ABSTRACTS

Effect of Weight Gain During Adult Life on Serum Triglyceride Concentrations of Normal Man. MARGARET J. ALBRINK,* J. WISTER MEIGS AND MORRIS GRANOFF, New Haven, Conn.

The finding of high serum triglycerides in patients with coronary artery disease prompted a study of the association between serum lipids and various traits of 215 apparently healthy male factory employees between 30 and 70 years of age. Significant elevations of serum triglycerides were associated with advancing age, obesity, family history of diabetes or coronary artery disease, and weight gain during adult life. The mean serum triglyceride concentration of 78 men who had gained less than 10 pounds since age 25 was 4.6 mEq per L compared to 7.1 mEq per L for the 137 men who had gained more than 10 pounds, a highly significant difference. The weight-stable group did not show an increase in later decades as did the weight gainers. Although stocky men of stable weight had somewhat higher triglycerides than lean men, those who achieved a moderate degree of corpulence [estimated as height/(weight)$^{-3}$ index] through weight gain during adult life had a mean triglyceride concentration nearly twice that of men who reached young manhood with this degree of adiposity but gained no further weight during adult life. The tendency for low triglycerides in men with negative family histories noted for the entire group was not found in the weight-gainers. Among the weight-stable men, however, those with a negative family history had significantly lower triglycerides than those with a positive family history and as a group had the lowest triglycerides except for the leanest 7 per cent in whom not a single instance of triglyceride concentration above 5.5 mEq per L was encountered regardless of family history. Serum cholesterol varied roughly with triglycerides but in general the differences between the various groups was of a lower degree of significance. It was concluded that weight gain during adult life was the single most important factor associated with hyperglyceridemia in normal men.

Metabolic and Circulatory Effects of Epinephrine in the Forearm of Man. REUBIN ANDRES,* MARCEL BALTZAN, GORDON CADER AND KENNETH L. ZIERLER,* Baltimore, Md.

Consensus of recent literature is that infusion of epinephrine i.v. in man causes sustained increase in forearm blood flow and in systemic blood lactate concentration, but intra-arterial infusion causes only transient increase in forearm blood flow and no increase in lactate production by peripheral tissues. It has therefore been postulated that epinephrine itself lacks sustained vasoactive effect and even glycogenolytic potency in man and that some active intermediate is formed by it to explain its i.v. effects. Furthermore, the effect of epinephrine on net postassium movement in muscle is in dispute, and other studies in a variety of preparations suggest that epinephrine inhibits glucose uptake by skeletal muscle. We have tried to resolve these reports, which are either internally inconsistent or at variance with reports of epinephrine effects in laboratory animals or in isolated systems, by carefully controlled observations in 17 subjects in whom epinephrine was administered into the brachial artery (0.002 μg per kg body wt per minute for 25 minutes) to achieve an arterial concentration of about 4 μg per L. This dose exerted no systemic effect (constant arterial glucose, lactate, K and free fatty acids) but it produced: 1) sustained doubling of forearm blood flow (so that no vasoactive intermediate need be postulated), 2) 10-fold increase in forearm lactate production (so that it is glycogenolytic in man), 3) small increase of no statistical significance in glucose uptake, 4) large K uptake, and 5) the anticipated increase in FFA release by forearm adipose tissue.

The Effect of Blood Glucose on Circulating Bound and Free Insulin. HARRY N. ANTONIADES, KARE GUNDERSEN AND HUGH PYLE, Boston, Mass. (introduced by Charles A. Janeway).

Earlier reports have presented evidence that insulin circulates in the peripheral blood in two forms—a biologically active "free" insulin and a biologically inactive "bound" form. The bound insulin is complexed to basic proteins and can be dissociated *in vitro* into biologically active free insulin by centrifugation at pH 10 or by incubation with adipose tissue extracts. Examination of the ratio of free and bound insulin in nondiabetic subjects shows that this ratio does not depend upon a pure physicochemical equilibrium. Present studies suggest that the ratio is related to the blood glucose level. Examination of free and bound insulin before and after glucose administration shows that a rise in blood glucose results in a dissociation of the bound insulin. Following the intravenous administration of glucose, there is a rapid dissociation of bound insulin resulting in an increase of free insulin. The free insulin falls with the decreasing blood glucose level. At the end of 4 to 5 hours, the ratio of "bound" insulin to "free" again returns to preglucose administration levels. In diabetic patients, however, the rate of dissociation of bound insulin, following glucose administration, is significantly slower and does not reach the nearly complete dissociation found in nondiabetic subjects.

* Member.

The Effect of 17-Ethyl-19-nortestosterone and Icterogenin on the Hepatic Metabolism of Bilirubin in Normal and Gunn Rats. IRWIN M. ARIAS, SIDNEY GOLDFISHER, ALEX B. NOVIKOFF AND EDWARD ESSNER, New York, N. Y. (introduced by David J. Hamerman).

Conjugated hyperbilirubinemia, bilirubinuria and relatively normal hepatic histology have been regularly observed in humans after 17-ethyl-19-nortestosterone (Nilevar) administration and in sheep and rabbits after treatment with Icterogenin, an alkaloid obtained from *Lippia rehmanni.* The administration of Nilevar or Icterogenin to Wistar rats resulted in significant reduction in the maximal rate of excretion of bilirubin in the cannulated bile duct following a constant intravenous infusion of unconjugated bilirubin. The maximal rate of excretion of bilirubin in the cannulated bile duct was similarly reduced in Wistar and homozygous Gunn rats following constant intravenous infusions of conjugated bilirubin. Hepatic uridine diphosphoglucose dehydrogenase and glucuronyl transferase activities were unaffected by treatment with either Nilevar or Icterogenin. Although the livers of treated animals appeared normal in routine histological sections, marked changes were apparent in both cytochemical and electron microscopic preparations. The bile canaliculi (excretory surfaces) showed dilatation and fragmentation accompanied by decreased ATPase activity, increased alkaline phosphatase activity and considerable loss of microvilli. Changes also occurred elsewhere in the cell membrane and in acid phosphatase-rich lysosomes. These observations suggest that Nilevar and Icterogenin interfere with the mechanism by which conjugated bilirubin is transported from the liver cell into the bile.

Alterations in Granulocyte Kinetics Induced by Epinephrine, Exercise, Endotoxin and Prednisone. JOHN W. ATHENS,* SPENCER O. RAAB, OTTO P. HAAB, HELEN ASHENBRUCKER AND GEORGE E. CARTWRIGHT,* Salt Lake City, Utah.

The influence of epinephrine, exercise, endotoxin and prednisone on the total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), marginal granulocyte pool (MGP), half-time disappearance of granulocytes from the TBGP ($T_{\frac{1}{2}}$), and the granulocyte turnover rate (GTR) has been studied in normal subjects. Granulocytes were labeled *in vitro* by the method described previously. Epinephrine infusion (5 subjects) or acute exercise (4 subjects) caused a shift of granulocytes from the MGP to the CGP with no change in TBGP, $T_{\frac{1}{2}}$ or GTR. In 2 of 6 subjects given bacterial endotoxin a neutropenia occurred 1.5 hours after endotoxin injection, but the TBGP was unchanged. The neutropenia thus reflected a shift of granulocytes from the CGP to the MGP. A neutropenic phase was not detected in the remaining 4 subjects. Five hours after endotoxin injection all 6 subjects had a leukocytosis and the TBGP increased twofold. Endotoxin injection did not alter the granulocyte radioactivity curve during the neutropenic period, but coincident with the rise in granulocyte count a precipitous decrease in radioactivity occurred. These findings suggest that endotoxin produced neutrophilia by causing an influx of unlabeled granulocytes from the bone marrow into the TBGP. It may also be inferred that in subjects who develop leukopenia there is a transient shift of granulocytes from the CGP to the MGP. Five subjects were given prednisone for 11 days. The TBGP increased 67 per cent and the $T_{\frac{1}{2}}$ was prolonged. The GTR was unchanged. Rapid infusion of hydrocortisol produced a marked neutrophilia but there was no sharp decrease in the radioactivity curve. These data suggest that steroid neutrophilia does not result from an influx of granulocytes from the marrow.

