nearing completion, including one of normal families (children and adults). These data suggest that Eaton antibodies are either relatively infrequent or short-lived in the general population.

Characterization of Nephron Function in Acute and Chronic Hydronephrosis. WADI SUKI, GARABED EKNOYAN, FLOYD C. RECTOR, JR.,* AND DONALD W. SELDIN,* Dallas, Texas.

The effects of acute and chronic unilateral hydronephrosis on renal function and urinary composition were studied in dogs with a split bladder, during both water diuresis and hypotonic saline (0.1 M HaCl) diuresis, using the contralateral kidney for comparison. In acute hydronephrosis during water diuresis there was a reduction in urine Na concentration, Na excretion ($U_{Na}V$), per cent of filtered Na excreted, C_{H_2O}/C_{osm}, and C_{H_2O} per 100 ml GFR, and an elevation of urine osmolality (U_{osm}), in comparison to the control side. The infusion of hypotonic saline reduced U_{osm} below control and increased C_{H_2O}/C_{osm}. This pattern of alteration in renal function is that of nephron hypoperfusion and is identical with that seen during renal artery constriction. Chronic hydronephrosis, in contrast, resulted in elevation of U_{Na}, $U_{Na}V$, per cent of filtered sodium excreted, U_{osm}, and C_{H_2O} per 100 ml GFR and a fall in C_{H_2O}/C_{osm} during water diuresis when compared to the control side. Hypotonic saline infusions did not alter any of these findings qualitatively. This pattern of altered renal function is that of nephron hyperperfusion and is akin to osmotic diuresis. Studies of Tm_{PAH} revealed a loss of renal mass in both stages of hydronephrosis. In acute hydronephrosis, however, GFR/Tm_{PAH} was low, whereas in chronic hydronephrosis GFR/Tm_{PAH} was high compared to the control kidney, thus confirming the findings of hypoperfusion in acute hydronephrosis and hyperperfusion in chronic hydronephrosis. In summary, renal hemodynamic studies and studies of urine composition demonstrate that the residual nephrons of the acutely hydronephrotic kidney are hypoperfused, whereas the residual nephrons of the chronically hydronephrotic kidney are hyperperfused.

Gastrointestinal Urease Activity in Man. W. H. J. SUMMERSKILL, T. AOYAGI, W. B. EVANS, AND G. W. ENGSTROM, Rochester, Minn. (introduced by Hugh R. Butt †).

The hyperammonemia sometimes associated with hepatic disease is supposed in part to be derived from gastroin-

testinal urease activity. The location and amount of urease in the gut in humans, together with its relationship to ammonemia, were therefore investigated by *in vitro* and *in vivo* methods. For the latter, 107 specimens of mucosa were obtained from different areas of the gastrointestinal tract by operative or nonoperative biopsy. Urease activity was measured from quadruplicate determinations on individual samples. A gradient from upper to lower levels was apparent, values being higher in the stomach than small bowel ($p < 0.01$) and greater in the small than in the large intestine ($p < 0.01$). For *in vivo* studies, changes in blood ammonia concentration (peak less fasting value) were measured in 12 patients with cirrhosis, after the administration of ammonium acetate or urea (of comparable NH_3N content) by mouth or by rectum. Data from the same patient were compared. Significant increases followed each procedure, but ammonemia after oral urea was less ($p < 0.001$) than under other test circumstances, all of which gave similar results. The studies were repeated after the administration of neomycin. Significantly less ammonemia followed the administration of urea by rectum ($p < 0.001$), but other results were unaffected by the antibiotic. It is concluded that urease activity capable of causing hyperammonemia is present throughout the gastrointestinal tract in man, being greatest in the large bowel. By contrast, mucosal urease content is greatest in the upper gastrointestinal tract. Large bowel urease activity probably is largely bacterial in origin, since it was decreased significantly by neomycin, which did not affect the other aspects of urea and ammonia metabolism studied.

DNA in the Serum of Patients with Systemic Lupus Erythematosus (SLE). E. M. TAN, P. H. SCHUR, AND H. G. KUNKEL,† New York, N. Y.

Despite the finding of antibodies to DNA and other cell constituents in the sera of patients with SLE, mechanisms of tissue injury involving such antibodies have not been clear. In a search for antigens that might interact with such antibodies to produce harmful antigen-antibody complexes, the observation was made that free DNA may appear in the serum of these patients. Analysis by double diffusion in agar, utilizing SLE sera containing DNA antibodies, proved a sensitive and specific indicator for DNA. Quantities as low as 5 μg could be determined readily. Seven different patients with SLE were found who showed the presence of DNA in the serum at some time in their course, particularly during the febrile periods. In each instance the precipitin line showed a reaction of identity with purified DNA and was eliminated with DNAase. The highest levels could be confirmed by chemical assay for DNA. Studies in one patient were particularly revealing. On admission large amounts of DNA antibodies were found in the serum, which persisted for several weeks, and then declined, and were directly followed by the appearance of free DNA, which was found in six consecutive samples taken over a 5-week period. The latter sera containing free DNA gave strong precipitin reactions with the earlier sera containing DNA antibodies. It appeared likely that at some point overlap between circulating DNA and DNA antibodies occurred. Analyses for DNA in the sera of normals and patients with other diseases have been initiated; of 110 sera examined, three from seriously ill patients have been positive. The results obtained demonstrate the presence of one potential antigen in the blood, which in patients with SLE might complex with specific antibody and, together with complement, constitute a mechanism for renal injury in this disease.

Protection against Lethality of *E. coli* Endotoxin with O-Antiserum. WILLIAM TATE, HERNDON DOUGLAS, AND ABRAHAM BRAUDE,† Pittsburgh, Pa.

Passive transfer of serum from resistant animals protects against pyrogenic doses (0.0001 mg) of endotoxin in rabbits but not consistently against lethal doses (0.1 mg) in mice. Since the ratio of lethal to pyrogenic dose is approximately 1,000 to 1, it is possible that the protective power of immune serum is overcome by excess endotoxin in normal mice. A study was therefore made to determine if antiserum would protect against the minute doses (0.0001 mg) of endotoxin that kill adrenalectomized mice. Rabbit O-antiserum was obtained 10 days after 1 intravenous injection of 10^8 boiled cells of *E. coli* O:113 and found by density gradient analysis to contain exclusively 19 S O-antibody giving 1 agar-precipitin band with O:113 endotoxin. One ml of antiserum was inoculated subcutaneously into 28 adrenalectomized CF1 mice, and the animals were challenged 18 hours later with 1 μg *E. coli* O:113 endotoxin intravenously. Only 4 of the 27 mice given O-antiserum died, whereas 25 out of 28 controls given nonimmune rabbit serum died. In adrenalectomized SPF (specific pathogen-free) mice the MLD_{100} was < 0.25 μg in controls given saline or nonimmune serum and > 1.0 μg in mice given O-antiserum. A delay in mortality was even more striking than over-all protection; no deaths occurred in the protected animals in the first 9 hours when 45% of the controls had died. These results indicate that O-antiserum containing 19 S antibody can protect against the small lethal doses of endotoxin to which adrenalectomized mice are susceptible.

Parathyroid Hormone and Bone Mobilization *In Vitro*. ALAN TENENHOUSE, RICHARD MEIER, AND HOWARD RASMUSSEN,* Madison, Wis., and Philadelphia, Pa.

One of the most characteristic effects of parathyroid hormone administered *in vivo* is the mobilization of calcium from bone. The study of this phenomenon *in vitro* has been complicated by the complexities of the system employed and the long periods of incubation required. It has been possible to develop a simple *in vitro* system to study this phenomenon by incubating Ehrlich ascites tumor cells with dead Ca^{45}-labeled bone chips prepared from mouse calvaria. The incubation of the cells with the bone leads to Ca^{45} release that reaches a constant level

after 3 to 5 hours. Addition to parathyroid hormone (60 μg per ml) leads to a significant (75 to 200%) increase in Ca^{45} release. This effect of hormone requires the presence of viable cells. It is possible to separate the response into two phases. Initial incubation of the cells with hormone in the absence of bone (phase I) leads to the release of a heat-stable substance, which when incubated with the bone chips in the absence of cells (phase II) leads to calcium mobilization. The substance is neither citrate nor lactate. Concentrations of hormone as low as 7 μg per ml are sufficient to bring about these effects. The induction phase (phase I) requires inorganic phosphate and 1 to 2 hours incubation for maximal production of the bone mobilizing substance. The mobilization phase (phase II) is a pH dependent response with a pH optimum of pH 9.1, which requires 15 to 30 minutes for maximal calcium mobilization. A number of other peptide hormones have no effect in this system. The studies thus suggest that Ehrlich ascites cells respond to parathyroid hormone with the release of a heat-stable factor that can lead to the mobilization of calcium from bone.