Leukocyte D-Antigenicity Studied by Means of a Specific I^{131}-labeled Anti-D Antibody. ARTHUR E. BARNES, HERNANDO SARASTI AND WALLACE N. JENSEN,* Pittsburgh, Pa.

Specific I^{131} anti-D antibody eluates (I*anti-D) were prepared from the globulin fraction of high-titered anti-D serum. The I*anti-D was isolated by adsorption to D-positive (ccDee) erythrocytes and by subsequent elution with citric acid buffer. Immunologic specificity of the I*anti-D was demonstrated by 70 to 80 per cent total uptake by D-positive erythrocytes as compared with 2 to 10 per cent nonspecific adherence to D-negative erythrocytes and test tube. The amount of erythrocyte D-antigen necessary for a significant I*anti-D uptake above nonspecific adherence varied and was estimated by the disappearance of differences between the I*anti-D uptake on equal numbers of D-negative and D-positive erythrocytes in serial dilution. A 3 per cent greater I*anti-D uptake by D-positive erythrocytes than by D-negative erythrocytes was the minimal difference considered significant. Leukocyte suspensions isolated by differential sedimentation from 7 normal and 2 leukemic D-positive donors showed no I*anti-D uptake not attributable to the I*anti-D bound by contaminant erythrocytes or by nonspecific adherence. The amount of I*anti-D bound by the contaminant erythrocytes and nonspecific adherence was estimated by the amount of I*anti-D bound by the same number of erythrocytes as measured in each leukocyte suspension. Leukocytes from 5 other normal and 1 other leukemic donor showed small I*anti-D uptakes not attributable to D-positive erythrocyte contamination or nonspecific adherence; however, the mean I*anti-D uptake of this group was only 3.6 per cent of the uptake by equal numbers of D-positive erythrocytes. In addition, paired leukocyte suspensions from three D-positive and three D-negative donors had similar I*anti-D uptakes. Furthermore, serial dilutions of these leukocyte suspensions failed to show the attenuation of the total I*anti-D uptake expected when specific D-antigen sites were decreased in number. These investigations strongly suggest that leukocytes have very few or possibly no specific D-antigen sites.

Metabolic Functions of Vitamin B_{12}: The Possible Relationship of Megaloblast Formation to Unbalanced Growth in Bacteria Requiring Vitamin B_{12}. WILLIAM S. BECK,* Boston, Mass.

Although the megaloblast is a familiar morphological entity, few precise statements can be made about the mechanism of megaloblast formation. This shortcoming is attributable to 1) technical difficulties in obtaining homogeneous megaloblast populations; 2) faulty conclusions from existing data; and 3) incomplete knowledge of the nutritional basis of megaloblast formation in different species, of the means by which nutritional repletion reverses the process, of the biochemical functions of vitamin B_{12} and pteroylglutamic acid, and of the nutritional interactions of bone marrow elements. Our studies of the role of vitamin B_{12} in bacterial nucleic acid metabolism have implicated B_{12} in the biosynthesis of deoxyribonucleosides or deoxyribonucleotides. It appears that B_{12} participates (directly or indirectly?) in the reduction of ribosyl to deoxyribosyl groups, a reaction resembling other B_{12}-linked reductions (e.g., methyl synthesis) but differing from the B_{12}-linked isomerases (methylmalonyl CoA and glutamate) in which there is no net change in oxidation level and B_{12} participates directly as a coenzyme. Evidence implicating B_{12} in this conversion includes the nutritional sparing of vitamin B_{12} by deoxyribosides in lactobacilli and the observed effect of B_{12} on the production of acid-soluble deoxyribonucleosides or deoxyribonucleotides and DNA. Indications were also obtained that vitamin B_{12} has no direct role in thymine synthesis. In addition, the unbalanced growth phenomenon was observed in *Lactobacillus leichmannii* cultivated in limiting vitamin B_{12} or deoxyriboside. This is characterized by long filamentous forms in which cell division and DNA synthesis are defective while RNA and protein synthesis proceed normally. Similar results were obtained with B_{12}-starved *Euglena gracilis*. It will be shown that the biochemical characteristics of these filaments correspond with those described in the megaloblasts of pernicious anemia. They may therefore serve as useful models of the megaloblastic growth of B_{12} deficiency which is thus visualized as unbalanced growth due to diminished DNA synthesis resulting from impaired deoxyribosyl synthesis. The implications of this hypothesis will be examined.

Histochemical Topography of Anterior Pituitary: Correlations Between Enzyme Activities and Endocrine Function of Individual Cells. S. BLEICHER, M. KARNOVSKY AND N. FREINKEL,* Boston, Mass.

The responsiveness of adenohypophyseal carbohydate metabolism to insulin (Goodner and Freinkel) prompted further assessment of correlations between endocrine function and intermediate metabolism in anterior pituitary. Cryostat sections of male rat pituitary were incubated with Nitro-BT for histochemical localization of *a*) DPN- and TPN-diaphorases (DPND, TPND) as indices of electron transfer, and *b*) glyceraldehyde-3-P dehydrogenase (G3PD) and glucose-6-P dehydrogenase (G6PD) as representative enzymes of glucose disposition by glycolysis or direct oxidation, respectively. In adenohypophyses from normal animals, all cells displayed distinct TPND activity; less uniform reactivity obtained for DPND and G3PD; G6PD staining was patchy and variable. The reactions were most pronounced in the ovoid cells around the portal vessels which have been previously implicated in the secretion of gonadotropin. Their gonadotropic character was corroborated by orchiectomy. Within 3 to 4 days following castration, histochemical reactions were intensified in these cells (TPND > G6PD > G3PD > DPND) although other pituitary components were unaltered. Eventually, coincident with diffuse increase in "gonadotropes" (as judged by conventional histology), cells with augmented histochemical activity also became more numerous. Maximal reactions were seen in the signet-ring "castration cells" which appear several weeks after orchiectomy. The data indicate that *a*) pituitary cells may be differentiated by histochemical enzyme reactions; *b*) focal enzymatic changes may be associated with specific hormogenic challenges; and *c*) such localized metabolic realignments may be among the earliest indices of altered function. With regard to *c*, the heightened enzymatic activity of normal "gonadotropes" may be involved in the disparate vulnerability of gonadotropic function to diverse metabolic insults.

The Effect of Serum Albumin Infusion upon Lipid Mobilization in the Nephrotic Syndrome in Man. M. D. BOGDONOFF, J. LINHART, R. F. KLEIN AND E. H. ESTES, JR., Durham, N. C. (introduced by W. M. Nicholson).

In order to test the thesis that the interrelationship of serum albumin and the serum lipids is of specific import to the plasma lipid abnormality of the nephrotic syndrome, the following studies of lipid mobilization in man were conducted. Lipid mobilization was effected by a standardized (15 minutes; total dose, 150 μg) infusion of norepinephrine, and the pattern of change of the plasma free fatty acid (FFA) was followed. All subjects were observed in the resting, fasting state. The peak rise in FFA level is used as an index to the magnitude of lipid mobilization. Pulse, blood pressure, hematocrit, serum cholesterol, triglyceride, protein, albumin and glucose were also measured. In 20 metabolically normal individuals the mean peak FFA rise was +600 μEq per L; in 6 patients with the nephrotic syndrome the peak FFA rise was +54 μEq per L ($p < 0.01$). Blood pressure and pulse changes were similar. Following the infusion of 50 g of salt-poor human serum albumin (over a 30 minute period), the peak rise in normal subjects was further heightened to +1,000 μEq per L; in the nephrotic patients peak values were increased to +300 μEq per L. (Difference between the means, $p < 0.01$.) In two of the nephrotics serum triglycerides decreased following albumin and norepinephrine beyond the decrement accounted for by the expansion of plasma volume. In two other individuals with non-

nephrotic hypoalbuminemia, peak FFA rises were also low (+40, +50 μEq per L), and were similarly enhanced following the infusion of albumin (+250, +300 μEq per L). These studies are interpreted to indicate that the process of lipid mobilization is significantly modified by the nephrotic syndrome, and that this decreased responsiveness may be, in part, temporarily reversed by the infusion of serum albumin. It is further suggested that the hypertriglyceridemia may reflect a response to the loss of plasma FFA binding sites.