Tetany Induced by Hyperventilation in Anxious Subjects and the Effect of Adrenergic Blockade. W. C. THOMAS, JR., F. C. SCHWALBE, JR., J. R. GREEN, JR., AND A. M. LEWIS, Gainesville, Fla. (introduced by S. P. Martin †).

A biochemical explanation for the susceptibility to hyperventilation tetany of certain anxious, but otherwise healthy, subjects is unknown. The present study was initiated to determine whether or not such individuals are latent, chronic overbreathers who are thus rendered unduly responsive to overt hyperventilation. Analyses of arterial plasma obtained before and after induced hyperpnea from 20 normal subjects and 11 patients having anxiety and a tendency to hyperventilate revealed essentially identical basal values for pH, P_{CO_2} phosphorus, and potassium with comparable changes in these components on overbreathing by all individuals tested. However, only the anxious patients developed tetany during the brief (maximum, 3 minutes) hyperpnea, and this occurred at a higher P_{CO_2} than has been determined to be critical for development of tetany during hyperventilation by normal subjects. That catecholamines possibly promoted tetany in the above patients was suggested by ancillary observations of isolated mammalian nerves indicating that spontaneous, repetitive discharges (tetany) occurred on addition of 1 μg per L epinephrine to an incubation medium maintained at pH 7.60 and containing 6 mEq per L potassium. However, arterial plasma concentrations of epinephrine did not differ in tetany prone patients and normal subjects either before or after hyperventilation. In addition, vanillyl mandelic acid excretion was not significantly increased in the anxious subjects. Despite these findings, administration of guanethidine (20 mg per day) to five anxious subjects reduced to normal their susceptibility to tetany on overbreathing. Also, in a single patient with hypocalcemia, all manifestations of tetany disappeared on treatment with guanethidine. The foregoing observations indicate that certain anxious patients constitute a biochemically distinct group in whom hyperventilation induced tetany occurs at higher P_{CO_2} values than in normal subjects. Although no evidence of unique catecholamine metabolism was detached in the tetany prone patients, the observations suggest that epinephrine does contribute to tetany of various causes.

A Comparison of Medium and High Intakes of Sodium as a Sole Treatment for Severe Postural Hypotension. LOUIS TOBIAN * AND JUDITH MEULI, Minneapolis, Minn.

Dietary salt was varied during four periods in a patient with severe tabetic postural hypotension. The patient was progressively tilted each day to find the angle of tilt that lowered his systolic pressure to 65 mm. During 14 days of period I, the patient ate 7 g NaCl daily and required an average tilt of 7.0 degrees to reach 65 mm. During 18 days of period II, he ate 30 g NaCl daily and required an average tilt of 26.1 degrees. During 19 days of period III, he ate 7 g NaCl daily plus placebo pills and required 12.5 degrees. During 20 days of period IV, he ate 37.2 g NaCl daily and required 38.0 degrees of tilt (significantly higher than periods I, II, and III). Symptoms closely paralleled tilt data. He could walk without fainting during periods II and IV but not during periods I and III. His daily supine blood pressures averaged 98/59 in I, 144/83 in II, 120/69 in III, and 154/85 in IV. Plasma volume was 3,422, 4,125, 3,326, and 3,700, and blood volume was 4,720, 5,156, 4,964, and 5,136 in successive periods. Supine cardiac output determined by an impedance method averaged 5.1, 5.9, and 5.0 L in periods I, II, and III. If peripheral resistance is mean arterial pressure divided by cardiac output, his supine peripheral resistance averaged 17.4, 18.0, and 19.6 U in periods I, II, and III. A high salt intake greatly alleviated symptoms and objectively increased tolerance to tilting when compared to a normal salt intake. It also significantly raised supine blood pressure. The effects of a high salt intake can be partially explained by an increase in blood and plasma volume and an increase in cardiac output. The high salt intake is an effective treatment for severe postural hypotension.

Role of Sympathetic Nervous System in Lipid Mobilization during Exercise. W. G. TROYER, JR., S. J. FRIEDBERG, AND M. D. BOGDONOFF,* Durham, N. C.

Previous work has demonstrated that plasma free fatty acid (FFA) mobilization is markedly increased during exercise. In order to assess the role of the sympathetic nervous system in this process, volunteer subjects were exercised for 45 minutes at 65 w during continuous blockade of beta adrenergic receptor sites induced by propranolol and during a control session. The dose of propranolol used has been previously shown to inhibit the

FFA mobilizing action of a norepinephrine infusion (8.8 μg per minute) for 60 minutes. Serial determinations of pulse rate, blood pressure, and arterial FFA were obtained before, during, and after exercise. Adrenergic blockade was achieved in all subjects as evidenced by the diminished rise in pulse and blood pressure during exercise. Mean control and experimental FFA values obtained before exercise were not significantly different. During and after exercise the control FFA values were always greater than the blockade determinations. Analysis of variance of the differences was highly significant ($p < 0.001$). During exercise the mean percentage increase of plasma FFA was + 50% (control), whereas the blockaded group showed a mean percentage decrease of − 27% from pre-exercise base-line values ($p < 0.01$). These results have been interpreted to indicate that the sympathetic nervous system has a major role in the magnitude of lipid mobilization that is stimulated by exercise.

Augmented Natriuretic Response to Acute Sodium Infusion in Normotensive Patients with Chronic Pulmonary Disease. CARLOS A. VAAMONDE, LILIANA S. VAAMONDE, HUGO J. MOROSI, AND SOLOMON PAPPER,* Albuquerque, N. M.

There is evidence that elevation of blood pressure itself may somehow be responsible for the well-established exaggerated natriuresis that hypertensive patients exhibit in response to acute administration of sodium. Although the mechanism for this remains unknown, one of the possibilities is that elevation of the blood pressure "elsewhere" in the body, not requiring elevation of pressure in the systemic or renal circulation, might stimulate an indirect mechanism for sodium excretion. To examine this possibility the natriuretic response to acutely administered sodium was studied in three male patients with chronic pulmonary disease (bronchitis and emphysema), normal systemic blood pressure, and evidence of pulmonary hypertension and compared to three male normotensive control subjects without pulmonary disease. No subject had evidence of hepatic, renal, or cardiac disease. They received a diet containing 150 mEq of sodium daily. They remained recumbent from 8:00 a.m. until 3:00 p.m. and voided spontaneously at 30-minute intervals. The subjects were studied on two separate occasions: *1*) blank day—no manipulation was undertaken other than venipuncture to collect blood; *2*) saline day— 2 L of hypotonic (125 mEq per L) sodium chloride-lactate solution was infused intravenously from 10:30 a.m. to 12 noon. The maximal increase of sodium excretion from the mean preinfusion rate on the saline day, as compared to the blank day, was an average of 443% greater in the patients with chronic pulmonary disease than in the control subjects. The data demonstrate that patients with normal systemic blood pressure and chronic pulmonary disease with evidence of pulmonary hypertension exhibited an augmented natriuretic response to the acute administration of sodium when compared with control subjects without pulmonary disease. The mechanism whereby this augmented natriuresis occurs and the possible relationship to the mechanisms in systemic hypertension are not known.

Heme and Porphyrin Synthesis in Refractory Sideroblastic Anemia. J. D. VAVRA AND S. A. POFF, St. Louis, Mo. (introduced by Elmer B. Brown*).

Refractory sideroblastic anemia, a disease seen predominantly in elderly males, is characterized by normochronic or hypochromic anemia, normoblastic hyperplasia of the marrow, elevation of serum iron, accumulation of large quantities of iron within mitochondria of erythroblasts, and refractoriness to therapy. Previous reports suggest that defects in several steps of heme synthesis may be important in the pathogenesis of the anemia. Eight patients with this disease have been studied in an attempt to identify such a defect. The capacity of blood from these patients to synthesize heme and porphyrins from optimal concentrations of glycine, aminolevulinic acid (ALA), porphobilinogen, and protoporphyrin was evaluated by incubating hemolysates with these C^{14}-labeled substrates or Fe^{59} for 4 hours and quantitating the uroporphyrin (Uro), coproporphyrin (Copro), protoporphyrin (Proto), and heme formed. The quantity of heme formed determined by C^{14}-substrate incorporation was 3- to 10-fold greater than that measured by Fe^{59} incorporation, probably reflecting the iron overload of erythroblast mitochondria. All hemolysates synthesized 20- to 50-fold more porphyrins than heme from ALA. Of these porphyrins only 1 to 8% were Proto, the remainder being Uro and Copro. The capacity of bone marrow hemolysates to synthesize a higher percentage of Proto (24 to 50% of total porphyrin) indicated that conversion of Copro to Proto was not defective. Although heme was apparently synthesized from all substrates in quantities comparable to other patients we have reported, the total quantity was small, suggestive of depressed conversion of Proto to Heme. However, assays of heme synthetase in the erythrocytes of these patients were statistically similar to other patients with comparable numbers of reticulocytes. Assays of ALA dehydrase (reported by others to be depressed) were also normal. These studies fail to demonstrate directly a defect in heme synthesis between ALA and heme. Assays of the remaining step, ALA synthetase, are being made.

Regional Vasomotor Activity of the Pulmonary Vasculature. HENRY N. WAGNER, JR.,* DONALD E. TOW, AND VINCENT LOPEZ-MAJANO, Baltimore, Md.

In the past, demonstration of vasomotor activity of the pulmonary vasculature in man has been difficult, primarily because of technical complexity and problems associated with accurate measurement of the pressure gradient across the pulmonary vascular bed. We have used radioisotope scanning to measure regional pulmonary blood flow in man and dog and have been able to determine the effect of local hypoxia and vasoactive agents. At appropriate times following the intravenous injection of I^{131}-labeled macroaggregated human serum

albumin, lung scanning was performed. The albumin particles were sufficiently large (10 to 100 μ) to be lodged in the pulmonary capillary bed before being metabolized. The regional concentration of radioactivity has been shown to be directly related to local blood flow and can therefore be used for its measurement. The partition of pulmonary blood flow was quantified both by densitometry and by measuring the count rates in various regions. Unilateral hypoxia was produced in man by ventilating a single lung with 100% nitrogen for 7 minutes while the other lung was ventilated with 100% oxygen. This resulted in an average decrease of 42% in pulmonary blood flow to the involved lung. Vasoactive agents were administered to dogs through a cardiac catheter placed in various branches of the pulmonary artery. Local decreases in relative blood flow were measured in dogs after the local infusion of serotonin. Increases in regional blood flow followed infusion of acetylcholine. The scanning technique has provided a unique, technically simple, and safe approach for the delineation and quantification of local vasomotor activity of the pulmonary circulation.

Quantitation of Gastrointestinal Protein Loss with Copper^{67}-labeled Ceruloplasmin. THOMAS A. WALDMANN, ANATOL G. MORELL, R. DEAN WOCHNER, AND IRMIN STERNLIEB,* Bethesda, Md., and New York, N. Y.

Pathologic loss of serum proteins into the gastrointestinal tract resulting in hypoproteinemia has been demonstrated by several methods using labeled macromolecules. The possibility that a similar although quantitatively smaller loss of proteins may normally occur has been the subject of considerable controversy since available methods have not been sufficiently quantitative. We report here the results of studies indicating that Cu^{67}-ceruloplasmin can be used to accurately measure gastrointestinal protein loss. Absorption of the Cu^{67} moiety after oral administration of the labeled protein was found to be minimal in men, dogs, and rats. Distribution and survival of intravenously administered I^{131}-ceruloplasmin and Cu^{67}-ceruloplasmin were comparable in the dog with approximately 75% of the body pool of ceruloplasmin within the intravascular space and a mean of 21% of the circulating pool catabolized per day. These studies, considered with earlier observations that exchange of ceruloplasmin copper with ionic copper does not occur *in vivo*, indicate that the Cu^{67} label may be used to study the metabolic fate of the entire ceruloplasmin molecule. In dogs infused with Cu^{67}-ceruloplasmin the daily fecal excretion of Cu^{67} represented 1.0 to 1.9% of the circulating pool of Cu^{67}-ceruloplasmin. This gastrointestinal loss accounted for only 6 to 11% of the over-all catabolism of ceruloplasmin. Thus, Cu^{67}-ceruloplasmin appears to be a satisfactory macromolecule for quantitating gastrointestinal protein loss for these reasons: *1*) Metabolism and distribution are not altered by the labeling procedure. *2*) The Cu^{67} is an integral part of the protein throughout the molecule's life. *3*) Absorption of the Cu^{67} from the gastrointestinal tract and secretion of ceruloplasmin copper into the gastrointestinal tract are both negligible. This study also demonstrates that bulk loss of serum protein into the gastrointestinal tract does not appear to be a significant factor in protein metabolism in the normal dog.

***In Vitro* Protein Synthesis in Isolated Rat Glomeruli.** W. GORDON WALKER,* Baltimore, Md.

In most diffuse renal diseases the glomeruli are usually more damaged than the tubular cells. This suggested that glomeruli may be more active metabolically, hence more susceptible. Accordingly the following studies of glomerular protein metabolism were undertaken. It was first necessary to devise a method for studying metabolism of isolated glomeruli. To accomplish this, glomeruli were isolated from rat renal cortical homogenates by screening through stainless steel wire mesh of graded sizes. Yields of 20,000 to 30,000 glomeruli per kidney were obtained. Viability, judged by O_2 consumption, was maintained for more than 4 hours after isolation. When incubated with C^{14}-lysine in Krebs-Ringer-phosphate, both glomeruli and washed cortical homogenate (mostly tubular cells) preparations incorporated lysine into soluble and particulate fractions of cell protein. Rate of incorporation into glomerular protein was always greater than in cortical homogenate. Average data for specific activities in nine experiments (3-hour incubation) were: glomeruli, 5,300 $\pm$ 255 (SEM) cpm per mg protein; tubules, 762 $\pm$ 40 (SEM) cpm per mg protein. These data suggest more rapid protein synthesis in glomeruli than in tubules. This finding is supported by demonstration of more rapid H^3-cytidine incorporation in ribonucleic acid (RNA) of glomeruli than in tubular cell RNA (specific activity glomerular RNA/specific activity tubular RNA = 700/75). RNA content of glomeruli per milligram of protein was significantly greater than that for tubular cells (0.071 $\pm$ 0.007 for glomeruli/0.028 $\pm$ 0.002 for tubules). All of the above data indicate more rapid protein synthesis by the glomeruli than by tubular cells. Puromycin inhibited amino acid incorporation into protein, but had no effect upon cytidine incorporation into RNA. The experiments point to greater protein synthesis, and perhaps greater metabolic needs by glomeruli than by tubules, suggesting that this may be related to glomerular susceptibility to disease.

Radiomagnesium Kinetics in Normal and Uremic Subjects. STANLEY WALLACH, JUAN E. RIZEK, AND ALEXANDRA DIMICH, Brooklyn, N. Y. (introduced by David M. Kydd †).

Analog computer analyses of plasma specific activity (SA) data obtained after the intravenous injection of Mg^{28} (200 μc per mEq) verified the relation: $SA = Ae^{-at} + Be^{bt} + Ce^{-ct}$. Consequently, a three compartment model with provision for irreversible removal of Mg^{28} from extracellular fluid by excretion and other processes was assumed for digital computer analyses of curves of

plasma magnesium specific activity of six normal, two normomagnesemic, and four hypermagnesemic uremic subjects. In the normal subjects, the extracellular magnesium of 0.31 mEq per kg equilibrated with a tissue magnesium compartment (Q_1) of 0.18 mEq per kg within 13 minutes of Mg^{28} injection. Two additional tissue magnesium compartments (Q_2 and Q_3), having sizes of 1.73 and 0.17 mEq per kg and exchange rates of 0.13 and 0.49 mEq per kg per hour, respectively, were indicated by the computer analyses. A fourth tissue magnesium compartment (Q_4) of indeterminate size with an influx rate of 0.035 mEq per kg per hour but a negligible efflux rate was also disclosed. The presence in renal tubular cells of a magnesium fraction having the characteristics of Q_4 and which exchanged with tubular urine magnesium was suggested by urine specific activities 20 to 40% higher than those of plasma throughout all studies. Similar results were obtained in normomagnesemic uremia. In hypermagnesemic uremia, the extracellular magnesium was 0.42 mEq per kg, Q_1 was increased by 200%, and Q_2 and Q_3 were of normal size. Fractional influx rates of Q_2, Q_3, and Q_4 were decreased by 50%, but because of hypermagnesemia, total influx rates were within the normal range. These data indicate that hypermagnesemia influences the mechanisms responsible for cellular transport of magnesium so that fractional influx of cell magnesium is reduced. In hypermagnesemic uremia, although one tissue magnesium fraction (Q_1) is increased, the fractional influx of the majority of exchangeable cell magnesium fractions is reduced, and cell magnesium concentrations are thereby maintained at normal levels.

The Nature of Adrenergic Influence on Sodium Transport in Frog Skin. CHARLES O. WATLINGTON, Richmond, Va. (introduced by G. Watson James III *).