Congenital Adrenal Hyperplasia due to Deficiency of 3β-Hydroxy Steroid Dehydrogenase. ALFRED M. BONGIOVANNI* AND WALTER R. EBERLEIN,* Philadelphia, Pa.

Three unusual infants with the salt-losing form of congenital adrenal hyperplasia have been studied (one a male and two female pseudohermaphrodites). All died within the first few months of life in spite of presumably adequate therapy. Two of the infants had siblings who died with similar symptoms. Postmortem examination was performed on one of the studied cases and on the sibling of another. The excretion of total neutral 17-ketosteroids and Allen chromogens in the urine of all three cases was elevated and fell during treatment with cortisol. Pregnane-3α,17α,20α-triol, pregnane-3α,17α-diol-20-one, and pregnane-3α,17α,20α-triol-11-one, generally found in large amounts in the urine in other subjects with this form of disease, were virtually absent. Urinary Porter-Silber chromogen excretion was extremely low and no metabolites of cortisol were detected by paper chromatography of suitably prepared extracts of urine. The major urinary steroid metabolites were present in the nonglucuronoside, digitonin-precipitable fraction, recovered by solvent extraction after solvolysis in tetrahydrofuran with 10^{-2} N perchloric acid or prolonged heat hydrolysis at neutral pH and reconstitution in acidic ethanol. The predominant steroid isolated was identified as Δ^5-pregnene-3β,17α,20α-triol. Smaller amounts of Δ^5-pregnene-3β,20α-diol were also detected. Dehydroepiandrosterone accounted for approximately 25 per cent of the total 17-ketosteroids. Other ketonic steroid metabolites, tentatively identified, were Δ^5-pregnene-3β,17α-diol-20-one and Δ^5-androstene-3β,16α-diol-17-one. Certain of these metabolites have previously been isolated from the urine of subjects with adrenocortical neoplasms, but this is the first reported instance of their excretion in subjects with congenital adrenal hyperplasia. These findings indicate a third specific defect in adrenal steroidogenesis in the latter syndrome, a primary deficiency of the adrenocortical enzyme, 3β-hydroxy dehydrogenase.

Procedure for Isolation and Determination of Human Blood Angiotensin. R. BOUCHER, P. BIRON AND J. GENEST, Montreal, Canada (introduced by Vincent P. Dole).

This procedure, modifying in several aspects Paladini's method, involves: 1) direct ethanol precipitation of 100 to 400 ml of arterial or venous blood, 2) ether extraction of the filtrate, 3) successive purification of the aqueous phase by column and paper chromatography. Angiotensin, located by its Rf value, is assayed in a rat preparation for pressor activity. This procedure is sensitive to 0.005 μg. Recovery of angiotensin is quantitative. Specificity is based on the following similarities with the standard valine-5 angiotensin II asparaginyl: 1) identical Rf values in two different paper chromatographic systems, 2) identical migration rate in one paper electrophoretic system, 3) identical pressor response curve in the rat assay, 4) angiotensin added to blood or isolated from blood during infusion of this substance, shows the same characteristics as mentioned in 1, 2 and 3; 5) the angiotensin isolated from human serum after incubation with a renin preparation from renal tissue of a patient with unilateral renal hypertension, shows the same characteristics as the material isolated from hypertensive patients; 6) inactivation by trypsin. Arterial angiotensinemia during angiotensin infusion given at hypertensive rates (30 mm Hg diastolic pressure above control levels) varies between 0.035 and 0.200 μg% of blood. So far, 20 normal subjects and 40 patients with various types of hypertension have been studied. Based on the above criteria, angiotensin is present in some hypertensive patients.

An Abnormal Serum Cholesterol Ester Fatty Acid Pattern in Liver Disease. C. Y. BOWERS, JAMES E. MULDREY, JAMES G. HAMILTON AND O. NEAL MILLER, New Orleans, La. (introduced by Grace A. Goldsmith).

The ratio of free cholesterol to its esters in human serum has been used as a test of liver function. To extend these observations the cholesterol ester fatty acid pattern was determined in sera of patients with various liver diseases. Serum lipids are chromatographed on silica gel-coated glass fiber paper. Chromatography in iso-octane (20 minutes) completely separates cholesterol esters into five fractions: S, saturates, O, oleate and palmitoleate; L, linoleate; A, arachidonate and linolenate; and P, higher polyenoates. Esters are visualized as charred spots after treating with sulfuric acid followed by heating and were determined quantitatively by densitometry of the charred spots. Representative values for S, O, L and A are 25, 70, 120 and 25 mg% in normal persons and 20, 75, 45 and 20 mg% in patients with cirrhosis of the liver. This reversal of the O/L ratio has been conspicuously absent in patients with endocrine abnormalities and various lipid disorders, including patients with hypercholesterolemia on varied dietary regimens. It is probable that studies of cholesterol esters in liver disease may elucidate some of the regulating factors which influence the metabolic turnover of the fatty acid moieties.

Heterogeneity of Serum Alkaline Phosphatases: The Influence of Race, Pregnancy, Growth and Disease. SAMUEL H. BOYER, RICHARD SCHULTZ AND ROBERT WEILBACHER, Baltimore, Md. (introduced by Victor A. McKusick).

A variety of investigations have established the presence of several phosphatases in human serum. We have demonstrated 13 serum alkaline phosphatases by starch gel electrophoresis and subsequent enzyme identification by histochemical methods. Less effective resolution was obtained by cellulose column chromatography. Ten components are α_2-globulins and three are β-globulins. No single individual exhibits all components. Patterns observed in normal white adults are generally quite similar and consist of two principal zones of activity. Approximately 4 per cent of American Negroes have a group of phosphatases not observed in whites. One of these latter components possesses a mobility identical to the principal alkaline phosphatase purified from human intestine. The alkaline phosphatases associated with a variety of diseases do not usually differ electrophoretically from the normal pattern, and when different are not specific for any particular disorder. From birth to adolescence the two principal zones of phosphatase activity differ slightly from the adult pattern. Patterns appearing in children with hypophosphatasia or rickets are electrophoretically indistinguishable from the normal childhood pattern. During pregnancy additional electrophoretic zones of phosphatase activity start to appear beginning at about Week 15 of gestation. By Week 31 nearly all women exhibit the additional components, which disappear shortly after delivery. One particular component, unique to pregnancy, appeared in 36 of 57 white women who were in Week 31 of their confinement. In a similar group of 59 American Negroes this component was present in only 14 individuals. The racially associated heterogeneity of certain serum alkaline phosphatases suggests a genetically determined polymorphism. The tissue source of the various enzymes can possibly be established by comparison with purified phosphatases from several tissues and by immunochemical methods.

Stimulatory Effect of Chlorobutanol on Uridine Diphosphate Glucose Dehydrogenase. GEORGE A. BRAY AND A. H. CONNEY, Bethesda, Md. and Rochester, N. Y. (introduced by Lawrence E. Young).