Epinephrine alters membrane excitation in cardiac tissue and smooth muscle and influences renal excretion of electrolytes. Its dual physiologic effects result from stimulation of alpha or beta adrenergic receptors. A difference in ion transport effect related to the two receptors is unknown. This study demonstrates such a difference in frog skin, using the technique of Ussing and Zerahn *in vitro* and a recently developed adaptation *in vivo*. In these methods, short-circuit current (I_s) measures net active ion transport, Na^{22} outflux is an index of Na^+ permeability, and influx is a function of both active transport and permeability. Skin resistance is the ratio of open circuit potential to I_s. Phenoxybenzamine was used for alpha blockade, pronethalol for beta blockade, and isoproterenol for beta adrenergic stimulation. Intravenous epinephrine increased I_s, but Na^{22} flux was unchanged. The I_s alteration was probably due to active Cl^- transport outward related to mucous gland stimulation, as reported by others in isolated skin. Intravenous pronethalol decreased I_s and net Na^+ transport equally. Skin resistance increased and Na^{22} outflux decreased. These effects occurred *in vitro* only if the inside of the skin was exposed to epinephrine as well as to pronethalol. They were reversed with phenoxybenzamine. Intravenous isoproterenol increased I_s and Na^{22} outflux and decreased resistance. Isoproterenol alteration of I_s and resistance was blocked *in vitro* by pronethalol. It is concluded that alpha adrenergic stimulation decreases and beta stimulation increases Na^+ permeability in frog skin. These findings suggest that similar mechanisms may mediate adrenergic effects in other ion transport systems.

Globin Synthesis in Thalassemia. D. J. WEATHERALL AND M. A. NAUGHTON, Baltimore, Md. (introduced by C. L. Conley †).

Indirect evidence has suggested that some forms of thalassemia result from inherited defects in the synthesis of either the α- or β-chains of the globin fraction of hemoglobin. In this study, to further characterize these defects, the *in vitro* incorporation of radioactive amino acids into the α- and β-chains has been compared in blood samples from thalassemic and nonthalassemic individuals. Blood rich in reticulocytes was obtained from 15 individuals with the following conditions: hereditary spherocytosis, acquired hemolytic anemia, congenital nonspherocytic hemolytic anemia, thalassemia major, sickle-cell thalassemia, and F-thalassemia. Washed cells were suspended in an amino acid mixture containing C^{14}-leucine or C^{14}-valine and samples removed at various intervals and immediately frozen. Hemoglobin fractionation was achieved by Sephadex filtration, dialysis, column chromatography, and starch gel and starch block electrophoresis. The α- and β-chains were separated by hybridization with canine hemoglobin, countercurrent distribution, or column chromatography; their purity was checked by fingerprinting; and their specific activities were determined. In all nonthalassemic samples the specific activities of the α- and β-chains of hemoglobin A were similar at incubation times of more than 3 minutes. In all thalassemic samples the specific activity of the α-chain of hemoglobin A was 2 to 5 times that of the β-chain at incubation times varying from 3 minutes to 5 hours. The specific activities of the α-chains and γ-chains of associated hemoglobin F in thalassemia major were similar to each other, as were the α-chains and β^S-chains of hemoglobin S in sickle-cell thalassemia. Since the specific activities of the α- and β-subunits of hemoglobin A do not equalize after a relatively long period of incubation of thalassemic cells there must be a marked defect in β-chain synthesis with a defect in association of completed β-chains with α-chains. These findings are more in keeping with a structural rather than rate-controlling genetic defect as a basis for this disorder.

The Role of Cellular Hemoglobin Concentration in the Control of Human Erythrocyte Volume in Plasma. ROBERT I. WEED AND ANTHONY J. BOWDLER, Rochester, N. Y. (introduced by S. N. Swisher †).

It is known that the distribution curve of red cell volumes, as measured by an electronic particle counter, is nongaussian, being skewed toward the larger cells. Since hemoglobin concentration within some red cells may reach

as high as 50 g per 100 ml at the tonicity of plasma, this investigation was undertaken in order to evaluate whether the anomalous osmotic coefficient of hemoglobin might be of importance in determination of red cell volume. Probability analysis of human erythrocyte volume distribution at the tonicity of plasma suggests the presence of two major populations. However, no larger population with a distinct second modal peak was seen unless some cells underwent swelling after standing in the diluent more than 20 minutes. Swelling of red cells with hypotonic salt solutions alters the volumes to that of a normal distribution pattern. Similarly, the study of hemoglobin-free ghosts reveals a volume distribution curve that is very close to gaussian. It is concluded, therefore, that the cells within a red cell population that have a high hemoglobin concentration are larger in size than would be predicted from a normal volume distribution pattern because of the anomalous osmotic coefficient of hemoglobin, and actually appear as a second population at the tonicity of plasma. Thus, the asymmetry of the RBC volume distribution curve resides with the small, older cells, rather than the larger cells, as has been suggested.

A Direct Effect of Steroids on the Permeability of Lipid Membranes (Liposomes). GERALD WEISSMANN,* GRAZIA SESSA, MALCOM STANDISH, AND A. D. BANGHAM, New York, N. Y., and Cambridge, England.

Cortisone, cortisol, and chloroquine stabilize lysosomal membranes. In direct contrast, diethylstilbesterol, progesterone, and most 5 β-H steroids (e.g., etiocholanolone), but not their 5 α-H isomers (e.g., androsterone), render lysosomes permeable. Lysosome-disruptive steroids are also hemolytic; a model system was therefore devised to test whether steroids interacted with lipids common to several biologic membranes. Lecithin, dicetylphosphate, and cholesterol (70:20:10) were induced to form concentric, lamellar spherulites in aqueous dispersion. These artificial liposomes were formed to sequester cations (K^+, Na^+), anions (Cl^-, $Cr_2O_4^=$, $PO_4^=$), glucose, and glycine from 0.145 M solutions in water. Leakage of sequestered marker molecules was determined after dialysis had removed any unenclosed excess from the liposomes. Hemolytic and lysosome-disruptive steroids (etiocholanolone > progesterone > lithocholic acid > pregnanolone), but no 5 α-H isomer, induced concentration-dependent leaks of marker molecules (e.g., 78% of total cation, 37° C, 60 minutes, at 5×10^{-4} M etiocholanolone). Diethylstilbestrol, Triton X-100, streptolysin S, lysolecithin, gramicidin, and icterogenin also rendered liposomes more permeable, whereas addition of cortisone or cortisol acetate, or chloroquine, retarded leakage of marker molecules. Cortisone could be preincorporated into liposomes: membranes containing an optimum of 1% cortisone proved more resistant to subsequent disruption. To determine whether these effects of steroids might regulate enzyme activity, liposomes filled with glucose were placed in glucose-free, isotonic medium containing excess glucose oxidase, peroxidase, and chromogen. Addition of disruptive steroids, with leak of substrate, visibly activated the coupled enzyme system. These studies *1*) describe synthetic lipid spherulites, the membranes of which resemble those of lysosomes or erythrocytes in their response to steroids or other agents, and *2*) suggest that effects of steroids on the permeability of cells and organelles may be explained by their direct interaction with surface lipid, independent of sugars, proteins, or cell metabolism.

Mechanism of Action of Dextran. ROE E. WELLS, JR., Boston, Mass. (introduced by Eugene C. Eppinger †).

Studies of the effects of dextran upon erythrocyte aggregation and blood viscosity *in vitro* were conducted to determine whether the ability of dextran to improve flow within small vessels is due to hemodilution or to the dispersion of aggregated red cells or both. Samples of freshly drawn heparinized whole blood were studied before and after the addition of two different dextran solutions (6% in saline, average mol wt 75,000, and 10% in saline, average mol wt 40,000) to produce a 20% concentration of dextran. Dextrans were added to samples before and after addition of fibrinogen (final fibrinogen concentration, 750 mg per 100 ml). Measurements were made of viscosity of whole blood and plasma at low rates of shear (0.1 to 20 sec^{-1}) sedimentation rate (ESR), total protein, and fibrinogen concentration. Photomicrographs were taken of the cell sedimentation forms. Control studies were made with equivalent volume dilutions of isotonic NaCl and albumin solutions (7.5 g per 100 ml). The addition of dextran to blood reduced viscosity values by 25 to 40% with similar reductions in total protein and hematocrit. ESR were slightly increased. The addition of fibrinogen to whole blood caused a 20 to 30% increase in viscosity and a 50 to 60% increase in ESR. Dextran added to the fibrinogen elevated sample and reduced viscosity 5 to 20%, but ESR varied $\pm$10% of initial values. Fibrinogen added to samples of dextran in whole blood showed viscosity increases of 25 to 50%, and ESR increased an additional 5 to 15%. Albumin decreased viscosity to a greater extent than dextran, but less than saline. Photomicrographs did not reveal dispersion of any aggregates produced by fibrinogen or their prevention when dextran preceded fibrinogen. Dextran appeared to interact with the fibrinogen to form amorphous coagula and sometimes frank clots.