Patients with essential pentosuria have a metabolic block in the glucuronic acid pathway leading to the excretion of large amounts of L-xylulose. Aminopyrine stimulates the excretion of L-xylulose in these patients presumably by stimulating this pathway. Administration of aminopyrine, chlorobutanol, barbiturates and several other drugs to rats has the dual property of accelerating the formation of ascorbic acid through the glucuronic acid pathway and of increasing the activity of certain drug-metabolizing enzymes in liver microsomes. *In vitro* studies with rat liver preparations have shown that the effect of chlorobutanol administration *in vivo* cannot be explained by altered activities of hepatic glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucomutase, uridine diphosphate glucose (UDPG) pyrophosphorylase or by altered activities of the enzyme systems that convert D-glucuronolactone to L-gulonolactone or which convert L-gulonolactone to L-ascorbic acid. However, chlorobutanol administration increases the activity of UDPG dehydrogenase by about 100 per cent. Chlorobutanol administration also has a marked effect in stabilizing UDPG dehydrogenase. This enzyme, when obtained from control rats, is unstable if allowed to age at 4° C for 5 days but is stable when obtained from chlorobutanol-treated rats. The enzyme preparations obtained from control rats can be stabilized by the addition of UDPG or uridine diphosphate glucuronic acid (UDPGA) but not by the addition of ATP or chlorobutanol. It is possible that increased activity of UDPG dehyrogenase may make more UDPGA available for ascorbic acid synthesis and for drug glucuronide formation. Administration of chlorobutanol and other drugs that stimulate ascorbic acid synthesis via the glucuronic acid pathway also stimulates the activity of enzyme systems in liver microsomes that metabolize drugs. The mechanism by which drug administration elicits these adaptive responses is being investigated.

Biosynthesis of Urea in Kidney Tissue Without Exogenous Substrate. WILLIAM A. BRODSKY* AND H. C. HUANG, Louisville, Ky.

Urea and ammonia concentrations of kidney cortices of dogs were observed under *in vitro* conditions. Homogenates or slices of cortex were immersed in K-rich Ringer's solutions with and without added substrates. Urea concentration increased from initial levels of ca. 15 to 19 μmoles per g in 1 to 2 hours. Free ammonia, measured simultaneously in the same aliquots increased from 3 to 8 μmoles per g. Rate of formation of urea in slices was increased slightly by addition of citrulline, glutamate, Mg^{++}, and cytochrome C. In one case, urea concentration remained fixed when the tissue was incubated in Na-rich Ringer's. In another, urea concentrations remained fixed when tissue was incubated in a citrulline glutamic acid Ringer's of pH 4. Findings confirm and extend those of Krebs, Borsook and Cohen who found urea or arginine formation in kidney slices and homogenates, but only with added substrate. This might explain changes of urea clearance in sheep on high and low protein diets (Schmidt-Nielsen), as well as the high urea/inulin ratios in micropuncture samples of distal convoluted tubules (Lassiter, Gottschalk).

Chromatographic Studies of Endogenous Pyrogen. NORMAN K. BROWN AND ROBERT G. PETERSDORF, Seattle, Wash. (introduced by William M. M. Kirby).

Endogenous pyrogen (EP) has been found in the blood stream of animals given bacterial endotoxins,

myxoviruses, tuberculin-sensitive animals challenged with old tuberculin, and in experimental pneumococcal, streptococcal, and staphylcoccal infections. EP is presumably derived from injured leukocytes, and leukocytic exudates have been found pyrogenic for animals of the homologous species. Precise biochemical characterization of EP has not been accomplished although available data indicate that it is a protein. In the present studies column chromatography was employed to purify EP, to delineate its biochemical properties and to determine any possible biochemical relationship between serum and leukocytic EP. EP was produced by intravenous administration of typhoid vaccine to rabbits. Two-hour serum was obtained and dialyzed to 0.01 M ionic strength. Dialyzed serum was placed on columns of diethylaminoethyl-cellulose (DEAE) in Tris-succinate buffer at pH 8.2 or in acetate buffer at pH 4.5 to 5.4. Stepwise elution was accomplished with increasing concentrations of NaCl. Tests for pyrogenicity in rabbits, paper electrophoresis, and biuret protein determinations were performed on each eluted fraction. EP was consistently eluted from columns at pH 8.2 with salt concentrations of 0.05 to 0.1 M. Under these conditions, only fractions containing α- and β-globulins were pyrogenic. More efficient purification was obtained with columns at pH 4.5, where EP was adsorbed in association with α-globulins. The remaining serum proteins, comprising about 80 per cent of the original sample, had no affinity for DEAE at this pH. These results suggest that serum EP resides in the electrophoretic α-globulin fraction. This method of selective absorption of EP from nonpyrogenic serum proteins may offer a valuable approach to further purification of EP and its characterization in various types of fever. Preliminary chromatographic studies of EP from leukocytic exudates have paralleled those of EP derived from serum.

A New Parameter in Evaluation of Normal and Abnormal Tissue Slice Metabolism. EDWARD A. BRUNNER AND M. A. SPIRTES, Philadelphia, Pa. (introduced by Joseph R. Di Palma).

The rate of anaerobic glycolysis of normal liver slices can be increased 5- to 10-fold by immersing the slices in hypertonic cofactor-enriched medium. This marked increase in the $Q^{N_2}_{CO_2}$ was found to be accompanied by a comparable increase in the production of lactate. Analysis of the various cofactors in the medium by exclusion experiments showed that hypertonicity, ATP and DPN were the agents responsible for the increased rate of glycolysis. By separating the slices from the surrounding medium following a 10-minute period of preincubation and then determining the glycolytic rate in the latter alone, it was demonstrated that a large part (about 50 per cent) of the increased glycolytic activity was present outside the slice if fructose 1,6-diphosphate was used as substrate. No glycolytic activity could be demonstrated in the surrounding medium when glucose was added as substrate. Thus, some leakage of glycolytic enzymes occurred into the hypertonic-enriched medium, but at least one of the first three enzymes of the glycolytic series either did not leak or was destroyed outside the slice. The possibility was next investigated that the hypertonic-enriched medium might unmask a metabolic change in an abnormal tissue slice which had not manifested itself in Ringer's solution. Liver slices from hyperthyroid animals are known to show a glycolytic rate similar to those from normal animals, in Ringer-type solutions. Utilizing the hypertonic cofactor-enriched medium it was possible to demonstrate a metabolic difference between the two types of slices, one which heretofore could not be demonstrated. The hyperthyroid slices resisted the stimulation to glycolysis which the enriched medium was able to induce in normal liver slices; they maintained a glycolytic rate similar to that which they demonstrated in the Ringer-type medium. In investigating the metabolism of tissues from animals with various physiological abnormalities, the possibility of using experiments similar to those outlined above should be considered. In this way it may be possible to show changes in other parameters where such changes have not in the past been found using a Ringer-type medium.

Changes in Erythrocyte Cation Concentration in Chronic Respiratory Insufficiency. C. E. BUCKLEY, III AND H. O. SIEKER,* Durham, N. C.

Patients with chronic respiratory insufficiency often tolerate marked hypercapnia but may develop uncompensated respiratory acidosis without striking change in the elevated arterial carbon dioxide tension. Serum electrolyte alterations in such patients are recognized, but changes in intracellular cation concentrations have not been investigated. To assess the intracellular changes in these ions, sodium and potassium concentrations were determined in erythrocytes and plasma. Observations were made on 14 normal subjects and on 62 patients with varying degrees of respiratory insufficiency. The mean erythrocyte potassium value for 14 controls was 97.4 ± 1.30 mEq per L of red blood cells. Patients with compensated respiratory acidosis (arterial pH above 7.34) had a mean erythrocyte potassium value of 107.0 ± 2.28 mEq per L, while those with uncompensated respiratory acidosis (arterial pH below 7.35) averaged 95.5 ± 2.52 mEq per L. Erythrocyte sodium concentrations in patients with severe respiratory acidosis and cor pulmonale ranged up to 74.6 mEq per L, as compared to a mean of 14.0 mEq per L for controls. Congestive heart failure and decompensated respiratory acidosis were associated with lowering of the erythrocyte potassium concentration in proportion to the severity of the heart failure. Low plasma sodium values were observed in some of the patients in this group. The total of the two cations, sodium and potassium, ranged as high as 181.5 mEq per L in this group as compared to a mean of 111.4 mEq per L for controls. These data suggest that the erythrocyte sodium and potassium concentrations portray the reten-

tion and distribution of cations secondary to bicarbonate retention in chronic respiratory insufficiency. The shift of sodium into the red cell and the associated potassium loss reflect decompensation with respiratory acidosis and congestive failure.

The Effect of Mineralocorticoids on Renal Handling of Potassium. ROBERT CADE AND ROBERT SHALHOUB, New York, N. Y. (introduced by Ralph E. Peterson).

According to Berliner, potassium excreted in urine enters the tubular fluid as a result of ion exchange in distal tubules and collecting ducts. Exchange of Na for K and H ions in the distal tubule can be increased by administration of mineralocorticoids. We have investigated the relationship between Na load and K excretion by infusing Na_2SO_4 into normal and adrenalectomized humans and dogs and into humans with spontaneously occurring adrenal insufficiency and have found that activity of distal ion exchange processes is related to dose of administered mineralocorticoid. In normal and in adrenalectomized animals with mineralocorticoid replacement, potassium excretion is directly proportional to Na load until a K excretory plateau is reached; further increases in Na load then have no effect on K excretion. The ratio UV_K/UV_{Na}, when UV_K is below the excretory plateau, is a function of mineralocorticoid levels. With large doses of steroids, ratios approaching 2 are obtained; with decreasing doses, the ratio decreases progressively to less than 0.1. Adrenalectomized animals without mineralocorticoid replacement do not vary K excretion with alterations of Na load; when the glomerular filtration rate is changed, however, excretion of K varies in direct proportion to the alteration of filtered K. In the absence of mineralocorticoid we interpret this data as indicating complete inactivity of distal ion exchange; all excreted potassium results from filtration and incomplete reabsorption. When mineralocorticoid is present, the activity of distal ion exchange is related to the steroid level. The maximal K excretory plateau is affected by body stores of K and other factors but cannot be raised by increasing steroid doses.

The Altered Distribution of the Bile Acid Pool in Obstructive Jaundice and its Correction, Including Relief of Pruritus, with a Bile Acid Sequestering Resin. JAMES B. CAREY, JR., Minneapolis, Minn. (introduced by C. J. Watson).

The hypothesis has long been held that the pruritus of jaundice is related to increased concentrations of bile acids in blood. The bile acid pool is normally confined almost entirely to the enterohepatic circulation; only small amounts escape in feces and even smaller quantities occur in systemic blood. To investigate the altered distribution and excretion of bile acids in pruritic jaundice and the effect of removing them from the enterohepatic circulation, total serum bile acid concentrations were measured spectrophotometrically and fecal deoxycholic acid was quantitated after isolation by column chromatography. Bile acids were removed from the enterohepatic circulation by feeding an anion exchange resin (MK-135) which binds them in a nonabsorbable complex. The fecal excretion of deoxylcholic acid was increased from 54 to 500 mg per day in a healthy adult fed 10 g daily. Resin administration to six jaundiced patients with severe pruritus and hyperbileacidemia (24 to 114 μg per ml) lowered serum bile acids and relieved pruritus in each patient. One, a 5 year old boy, with congenital biliary atresia and unrelenting pruritus had total serum bile acids of 115 μg per ml and excreted < 1 mg deoxycholic acid per day. Resin feeding lowered serum bile acids to 20 μg per ml and increased fecal deoxycholic acid to 15 mg daily. Pruritus ceased for the first time in his life. In two patients resin withdrawal increased serum bile acids and pruritus returned; refeeding again lowered serum bile acids and relieved pruritus. These observations demonstrate that the altered distribution of the bile acid pool in incomplete biliary obstruction can be partially corrected by a resin which removes bile acids from the enterohepatic circulation, thus increasing the fecal excretion and lowering serum concentrations. The latter is associated with relief of pruritus, indicating that bile acids play an important role in the pruritus of jaundice.

Transient Excretion of Phosphate after Injection of its Potassium Salt. GASPAR CARRASQUER, Louisville, Ky. (introduced by J. Murray Kinsman).

Renal secretion of phosphate was observed in dogs following injection of KH_2PO_4 into the renal artery, using the technique of Chinard. Nembutalized dogs were given priming and maintenance infusions of mannitol, creatinine, KH_2PO_4 and NaH_2PO_4, in amounts sufficient to maintain high steady-state levels of urine flow, and to maintain constant levels of creatinine (1 to 2 μmoles per L) and of phosphate (3 to 5 μmoles per L) in plasma. After 40 to 60 minutes of maintenance infusion, instantaneous injection of KH_2PO_4 and creatinine (500 μmoles each) was delivered into one renal artery. Following injection, 20 to 30 aliquots of urine were obtained at 6 to 18 seconds each. The pattern of phosphate excretion as compared with creatinine excretion was similar to that obtained when NaH_2PO_4 was injected into the renal artery with elevated plasma phosphate. In short, the transient clearance, or increment of phosphate excreted per unit injected per one renal circulation exceeded that of creatinine during both upsweep and downsweep of the excretory response.

Disorders of Potassium Metabolism in Liver Disease. T. H. CASEY, W. H. J. SUMMERSKILL AND A. L. ORVIS, Rochester, Minn. (introduced by H. R. Butt).

Isotope studies were made in 37 patients with cirrhosis and hepatitis to define the postulated importance of disordered potassium metabolism in complications associated

with liver disease. Conflicting relationships between serum potassium (K_S), exchangeable potassium (K_E), exchangeable sodium (Na_E), total body water (TBW), body weight (BW) and dry body weight (DBW), and variable responses to administration of potassium salts, hindered identification of potassium depletion. Serial changes in K_E and K_E/DBW (30 patients) were most valid in defining metabolic interrelationships. Potassium depletion (low K_E and K_E/DBW) and the ability to retain potassium quickly (12 patients) were related to severity of the liver disease, rather than to ascites or diuresis with its treatment (16 patients). An association between ammonia and potassium metabolism was indicated in 8 noncomatose patients by increasing fasting arterial ammonia concentrations (NH_4A) in relation to decreasing K_E/DBW. In hepatic coma, changes in the EEG were related either to changes in NH_4A or to changes in K_E, where one value remained relatively constant. Extracellular pH was not a factor. During chlorothiazide therapy, clinical and EEG changes of coma were associated with elevation of NH_4A without changes in K_E. A direct effect of chlorothiazide on ammonia metabolism was also demonstrated by significant elevation of NH_4A at 60 minutes after the drug was given in ammonia tolerance tests performed with and without chlorothiazide in 11 patients. In 14 patients with disorders of water and electrolyte metabolism, phases of dilution and azotemia were demonstrated. Hypokalemia was primarily due to dilution (shown by changes in serum sodium (NA_S) related to $Na_E + K_E$/TBW. In azotemia (8 patients), hyperkalemia and ECG changes developed without potassium retention (increase in K_E) and despite greater dilution. Since falls in Na_S and rises in K_S were greater than could be accounted for by changes in $Na_E + K_E$/TBW, hyperkalemia appear to result primarily from intercompartmental shifts of sodium and potassium.

External Secretory Function of Pancreas in Diabetes Mellitus. W. Y. CHEY, H. SHAY AND C. R. SHUMAN, Philadelphia, Pa. (introduced by Richard A. Kern).

Whether changes in external pancreatic secretion can occur in diabetes mellitus has long been debated. Responses of serum amylase and lipase and of the duodenal contents in the pancreozymin-secretin test were compared in 70 diabetic and 40 patients free of gastrointestinal disease. In 50 hospitalized adult diabetics selected by the method of randomization, 10 showed either elevated fasting serum enzyme values or an abnormal serum enzyme response after the hormones were injected and 18 patients showed abnormal values in the duodenal contents. Similar abnormal duodenal findings were obtained in all 6 cases of juvenile diabetes. The most common abnormality was a low amylase output. Diabetic patients with unexplained abdominal symptoms in whom other laboratory studies including gastrointestinal X-ray revealed no abnormality, yielded a higher incidence of abnormal results with the pancreozymin-secretin test than did those without such symptoms. Exocrine pancreatic function was further evaluated in selected patients by starch tolerance, I^{131}-triolein tests and stool examination for fat and undigested meat fiber. These results will be discussed. Duration or severity of disease or complications in the diabetics with abnormal external pancreatic function were not different from those with normal function. Involvement of the islets of Langerhans in primary chronic disease of the acini (chronic relapsing pancreatitis) is well known; the reverse process apparently also can occur in diabetes.