Determination of Serum Thyroxine Levels in Man by Thin Layer Chromatography. CHARLES D. WEST,* VIRGINIA J. CHAVRÉ, AND MARTHA WOLFE, Salt Lake City, Utah.

A specific method for measuring serum thyroxine (T_4I) levels has been developed that provides an extremely reliable and practical test for thyroid function, even in patients who have received iodinated or mercurial compounds. The average normal serum T_4I level (5.4 μg per 100 ml) and the normal range (3.2 to 7.9 μg per 100 ml) were established in 43 euthyroid control subjects. The

determination of serum T_4I levels was evaluated as a thyroid function test in a total of 339 patients of whom 66 were hypothyroid, 64 were hyperthyroid, and 209 were euthyroid. The T_4I levels were lower than the normal range in 98% of the hypothyroid patients and higher in 97% of the hyperthyroid patients. Ninety-four per cent of the euthyroid patients had normal values. The T_4I procedure has a great advantage over PBI and BEI methods in that neither iodine nor mercury in any form tested interferes significantly with the determination. Ninety-three per cent of 152 patients with elevated PBI levels resulting from the administration of KI, Telepaque, Hypaque, Dionosil, or Pantopaque had T_4I levels that agreed with the clinical evaluation of thyroidal status. Fifteen of the 152 patients were hypothyroid, 2 were hyperthyroid, and 135 were euthyroid. Persistently high PBI and normal T_4I levels were encountered in 8 euthyroid patients who had not received iodine. Thyroiditis was probably responsible for the PBI-T_4I dissociation in 2 patients, but no known cause was found in the others. With laboratory errors excluded, abnormal T_4I levels were found in euthyroid patients only when an abnormality in thyroxine binding by plasma proteins was very likely. Thus, high T_4I levels were observed in pregnancy and patients treated with estrogens, and low values, in chronically ill patients with hypoalbuminemia and macrogammaglobulinemia and patients treated with Dilantin.

Cholesterol Biosynthesis in Circulating Blood Cells. M. P. Westerman, N. Takeuchi, N. Iritani, and W. W. Wells, Pittsburgh, Pa. (introduced by J. D. Myers †).

Previous studies have shown that the *in vitro* synthesis of neutral lipid and phospholipid in circulating blood cells occurs predominantly in white cells with lesser degrees found in platelets and reticulocytes. In view of our recent demonstration that the bone marrow is an important extrahepatic site of cholesterol synthesis, we have examined the *in vitro* and *in vivo* synthesis of cholesterol in the formed elements of whole blood with various types of hematopoietic stimulation. *In vitro* studies were performed by incubating whole blood obtained from phenylhydrazine-treated or phlebotomized rabbits with acetate-1-C^{14}. *In vivo* investigations were made on whole blood samples obtained from acetate-1-C^{14} injected rabbits previously treated with phenylhydrazine or phlebotomies. Blood was separated into red cell, white cell, and platelet-rich fractions, cholesterol isolated, and the specific activity determined. *In vitro* studies in which cell separation was obtained by differential centrifugation showed that the coefficient of correlation (r) between the leukocyte count and the cholesterol synthetic activity was 0.942 in control animals. In phlebotomized animals, $r = 0.674$ when comparing cholesterol synthesis to leukocytes, and $r = 0.687$ when cholesterol synthesis is compared to reticulocyte counts. The relative ability of leukocytes: reticulocytes:platelets to synthesize cholesterol was estimated at 428:4.3:1. Mature erythrocytes synthesized negligible cholesterol. These studies were confirmed by cell separation using dextran sedimentation. *In vivo* studies showed increased cholesterol specific activity in phenylhydrazine and phlebotomized animals that correlated well with the numbers of leukocytes and reticulocytes observed.

Two Different Interferons Induced in Human Leukocyte Cultures by Viral Infection and Phytohemagglutinin. E. Frederick Wheelock, Cleveland, Ohio (introduced by John H. Dingle †).

During attempts to culture peripheral leukocytes from persons with and without viral infections it was observed that phytohemagglutinin (PHA), an extract of the kidney bean, *Phaseolus vulgaris,* used to agglutinate red blood cells in the preparation of cultures, induced the synthesis of a virus-inhibitor in leukocytes from normal individuals. The properties of this inhibitor were determined and compared with the virus inhibitor, i.e., interferon induced in leukocytes by infection with Newcastle disease virus (NDV). Both inhibitors when incubated with cultures before virus inoculation protected human lung cells but not hamster kidney cells against Sindbis virus cytopathic effects. Neither inhibitor had a direct virus neutralizing effect. Both inhibitors were nondialyzable, nonsedimentable proteins destroyed by trypsin but unaffected by ribonuclease, desoxyribonuclease, or periodate. The NDV-induced interferon was stable at pH 1 to 11 and at 56° C, but the PHA-induced inhibitor was stable only at pH 3 to 9 and was destroyed at 56°. Thus the PHA-induced virus inhibitor is an interferon that is labile to heat and to extremes of pH. The interferon-inducing property of PHA was not affected by either trypsin or nucleases, alone or in sequential combinations, but was destroyed by periodate and 100° C. It was associated with the leukoagglutinating factor of PHA and known to stimulate RNA synthesis and induce formation of blastoid cells and mitosis in leukocyte cultures. Interferon production was detected in leukocyte cultures 2 to 3 hours after treatment with PHA, and maximal titers were reached by the fourth hour. The relationship between PHA-induced interferon and RNA synthesis is being studied to determine whether or not the interferon is produced in response to stimulation of RNA synthesis. Since viral interferon is known to inhibit virus replication by blocking RNA synthesis, PHA-induced interferon may be a cellular mechanism for regulation of RNA synthesis.

Erythropoietin and Erythropoiesis Inhibitor Activity in Man. W. H. Whitcomb, M. Moore, R. Dille, L. Hummer, and R. M. Bird, Oklahoma City, Okla. (introduced by R. H. Bayley †).

The humoral regulation of erythropoiesis has been studied since Erslev's observations in 1953. It is acknowledged that erythropoietin (ESF) induces an increase in erythropoiesis, but the precise role of this factor in the

over-all control of erythropoiesis is not clear. In the present study evidence of erythropoietin activity was identified in healthy subjects exposed to hypoxia and in anemic subjects breathing normal air. Evidence of the presence in blood of an inhibitor of erythropoietin activity was found in polycythemic subjects. In healthy humans exposed to 12,000 feet simulated altitude for 96 hours, plasma erythropoietin reached a peak at 36 hours equivalent to one-third of an ESF U per ml of whole plasma when assayed concurrently with erythropoietin standard B in the plethoric mouse system. This activity then subsided, despite continued exposure to altitude. With hypoxia used for the production of endogenous erythropoietin in the plethoric mouse, normal plasma and saline did not alter the erythropoietic response. The plasma from anemic patients potentiated the response, however, and the plasma of patients with primary polycythemia vera strikingly inhibited it. The ranges of erythrocyte iron incorporation in groups of assay animals receiving *a*) saline or normal plasma, *b*) anemic plasma, and *c*) polycythemia vera plasma were 8.6 to 10.8%, 11.5 to 22.1%, and 3.4 to 7.8%, respectively. Subjects phlebotomized at 24-hour intervals showed lesser amounts of inhibitory activity as the polycythemia subsided. These data indicate that a wave of erythropoietin activity occurs in man during exposure to 12,000 feet of simulated altitude for 96 hours and that erythropoietic inhibitory activity occurs in untreated subjects with polycythemia vera.

Calcitonin Activity in Hereditary Osteopetrosis. J. Earle White and Thomas M. Ahmann, Columbia, Mo. (introduced by C. Thorpe Ray †).

Four generations of a kindred having radiographic osteopetrosis manifested as a nonsex-linked dominant trait are undergoing studies of pathogenesis and treatment. None have visible deformities of bone nor referable symptoms except the four affected females, all of whom report symptoms suggesting hypocalcemia. Normal levels of plasma calcium in the morning fall gradually to a low of 6 mg per 100 ml by midnight, just before seizures in the propositus. Fluoride and heavy metal concentrations in water supplies and urine were repeatedly normal. In normal and affected kindred, parathyroid activity on bone and kidney was estimated by urinary hydroxyproline excretion and phosphate clearances, respectively. Plasma calcitonin levels were assayed in rats. On a gelatin-free diet, hydroxyproline excretion on control days was normal except for low values in affected adult males. Normal diurnal changes in phosphate clearance occurred in all subjects, although consistently high values, and lower plasma calciums and phosphates, distinguished the affected adult males. Parathormone infusion induced phosphodiuresis in all subjects, but only those with osteopetrosis responded also with an immediate and prolonged fall in plasma calcium, phosphate, and lactate. Initially low urine calcium excretions were unchanged. Calcitonin activity in plasma from the propositus was minimal in the morning but increased progressively, becoming maximal at midnight. Most marked activity was found after calcium infusion (10 mg per kg per 4 hours), although it failed to induce hypercalcemia. Renal responsiveness to parathyroid hormone diurnally and exogenously and to calcium infusions was normal in all kindred tested. Abnormal hypocalcemic responses to the same challenges, together with failure to alter urinary hydroxyproline, suggest that bones of the osteopetrotic subjects respond excessively to calcitonin and are refractory to parathyroid hormone.