Demonstration of an Essential Role of the Anterior Pituitary in Cushing's Syndrome Associated with Bilateral Adrenal Cortical Hyperplasia. NICHOLAS P. CHRISTY* AND WILLIAM D. DRUCKER, New York, N. Y.

Many attempts have been made to detect adrenocorticotropin in blood of patients with Cushing's syndrome associated with bilateral adrenocortical hyperplasia in order to implicate the pituitary in etiology of the disease. Adrenocorticotropin has not been demonstrable except after adrenalectomy. Earlier work by Jailer and coworkers showed an adrenocorticotropin of obscure nature and origin in plasma of patients with the untreated disease. To characterize this adrenocorticotropin further, the observations have been extended using an accurate and sensitive bioassay: prevention of adrenal atrophy in hypophysectomized rats by 9-day injection of patients' plasma, endpoint being comparison between average adrenal weights (AAW) of groups of 6 to 8 injected rats vs those of 6 to 8 uninjected controls. In an untreated Addisonian, adrenocorticotropin was found: AAW of injected animals 9.5 mg $\pm$ SD 1.27 vs 7.5 ± 1.18, $p < 0.02$. In 4 patients with Cushing's syndrome associated with presumably autonomous adrenal tumor, adrenocorticotropin was never found: AAW 8.9 mg $\pm$ 0.66 vs 8.9 ± 1.14. In 11 of 13 patients with Cushing's syndrome associated with bilateral adrenocortical hyperplasia (without adrenocortical or pituitary tumor) adrenocorticotropin was detected: for the group, AAW was 10.8 mg $\pm$ 1.27 vs 8.3 ± 0.94, $p < 0.001$. Of significance was the finding in 3 such patients that adrenocorticotropin (AAW 11.2 mg $\pm$ 0.87 vs 8.7 ± 0.78, $p < 0.001$) disappeared after pituitary irradiation and clinical remission (AAW 10.3 mg $\pm$ 1.51 vs 9.6 ± 1.30, $p > 0.10$). The detection in Addison's disease of adrenal weight-maintaining activity suggests that this adrenocorticotropin is ACTH. Disappearance of adrenal weight-maintaining activity (conceivably ACTH) from plasma of patients with Cushing's syndrome associated with bilateral adrenocortical hyperplasia after successful pituitary irradiation suggests that the adrenocorticotropin is of pituitary origin, and that the pituitary plays an essential or primary role in maintaining the hyperadrenal state.

Experimental Staphylococcal Infection: The Role of α-Hemolysin and the Effect of Bacterial Endotoxin. LEIGHTON E. CLUFF* AND RICHARD C. CONTI, Baltimore, Md.

Experimental infection of normal animals and man with coagulase-positive staphylococci can be accomplished readily only by inoculation of large numbers of bacteria, indicating the high degree of natural resistance to infection by these microorganisms. For this reason it has been difficult to examine the mechanisms involved in production of host injury in stapyhlococcal disease. Bacterial endotoxin injected intradermally or intravenously in a pyrogenic but nonlethal dose induces in rabbits a striking increase in susceptibility to experimentally induced staphylococcal skin infection. This increased susceptibility is demonstrable only within the first 4 hours after injection of the endotoxin, and infection in endotoxin-prepared animals can be induced only with viable pathogenic staphylococci. The infection in endotoxin-prepared animals suggests that elaboration of α-hemolysin by multiplying staphylococci is responsible for production of hemorrhagic, necrotic and suppurative lesions. Immunity to α-hemolysin (active or passive) provides protection against infection by staphylococci in endotoxin-prepared rabbits. The effect of endotoxin upon staphylococcal infection cannot be inhibited with dibenamine, chlorpromazine or cortisone but is prevented by rendering rabbits tolerant to the pyrogenicity of the endotoxin. The critcial period during which endotoxin affects staphylococcal infection suggests that its action is mediated by an impairment of normal leukocyte function.

Metabolic and Hemodynamic Responses to Prolonged Upright Exercise: Observations on Trained and Untrained Men. LEONARD A. COBB, WILLARD P. JOHNSON AND ROBERT A. BRUCE,* Seattle, Wash.

Effects of walking at a steady, strenuous pace (3 mph, 18 per cent grade) were examined in 9 sedentary young men and in 3 well trained mountain climbers, 1 of whom was studied twice. Serial measurements of cardiac output (indicator dilution), heart rate, $\dot{V}o_2$, and arterial levels of lactate, pyruvate, glucose and free fatty acids were made while the subjects were exercising on a treadmill for periods of up to 75 minutes. Obvious fatigue was regularly observed in the untrained subjects. Excess lactate (XL) was used as an index of anaerobic metabolism (Huckabee). In 6 untrained subjects where XL was measured, it steadily rose to 5.5 to 7.5 μmoles per L at the termination of exercise. In mountain climbers, XL peaked at 4 to 6 minutes (1.0 to 2.3 μmoles per L) and fell to a low plateau through the remaining period of exercise. Plasma unesterified fatty acids fell initially in all subjects and rose in recovery; the highest "rebounds" occurred in the climbers (up to 1,950 μmoles per L). Heart rate during exercise increased steadily in all subjects and was consistently higher in the untrained men (+22 per minute). The average cardiac index during each of 3 temporally equivalent periods was higher in the 7 sedentary subjects (10.4 vs 9.3 L per minute per m^2). Calculated arteriovenous oxygen difference was significantly greater (+27 ml per L, $p < 0.001$) in the climbers, whereas there was no apparent difference in stroke index between the two groups (59 vs 60 ml per beat per m^2). During submaximal upright exercise, greater endurance for prolonged exertion in mountain climbers appears related to metabolic adaptations which permit more effective utilization of oxygen and prevent the accumulation of a large oxygen debt. Under these conditions, improvement of physical performance was not dependent upon enhancement of cardiac output or stroke volume.

Maturation of Liver Enzyme that Conjugates Sulfobromophthalein Sodium (BSP) and Glutathione. BURTON COMBES AND GENEVA SUE STAKELUM, Dallas, Tex. (introduced by Ralph Tompsett).

BSP removal from blood is delayed in premature and newborn infants soon after birth. Thereafter it gradually becomes normal. It has been suggested that this improvement in BSP removal from blood may be due to increased perfusion of the liver with blood and/or to maturation of hepatic function. The present studies were undertaken to determine whether this change in BSP removal might be related to alterations in BSP metabolism after birth. Recent studies have shown that the major pathway of BSP metabolism in several species involves intrahepatic conjugation with glutathione, a process catalyzed by a cytoplasmic enzyme. BSP metabolism was appraised, therefore, by measuring BSP-conjugating enzyme activity and glutathione levels (nitroprusside method of Grunert and Phillips) in livers obtained from rats *in utero* and at various periods after birth. BSP-conjugating enzyme activity, low prior to delivery, increased rapidly soon after birth and then gradually approached adult levels. Five and 3 days before the expected date of delivery, 0.15 mg BSP was conjugated per g liver per 5 minutes. This value was 0.4 mg 1 day prior to delivery, 0.5 to 0.6 mg 1 day postpartum, 1.25 mg at 3 to 4 days, 1.5 mg at 2 weeks, 2.0 mg at 5 weeks and 2.3 mg in adult rat liver. Mixing equal amounts of fetal and adult liver yielded values for enzyme activity equivalent to the arithmetic mean of fetal and adult activity, suggesting that low enzyme activity *in utero* was not due to the presence of an inhibitor in fetal liver, nor was increased activity related to the presence of an enzyme activator in adult liver. Glutathione levels in liver were also low prior to delivery, then gradually approach adult values. There was approximately 1.0 mg glutathione per g 2 to 3 days prior to delivery, 1.0 to 1.35 mg during the first and second weeks post partum, 1.5 mg at 3 weeks, then 1.5 to 2.0 mg during the fourth and subsequent weeks. The above data in the rat suggest that delayed BSP removal from blood soon after birth is related to impaired metabolism of BSP. Attainment of normal BSP removal could be

well correlated with maturation of BSP-conjugating enzyme and increased glutathione levels in liver.