Myelomas Deficient at the Pepsin Site, a Buried Determinant of Human IgG. Ralph C. Williams, Jr., and Thomas G. Lawrence, Minneapolis, Minn. (introduced by Samuel Schwartz †).

The antigen within human IgG uncovered by enzymatic digestion with pepsin constitutes a true buried determinant since anti-γ-globulin factors reacting with this pepsin site can be inhibited by pepsin-digested human IgG but not by whole Fr II. Some inhibition has also been achieved with isolated IgG H-chains. Twenty isolated IgG myeloma proteins and 6 IgA myeloma proteins were digested with pepsin at pH 4.1 and studied for their inhibitory capacity of 8 different 7 S anti-γ-globulin factors from human sera known to react with the pepsin site. Five IgG myeloma proteins and all 6 IgA myeloma proteins showed marked deficiency of the buried determinant uncovered by pepsin-digestion. The pattern of deficiency was not uniform, one agglutinator showing deficiencies in inhibiting capacities of the various myeloma proteins that differed from the rest. The IgG myeloma proteins deficient at the pepsin site could be predicted by Ouchterlony experiments using antipepsin-S fragment antisera absorbed with papain S fragment. The pepsin site was markedly diminished by 0.1 M mercaptoethanol treatment of the 5 S pepsin fragment; some reduction in antigenicity was noted after 0.01 M mercaptoethanol. Reduction with 0.1 M mercaptoethanol followed by reoxidation in 100% O_2 regenerated full reactivity of the pepsin-site antigen. Integrity of at least one interchain disulfide bond would seem to be essential for full expression of this buried antigenic determinant of IgG.

Intranuclear Localization of Testosterone-1,2-H^3 in the Preen Gland of the Duck. Jean D. Wilson * and Peter M. Loeb, Dallas, Texas.

Considerable evidence has been accrued to indicate that the anabolic action of testosterone is secondary to an acceleration of protein synthesis and that this enhancement is the result of an increased rate of synthesis of ribonucleic acid (RNA) within the nuclei of the cells of the target organs. To investigate the means by which this action is mediated the intranuclear localization of testosterone-1,2-H^3 has been studied in the duck preen gland, a large accessory sex tissue under the trophic control of testosterone. Adult ducks were injected intravenously with 0.5 mc testosterone-H^3; the preen glands

were removed and homogenized, and the nuclei were purified by centrifugation in sucrose gradient densities. The washed nuclei were shown to contain testosterone-H^3 that was not displaceable by cold testosterone. To determine the intranuclear localization of the testosterone-H^3 the nuclei were ruptured by sonication and separated by differential ultracentrifugation into heterochromatin, euchromatin, and soluble fractions. The identity of the various fractions was confirmed by electron microscopy. Although the euchromatin fraction contained only a small portion of the DNA, it contained a major portion (60 to 100%) of the testosterone-H^3. Furthermore, in four studies the nuclei and nuclear fractions were isolated from animals that had been injected with $NaHP^{32}O_4$, and as previously demonstrated in calf thymus by Frenster, Allfrey, and Mirsky, the euchromatin was shown to be the major site of synthesis of the nuclear RNA in this tissue. Finally, euchromatin from animals previously injected with testosterone-H^3 was centrifuged in cesium chloride gradient densities and separated into histone and desoxynucleic acid fractions; in these studies the testosterone-H^3 was found to be bound solely to the histone fraction. It has been concluded that testosterone is physically attached to the sites of active RNA synthesis and that this attachment appears to be to the histone portion of the chromosome.

A Unifying Concept for the Pathogenesis of the Syndrome of Iron Deficiency Anemia, Hypocupremia, and Hypoproteinemia in Infants. JOHN F. WILSON AND M. E. LAHEY, Salt Lake City, Utah (introduced by P. D. Hoeprich*).

Since 1956, reports from four centers have described 25 infants with the above-listed syndrome under varied titles: "transient dysproteinemia," "hypocupremia," "copper deficiency," or "copper and protein depletion complicating hypoferric anemia." Factual observations included *1*) an almost universal subsistence on homogenized milk (WCM), *2*) increased plasma protein catabolism with possible faulty synthesis, and *3*) a universally present iron-responsive anemia. Although two groups mentioned the possibility of GI loss of blood or plasma proteins per se, there was no unanimity of opinion concerning the pathogenesis. In this clinic the syndrome has been recognized in 21 additional infants, in some of whom fecal blood loss (FBL) was strongly suspected because of the severity of the anemia or from serial hematologic observations. By means of the Cr^{51}-RBC technique, however, 7 of 10 subjects (from the last 11 seen) showed definitive evidence of WCM-induced, pathologic FBL (mean: 1.95 ml per day). The FBL *1*) could be reduced significantly (mean: 0.41 ml per day) by dietary substitution of either soybean or heat-processed milk formulas; *2*) was related in one subject to the amount of WCM ingested; and *3*) continued in others despite blood transfusions or iron therapy. Of the three remaining subjects one showed transient FBL, unrelated to dietary changes, whereas the remaining two were consistently negative for abnormal FBL. A blood-losing enteropathy, frequently milk-induced, now emerges as a major pathogenetic mechanism in this previously enigmatic syndrome.

Renin in Pregnancy and the Menstrual Cycle. BERTRAM M. WINER, Boston, Mass. (introduced by Benjamin Alexander †).

Renin secretion increases in response to salt depletion, the erect posture, and renal artery stenosis, conditions reducing perfusion of the renal arterial bed. In the present studies effects of pregnancy, the menstrual cycle, and administration of progesterone on plasma renin were examined. Renin was assayed by use of rabbit aortic strips after dialysis, addition of salt, pH adjustment to 5.5, and incubation of plasma 1 hour at 37° C. In comparison with 20 control subjects vasoconstrictor activity of brachial vein plasma was increased 2- to 8-fold in 21 pregnant patients; values were high from the sixth week to the end of pregnancy with a gradual fall to control levels within a week after delivery. Column and paper chromatography by the method of Boucher and associates confirmed that the vasoconstrictor activity was due to angiotensin II, elaborated by renin during incubation. Plasma renin activity was not higher in three patients with toxemia than in normal pregnancy. To determine whether the uterus or placenta contributed a renin-like substance to the circulation, renin levels in renal, uterine, and brachial vein plasma were compared in pregnant dogs; levels were higher in renal than uterine or brachial vein plasma. In the menstrual cycle of 10 women plasma renin activity was low in the first few days of the cycle and increased gradually to reach a peak in the fourth week. Daily administration of progesterone caused a twofold rise in plasma renin activity within 4 days in three female dogs. Plethysmographic studies by others have shown reduced venous tone in pregnancy and after estrogen-progesterone administration. The increase in renin secretion during pregnancy and the menstrual cycle may be related to this effect of female sex hormones.

Studies on the Epidemiology of Hospital-acquired *Escherichia coli* Infections. RICHARD H. WINTERBAUER, MARVIN TURCK, ROGER P. KENNEDY, AND ROBERT G. PETERSDORF,* Seattle, Wash.

E. coli and other gram-negative enterobacteriaceae have become increasingly important causes of infections among hospitalized patients. By means of serologic grouping it has been demonstrated that among the 140 O groups, three, 04, 06, and 075, account for the majority of these infections. These strains are carried more frequently in the stools of hospitalized than nonhospitalized individuals, and tend to persist in the stool. They are usually also present in the stools of patients who develop infections. These data suggest that, in addition to their increased prevalence in the hospital, these strains may be more virulent for man. To prove this point, these studies were extended to a nonhospitalized population. Stool carrier rates of these strains outside the hospital were only 15%,

compared to 60% in hospitalized patients. However, in 70% of 50 patients who had acquired *E. coli* urinary infections outside the hospital, serogroups 04, 06, and 075 were the cause of infection. Identical strains were present in urine and stool in 80% of these patients. These results indicate that, despite low carrier rates, these strains are responsible for most *E. coli* infections both within and without the hospital and support the concept that these strains are epidemiologically more virulent. To study the mode of transmission of these *E. coli*, inanimate objects on medical and urological wards were cultured. *E. coli* 04, 06, and 075 were isolated in only 3% of cultures, far too rarely to account for the high frequency of infections with these strains. In contrast, *Klebsiella, Proteus,* and *Pseudomonas* were isolated in 22%, 8.5%, and 5.7% of cultures, suggesting that these infections often originate in extraintestinal foci. The carrier rate of *E. coli* 04, 06, and 075 in stools of hospital personnel was 40%, as opposed to 15% for nonhospitalized individuals and 60% for patients. These results incriminate human carriers in the spread of nosocomial *E. coli* infections.