Leukemic Cell Proliferation as Determined by In Vitro Deoxyribonucleic Acid (DNA) Synthesis. CHARLES G. CRADDOCK* AND GEORGE S. NAKAI, Los Angeles, Calif.

Assays of DNA synthesis by normal and leukemic cells (50 cases) were made under standard *in vitro* conditions employing both chemical extraction of P^{32} and tritiated thymidine (H^3T)-labeled DNA, as well as radioautography (H^3T). Points were obtained at 0.5 to 1 hour intervals for 3 hours and again at 19 hours for all parameters in selected instances. H^3T labeling was determined radioautographically both as per cent of labeled cells (per 600 cells) and the grain count distribution, counting immature cells only. The latter value was scored, points being assigned to each cell according to the intensity of labeling. The following observations were made: 1) The time course of H^3T uptake is quite different from that of P^{32} (or C^{14}-formate) in most cell systems; exogenous thymidine is rapidly polymerized with existing intracellular deoxynucleoside triphosphates of adenosine, guanosine and cytosine. This finding is consistent with the concept that endogenous thymidine formation lags behind that of the three other nucleosides. 2) In acute leukemic cells the pattern is often that of very low H^3T labeling (often not significantly above background by radioautography) by many cells. Here the chemical methods are necessary indices. 3) The patterns of labeling are quite distinctive for chronic myelogenous and chronic lymphocytic leukemias, where those cells which proliferate synthesize DNA at a rate comparable to normal myeloid and lymphoid precursors, respectively. The per cent of active cells in chronic lymphocytic leukemia is extremely small. The more blastic process tends to show less labeling on a per cell basis, especially in the lymphoblastic type. There are different patterns in the various types of acute leukemia. The evidence, when coupled with *in vivo* DNA labeling data, indicates a slow rate of proliferation per cell in some blastic leukemias, with a prolonged turnover of the leukemic tissue mass, and a prolonged cell survival. Alterations in the proliferative pattern during the progression of the disease will be described.

Idiopathic Hyperlipemia; the Behavior of the Isolated Chylomicrons as a Substrate for Lipoprotein Lipase. OSCAR B. CROFFORD, Nashville, Tenn. (introduced by Elliot V. Newman).

It is generally held that the rate-limiting step in the removal of chylomicrons from the plasma of patients with idiopathic hyperlipemia is the enzymatic hydrolysis of the plasma triglycerides. Some investigators have postulated an inherent abnormality in the chylomicrons which prevents their being readily attacked by the lipolytic enzymes. To study this possibility, comparisons were made between the isolated chylomicrons from a patient with idiopathic hyperlipemia and those isolated from a normal subject with postprandial lipemia. The comparative rates of lipolysis were determined by measuring the rate of glycerol production when the washed chylomicrons were incubated with lipoprotein lipase prepared from rat adipose tissue. The possibility of non-uniform affinity of the chylomicrons for the enzyme was explored by stepwise increases in the enzyme concentration, thus allowing the reaction to be carried to completion. It was demonstrated that the use of optical clearing as a quantitative index of triglyceride hydrolysis leads to serious errors if the substrate concentration is not adequately controlled. Optical clearing of an incubation mixture could be completely inhibited by the addition of nonturbid, triglyceride-rich plasma, even though lipolysis was proceeding at a linear rate. These results do not deny that optical clearing may be related to the degradation of chylomicrons which are large enough to scatter visible light, but if inequalities in the substrate concentration are produced by the addition of plasma which contains lipoproteins too small to cause visible turbidity, lipolysis can proceed at a maximal rate without visible clearing of the incubation mixture. The study shows that, with methods which are specific for lipolysis, there is no inherent abnormality of the chylomicrons which would explain the accumulation of triglycerides in the plasma of patients with idiopathic hyperlipemia and would place the metabolic defect at a site other than that which has been previously postulated.

Hemodynamic Effects of Oxygen Breathing. WALTER DALY AND STUART BONDURANT, Indianapolis, Ind. (introduced by John B. Hickam).

Despite common clinical usage of oxygen, conflicting data concerning its effects on cardiac index are available. An indocyanine green dilution method was used to evaluate this effect in 15 normal males. In random sequence, air or oxygen was inspired for 10 minutes before making observations. In 6 subjects, observations were made before and after release of arterial thigh tourniquets. In 7 other subjects, observations were made before and after atropinization. In nonatropinized subjects, oxygen breathing decreased the heart rate (69 ± 8 to 64 ± 9 per minute; $p < 0.001$), and cardiac index (3.01 ± 0.53 to 2.67 ± 0.46 L per minute per m^2, $p < 0.001$); stroke index remained unchanged (43 ± 7 ml per m^2); mean brachial pressure increased (93 ± 9 to 94 ± 8 mm Hg, p 0.005); systemic resistance increased (16.7 ± 3.1 to 18.1 ± 3.1 units, $p < 0.001$); circulation time increased (18 ± 3 to 21 ± 3 seconds, p 0.005). After atropinization, heart rate (96 to 97) and cardiac index (3.57 ± 0.48 to 3.50 ± 0.52 L per minute per m^2) were unaffected by oxygen; blood pressure increased (104 ± 8 to 108 ± 8 mm Hg, p 0.025); systemic resistance and circulation time were not affected. Arterial thigh tourniquet compression increased cardiac index

while air was breathed (2.77 ± 0.36 to 3.78 ± 0.38 L per minute per m^2, p 0.02); release of compression caused further increase to 4.59 ± 0.63 ($p < 0.01$). Thigh compression produced no increase during oxygen breathing, and release of compression produced an increase only to 3.74 ± 1.00 (p 0.01). Oxygen breathing (1 atm) produces a vagus-dependent decrease in heart rate and a rate-dependent decrease in cardiac index. In these observations, oxygen breathing abolished the effect of minor pain and anxiety on cardiac output and greatly decreased the effect of sudden release of arterial tourniquets. Oxygen effects must be considered in interpretation of physiologic data and are of obvious clinical significance.

The Effects of Antimitochondrial Sera on Mitochondrial Properties. JOHN S. DAVIS, IV AND ALFRED JAY BOLLET,* Charlottesville, Va.

Circulating factors which react with mitochondria and other cytoplasmic components have been reported in patients with systemic lupus erythematosus (SLE). The effects of these factors on properties of mitochondrial enzymes have been investigated and compared to those of antibodies produced in rabbits by injection of rat liver mitochondria in adjuvant. The rabbit antibodies and the lupus serum factors fixed complement with whole mitochondria, and with both the supernatant and sediment obtained after sonic disruption of mitochondria. The rabbit antisera caused precipitation of DPNH oxidase activity with sonicated mitochondria. Under appropriate circumstances, inhibition of DPNH oxidase activity of sonicated mitochondria by rabbit antisera could also be demonstrated. Sera from patients with SLE had a variable effect in these assays. The activity of these serum factors is being compared to the clinical status in the patients with SLE. The activity of several other mitochondrial enzymes was not inhibited by the antimitochondrial rabbit or lupus sera, including L-glutamic dehydrogenase, fumarase and "DPNH dehydrogenase," nor was acid phosphatase inhibited. However, antibodies produced by injection of rabbit with purified L-glutamic dehydrogenase did inhibit that enzyme in sonicated mitochondrial preparations. The rabbit antisera caused a release of L-glutamic dehydrogenase activity from isolated mitochondria into the supernatant solution, indicating an effect on the mitochondrial membrane. Sera from SLE patients had very little effect. The relative activity of various steps in the DPNH oxidase pathway in connective tissue harvested from polyvinyl sponges differed from the findings in liver. The DPNH-cytochrome C reductase/DPNH oxidase and "DPNH dehydrogenase"/DPNH oxidase ratios were considerably higher in the connective tissue. The influence of the antimitochondrial sera on the steps in the DPNH oxidase pathway and on ATP synthesis in different tissues is under study. These findings characterize some of the metabolic effects of antimitochondrial complement-fixing serum factors.