Exchange of Aldosterone between Plasma and Thoracic Duct Lymph in Man. MARLYS HEARST WITTE, NORMAN LEVINE, AND ALLAN E. DUMONT, New York, N. Y. (introduced by Currier McEwen †).

The transport and metabolism of aldosterone has been analyzed in terms of a 2-compartment model, in which the apparent initial distribution space of the tracer is less than total body water but greater than extracellular fluid. Direct measurement of radioactivity in the extravascular extracellular space has been limited to a few observations on ascites in dogs. Therefore, thoracic duct lymph, a portion of the circulating interstitial fluid readily accessible to sampling, was examined in patients with congestive heart failure, hepatic cirrhosis with and without ascites, and normals. A single dose of H^3-*d*-aldosterone was administered intravenously, and samples of plasma, thoracic duct lymph, and ascites were assayed for radioactivity as true aldosterone at frequent intervals between 2½ and 120 minutes. Radioactivity appeared in lymph as early as 2½ minutes and within 10 minutes reached a maximum close to the plasma value (protein content of lymph was much lower). After this peak, lymph and plasma radioactivity decreased at similar rates. Although metabolic clearance rates were reduced in some patients, the presence of massive extravascular fluid accumulation or severe liver dysfunction did not delay the appearance or peak of radioactivity in lymph. Analysis of the curves suggests that radioactive aldosterone distributes in an initial space that is probably extracellular fluid and that its disappearance from plasma may be better explained by a 3-component model. In several patients, additional studies were performed, which included lymph concentrations of endogenous aldosterone, urinary excretion of the 3-oxo conjugate, angiotensin and renin activity in lymph and plasma, and kinetics of distribution of Bromsulphalein.

Biochemical Determinants of Long Chain Fatty Acid Oxidation in the Newborn Heart. B. WITTELS AND R. BRESSLER,* Durham, N. C.

In recent years, long chain fatty acids (LCFA) have been shown to be the major metabolic fuel of the adult heart. In the developing mammalian heart, however, evidence has been obtained that carbohydrates are the primary source of energy. A study was undertaken to elucidate the biochemical basis of this age-dependent difference in cardiac metabolism. Cardiac homogenates from newborn rats (<1 day old) oxidized glucose-U-C^{14} at a rate 10-fold greater than those of adults ($N = 1.65$; $A = 0.17$ umoles per g protein per 30 minutes). The ability of the newborn heart to oxidize palmitate-1-C^{14}, however, was only 15% of the adult ($N = 0.94$; $A = 6.12$). The limited capacity of LCFA oxidation by the newborn heart was correlated with *1*) decreased LCFA activation (LCF acyl thiokinase activity), *2*) decreased LCF acyl CoA-carnitine transferase activity, and *3*) decreased concentration of carnitine. LCF acyl CoA-carnitine transferase and carnitine serve to effect a translocation of activated LCF acyl groups into mitochondria where the FA undergoes oxidation. Whereas LCFA oxidation was decreased in the newborn, incorporation of palmitate-1-C^{14} into glycerides and phospholipids greatly exceeded that in the adult. This indicated that LCFA activation was probably not rate limiting in palmitate oxidation. Correction of the carnitine lack *in vitro* tripled the palmitate rate of oxidation, but failure to attain the adult level of oxidation indicated that the transferase activity was probably the rate controlling step in LCFA oxidation by the newborn heart. Nondepressed rates of hexanoate-1-C^{14} and of succinate-2, 3-C^{14} oxidation indicated no impairment in activity of B-oxidation or in the activity of the Krebs cycle or the electron transport chain in the newborn heart. The observations imply an age-dependent evolution of substrate choice by the heart that is under enzymatic control.

A Vulnerable Period for Ventricular Tachycardia after Myocardial Infarction. GERALD A. WOLFF, FRANK VEITH, AND BERNARD LOWN,* Boston, Mass.

In the normal animal an electrical impulse delivered transthoracically during the vulnerable period (VP) of the cardiac cycle results in either a ventricular premature beat or ventricular fibrillation (VF). In the dog with myocardial infarction (due to ligation of the left anterior descending coronary artery) there also exists a distinct VP for ventricular tachycardia (VT). In 12 of 15 animals tested before and daily after coronary occlusion, a VP for VT was found only after myocardial infarction. VT was first induced within 2 hours after occlusion and could be induced thereafter for 7 to 10 days. In one animal the VP for VT was permanent. The mean duration of the VP for VT was 42 ± 19 msec. It coincided in location and duration with the VP for VF. The energy range of the VP for VT extended from a threshold of 0.3 ± 0.5 watt second (WS) to an upper limit of

10.0 ± 13.0 WS. The lower energy limit for VF was 1.2 ± 2.8 WS, and the upper limit was 114 ± 85 WS. In any one animal it required significantly less energy to produce VT than VF. Episodes of VT provoked through transthoracic stimulation of the VP were generally self-sustaining and associated with significant reduction in arterial pressure. In the group of 12 dogs the mean rate of the VT was 369 per minute. The arrhythmia was prefibrillatory. Stimulation of the VP after myocardial infarction provides a method for studying VT. These results suggest that in patients with coronary occlusion, ventricular premature beats that fall in the VP may trigger VT and thereby lead to VF.

The Total Blood Volume of the Forearm in Relationship to the Venous Pressure-Volume Curve. J. EDWIN WOOD,* Charlottesville, Va.

Interpretation of the venous pressure-volume curve of the extremity, as measured by the plethysmographic method, would be greatly enhanced by knowledge of the absolute volume of blood in the extremity and in the veins at the starting point of these curves. This series of experiments was designed to measure the volume of the blood present in the forearm when effective venous pressure is 1 mm Hg, related to the change in volume of the forearm caused by increasing local effective venous pressure (EVP) to 30 mm Hg, and to estimate the proportion of the total venous volume that was present in the veins at 1 mm Hg. Six subjects received intravenous radioactive iodinated albumin as usually administered for measurement of the blood volume. Radioactivity of the forearm was measured on 44 occasions with a columnated 2-inch scintillation crystal, spectrometer, and recording rate meter system. Background radioactivity was determined with the forearm in position beneath the crystal after serial inflation from wrist to elbow of three cuffs to 260 mm Hg. Cuff pressure was then released to a level of 15 mm Hg (EVP, 1 mm Hg), and radioactivity level was measured. EVP was then raised to 30 mm Hg with a final determination of radioactivity. Venous volume change induced by this degree of congestion was measured in a separate series of 15 plethysmographic studies on five subjects, average 2.6 ml per 100 ml of forearm. The volume of blood present in the forearm veins at EVP 1 mm Hg was estimated to be 0.7 ml per 100 ml. These data yield direct information indicating that the base-line venous volume utilized in the plethysmographic method for measuring venous distensibility is relatively small and constant.

The Hemolysis of Red Cells from Patients with Paroxysmal Nocturnal Hemoglobinuria by Isolated Subcomponents of the Third Complement Component. STANLEY YACHNIN, Chicago, Ill. (introduced by Leon O. Jacobson †).

Highly purified preparations of β_{1c} globulin (C'3a) can attach directly to erythrocytes of patients with paroxysmal nocturnal hemoglobinuria (PNHE). Formation of the intermediate complex, $PNHE_{\beta 1c}$, proceeds in the presence of Na_3HEDTA and is not affected by prior incubation of PNHE at 37° for 2 hours. $PNHE_{\beta 1c}$ form best at pH 7.5, and in contradistinction to PNHE will hemolyze in high dilutions (1:500) of human serum devoid of Ca^{++} or Mg^{++}. The optimal pH for $PNHE_{\beta 1c}$ lysis is 6.0 to 6.5. $PNHE_{\beta 1c}$ will hemolyze in serum lacking properdin, or the first, second, or fourth components of complement, but not in serum devoid of the third component of complement (C'3). Hemolysis of $PNHE_{\beta 1c}$ can be effected by purified subcomponents of C'3 (C'3b, C'3c), and the behavior of $PNHE_{\beta 1c}$ in all respects resembles that of their counterpart in classical immune lysis, $EAC'_{1,4,2,\beta 1c}$. Normal human red cells are also susceptible to hemolysis by purified subcomponents of C'3, but to a much lesser extent than PNHE. These findings confirm earlier speculation that in ordinary acid hemolysis in whole human serum, early fluid phase events in C' activation lead to direct attack of PNHE by C'3, without the mediation of cell-bound complement components. The difference between normal human red cells and PNHE would appear to be a difference involving the number or accessibility of membrane sites concerned with the attachment of β_{1c} globulin, which have long been recognized as participating in the phenomenon of immune adherence. Although these studies emphasize the role of fluid phase events in red cell membrane destruction by complement, similar mechanisms may participate in the injury of other tissues by complement. The presence of β_{1c} globulin on cells or in tissue should not be construed as meaning that cell or organ specific antibodies serve as the site for its attachment.

Biochemical Lesion in Dilantin-induced Erythroid Aplasia. A. A. YUNIS, G. K. ARIMURA, C. L. LUTCHER, J. BLASQUEZ, AND M. J. HALLORAN, Miami, Fla., and St. Louis, Mo. (introduced by W. J. Harrington *).

A 17-year-old Negro boy developed erythroid aplasia after the intake of 334 g of Dilantin over a 2½-year period. Prompt recovery followed discontinuation of the drug. The aplasia could be promptly reproduced with Dilantin. The administration of Dilantin and 1,500 ml of the patient's plasma to a normal recipient over a period of 3 days had no detectable effects on his serum iron level, plasma iron-binding capacity, Fe^{59} clearance, reticulocyte count, or number of nucleated red cells in his bone marrow. Large doses of vitamin B_{12} and folinic acid neither prevented nor reversed the aplasia, but the daily oral administration of 120 mg of riboflavin prevented the Dilantin effect. A 30% *in vitro* inhibition of uptake of C^{14}-formate into DNA and DNA thymine but not into RNA could be repeatedly demonstrated at a Dilantin concentration of 20 μg per ml of bone marrow suspension. No inhibition was demonstrated when the nucleated red cells were absent from the bone marrow. A similar pattern of inhibition could be demonstrated with the pre-

cursors adenine, glycine, orotic acid, uracil, and uridine, but the uptake of deoxyuridine and thymidine was not inhibited. With radioautography, there was a 65% inhibition of uptake of H^3-uridine into erythroid cells but not into myeloid cells. There was no inhibition of uptake of deoxyuridine or thymidine. Dilantin did not alter the uptake of C^{14}-glycine into heme or the incorporation of C^{14}-glycine or lysine into protein. Dilantin thereby appears to exert its toxic effect in this unusual patient through a specific inhibition of DNA synthesis in early erythroid cells, probably at the step of formation of deoxyribotides.

Observations on Hapten Specificity in Delayed Hypersensitivity. SOLOMON J. ZAK AND SIDNEY LESKOWITZ, Minneapolis, Minn., and Boston, Mass. (introduced by Wesley W. Spink †).

Previous studies have shown that immunization of guinea pigs with hapten conjugates of native proteins and of synthetic random copolymers that are immunogenic in complete Freund's adjuvant results in a state of delayed hypersensitivity whose specificity is directed towards determinant groups on the carrier. Studies by Leskowitz have shown that a hapten coupled to a nonimmunogenic synthetic carrier results in delayed hypersensitivity whose specificity is largely directed towards the hapten. The studies to be reported confirm the previous observations on hapten specific delayed hypersensitivity. The induction of hapten specific delayed hypersensitivity was most efficient when instead of the usual carrier, poly-L-tyrosine with a molecular weight of 10,000, the *N*-acetylated monomer was employed. Furthermore, an additional hapten, *p*-(acetoxymercuri)-aniline could function as an effective hapten in this regard, as shown previously for *p*-arsanilic acid. The delayed hypersensitivity induced against *p*-arsanilic acid could be transferred to nonsensitized recipient animals with living peritoneal macrophages from reactive donor animals. The histologic appearance of the reactions were compatible with those described by classical delayed hypersensitivity. These studies suggest that with pure synthetic compounds quantitation may be introduced into the field of delayed hypersensitivity.

The Effect of Ethanol on Hepatic Secretion of Triglycerides into Plasma. DAVID ZAKIM, DIANE ALEXANDER, AND MARVIN H. SLEISENGER,* New York, N. Y.

Ethanol ingestion raises plasma triglyceride (TG) concentrations in both man and rats, an effect that could result from ethanol inhibition of plasma TG clearance or stimulation of hepatic secretion of TG into plasma. In order to evaluate the *in vivo* effect of ethanol on hepatic TG secretion, we have measured the accumulation of plasma TG after iv Triton 1339 in rats fasted 12 to 14 hours and refed with ethanol or glucose. Four hours after ethanol feeding, and 2 hours after Triton injection, mean plasma TG accumulation was 11.1 mg per ml at plasma ethanol concentrations of 3.0 to 5.0 mg per ml. In isocaloric glucose fed rats mean plasma TG was 4.24 mg per ml 2 hours after Triton. It is perhaps more significant that the ratio of accumulated plasma TG to hapatic TG concentration was greater in the ethanol fed animals. Further, the increase in hepatic TG 4 hours after ethanol represented only 60% of the TG increase in the combined hepatic and plasma compartments, indicating that the amount of fat mobilized after ethanol feeding is greater than previously reported. Injection of Triton at intervals after ethanol feeding revealed that increased hepatic TG secretion persisted at least 14 hours. Plasma accumulation of phospholipids (PL) was also augmented by ethanol; thin layer chromatography of PL classes demonstrated that the increase was predominantly in the choline fraction. These experiments exclude interference with hepatic TG secretion as important in the pathogenesis of alcoholic fatty liver in the rat. They also indicate that the increase in TG turnover after the ingestion of ethanol is sufficient to overload plasma TG clearing mechanisms in the presence of an exogenous fat load. The increase in choline secretion may account for the increased choline requirement of rats maintained on ethanol-supplemented diets.

Phagocytosis of PPLO by Mononuclear Cells. DOROTHEA ZUCKER-FRANKLIN, MORTON DAVIDSON, BERTRAM GESNER, AND LEWIS THOMAS,* New York, N. Y.

Electron microscope studies have revealed that *Mycoplasma* is not only susceptible to phagocytosis by neutrophils, eosinophils, and macrophages, but also by cells that cannot be distinguished morphologically from lymphocytes. Cells obtained from human peripheral blood or rat thoracic duct lymph were incubated for 3 to 10 minutes with either *Mycoplasma gallisepticum* or *M. neurolyticum*. These organisms measured 300 to 800 mμ in diameter. Phagocytosis of PPLO by neutrophils was accompanied by degranulation as is the case with bacteria. In approximately 10% of cells with the morphological appearance, in thin sections, of lymphocytes, one or several intact organisms were seen within phagocytic vacuoles. In some preparations, the membranes of partially destroyed *Mycoplasma* were observed in the process of phagocytosis as well as within vacuoles. Many organisms were seen adherent to the plasma membrane, and all phases of engulfment were observed. Some of the mononuclear cells containing PPLO had a few profiles of rough endoplasmic reticulum. Under identical conditions, streptococci and latex particles (0.8 μ) were not taken up by lymphocytes, whereas typical macrophages and neutrophils avidly engulfed these objects. Two alternative interpretations of these observations are under consideration: *1*) the mononuclear cells that engulf PPLO are actually not lymphocytes despite their morphological resemblance to these cells in thin sections, or *2*) the uptake of PPLO by lymphocytes represents an exception to the generalization that these cells are not phagocytic. The first interpretation would suggest that there are more macrophages in thoracic duct lymph than previously suspected, the second

that the uptake of PPLO by lymphocytes may be analogous to the ingestion of virus particles known to occur in these cells in avian and murine leukemia.

Abnormal Estrogen Metabolism in Male Breast Cancer. BARNETT ZUMOFF, JACK FISHMAN, T. F. GALLAGHER, AND LEON HELLMAN,* New York, N. Y.

Unusual changes in the peripheral metabolism of estrogen have been demonstrated in men with breast cancer. Estradiol-C^{14} was given to four men with breast cancer (in five studies), four healthy men of comparable age, and nine men with miscellaneous cancers unrelated to breast. Compared with the healthy or sick control subjects, the men with breast cancer showed a sharp diminution in the formation of estrone [from 19 to 8%], 2-hydroxyestrone [19 to 3%], and 2-methoxyestrone [4 to 1%]. This was accompanied by a pronounced increase in the production of estriol [from 16 to 40%]. There was virtually no overlap between the control groups and the breast cancer patients. The altered metabolic pathways in the breast cancer patients are compatible with augmentation of the enzyme systems concerned with 16 α-hydroxylation and consequent depletion of the estrone pool. In view of the rarity of male breast cancer, the control subjects were carefully chosen to evaluate and hence exclude nonspecific factors such as age, state of nutrition, modalities of therapy, and the presence of chronic illness, which could conceivably have contributed to the metabolic differences observed. The results imply a relationship between estrogen biotransformation and an unusual steroid hormone related malignancy.