Fluorescence Spectrophotometric Studies of Metabolic Control in the Isolated Living Toad Bladder. ROBERT P. DAVIS AND MITZY CANESSA-FISCHER, New York, N. Y. (introduced by Quentin B. Deming).

Spectrophotometric studies of isolated mitochondria and unicellular organisms have revealed details of the processes of electron transfer and metabolic control. This is a report of the application of similar techniques to the study of active transport systems in an intact tissue in order to develop information on the metabolic regulation of transport. The fluorescence of reduced pyridine nucleotide (RPN), oxidized flavin nucleotides and tissue proteins was demonstrated in the living, intact, isolated toad bladder during and in the absence of active sodium transport. The quantitative response of the fluorescence intensities activated at 345, 325 and 290 mμ was observed in the resting state without added substrate, during transition from anaerobic to aerobic states, and after addition of glucose, adenosine diphosphate (ADP), pyruvate, succinate and amytal. Without disruption of biological structures, two fractions of RPN were demonstrated, one free and the other protein-bound. Study of these nucleotides under various conditions enabled identification of sites of inhibition of electron transfer and sites of coupling of energy changes of nucleotide reduction to phosphorylation of ADP. From the response to succinate, pyruvate and glucose addition, a succinoxidase system and a conventional pyridine nucleotide coupled electron transfer system were established. Very tight metabolic regulation by ADP has been demonstrated by the fall in fluorescent intensity of RPN following addition of ADP to the bladder. From the response to ADP, it is concluded that the bound RPN represents the special fraction of this nucleotide coupled to oxidative phosphorylation. The states of oxido-reduction of pyridine and flavin nucleotides in response to vasopressin have also been investigated. A marked increase in level of reduction and degree of protein-binding of nucleotides was shown. These data may provide information on the cellular biochemical mechanism by which energy is transduced to ion transport.

A Soluble Thyroidal Peroxidase-Iodinase System. LESLIE J. DEGROOT AND ANN M. DAVIS, Boston, Mass. (introduced by John B. Stanbury).

A soluble enzyme functioning as both iodide peroxidase and tyrosine iodinase has been extracted from sheep thyroids. It is released from mitochondria and microsomes by brief chymotrypsin digestion and extraction with N-butyl alcohol and acetone. It is nondialyzable, heat-labile, and is destroyed by prolonged enzymatic proteolysis. In standard conditions, enzyme (± 200 μg protein), buffer, I^{131}, 10 mμmoles KI, 1 μmole tyrosine, 1 μmole glucose, and 5 mg glucose oxidase are incubated 30 minutes, with formation of 8 mμmoles iodotyrosine. Enzymic activity can also be followed spectrophotometrically as an increase in absorbance at the iodo-

tyrosine peaks. The enzyme iodinates free tyrosine, monoiodotyrosine and certain proteins, and requires a hydrogen peroxide source. Specific activity (mμmoles of iodide bound to tyrosine per mg protein) 500 times that of the starting material has been reached. A similar enzyme can be solubilized from salivary and splenic tissue, but not from liver, kidney, or brain. It has no reduced pyridine nucleotide oxidase activity, no iodotyrosine deiodinase activity, and no cytochrome oxidase activity. In striking contrast to the enzyme prior to solubilization from cell particles, it is inhibited by reduced pyridine nucleotide. The enzyme can be saturated, but not inhibited, by increased concentrations of iodide. It is inhibited by thiocyanate cyanide, but not by perchlorate. Methimazole is a strict competitive inhibitor. The ratio of diiodotyrosine to monoiodotyrosine formed varies from 0.1 to 0.7 and is a direct function of the molar ratio of iodine to tyrosine present in the incubation mixture. The enzyme shares many of the properties of thyroid tissue studied *in vivo,* and may be a part of the complete *in vivo* iodinating system. It appears to be a peroxidase-iodinase fragment, split away from the reduced pyridine nucleotide-flavin system which may serve as its *in vivo* H_2O_2 generating source.

The Importance of Autonomic Integrity in Maintaining the Hyperkinetic Circulatory Dynamics of Human Hyperthyroidism. WILLIAM J. DEGROOT, JAMES J. LEONARD, HYMAN W. PALEY, JOHN E. JOHNSON AND JAMES V. WARREN,* Galveston, Tex.

Increased responsiveness to sympathetic nerve activity and endogenous circulatory catecholamines have been implicated as important mediators of the hyperkinetic circulatory state seen in thyrotoxicosis. This hypothesis has been formulated primarily from *in vitro* and *in vivo* animal studies. The advent of pharmacologic agents capable of interrupting the integrity of the autonomic nervous system at various sites has provided a tool for the re-evaluation of this thesis in human hyperthyroidism. The response to three drugs was evaluated: 1) the ganglionic blocking agent, trimethaphan camphorsulfonate (Arfonad); 2) guanethidine, a postganglionic sympathetic neuroplegic; and 3) reserpine, an agent causing tissue catecholamine depletion. Dose and duration of therapy were individualized. A total of 19 hyperthyroid patients was studied (mean age 32) and each patient served as his own control. Cardiac output was measured by the Hamilton indicator dilution method. Heart rate, blood pressure, carotid pulse tracing and phonocardiogram were recorded with each cardiac output. Comparable normal data were obtained from 29 young volunteers. Trimethaphan camphorsulfonate caused no change in heart rate but reduced cardiac output by 11 per cent (5.3 to 4.7 L per minute per m^2; $p < 0.02$), thus remaining 57 per cent above normal (3.0 L per minute per m^2). Diminution of cardiac output was not effected by guanethidine (5.6 to 5.2 L per minute per m^2) or reserpine (5.8 to 6.2 L per minute per m^2). Guanethidine caused a pulse slowing of 20 per cent (109 to 89; $p < 0.01$) and reserpine a slowing of 17 per cent (102 to 85; $p < 0.01$). The isometric contraction period (0.02 second) and the duration of ventricular ejection (0.23 second), short in hyperthyroidism, were not normalized by these drugs. Chemical autonomic blockade with three pharmacologically different agents failed to modify the basic cardiovascular response to increased metabolic demands. A mechanism other than increased sensitivity to catecholamines appears to be responsible for the hyperkinetic circulation of clinical hyperthyroidism.

The Influence of Parathyroid Hormone on Bone Matrix Hexosamine Synthesis. WILLIAM P. DEISS,* LEILA B. HOLMES AND C. C. JOHNSTON, Indianapolis, Ind.

The close temporal relationship between calcium mobilization by parathyroid extract (PTE) and the disappearance of bone matrix hexosamine suggests that the action of parathyroid hormone (PTH) on bone leads to a disruption of both the mineral and organic phases, which are normally intimately associated. The present studies were designed to evaluate further the dynamic state of bone matrix hexosamine as influenced by PTH by measuring *in vivo* and *in vitro* incorporation of C^{14} from glucose into hexosamine of femur matrix. In rats injected with glucose-C^{14} 6.5 hours after pretreatment (75 U PTE or equivalent volume of control diluent every 2 hours for 3 doses), the specific activity curves of the serum hexosamine were nearly identical in the PTE and control groups. There was, however, a distinctly impaired incorporation of isotope into bone matrix hexosamine throughout the 12 hours following the glucose-C^{14}. The peak specific activity of the matrix hexosamine occurred 5 hours after isotope injection and at this time there was a decrease in matrix hexosamine concentration and a 34 per cent decrease in specific activity of the PTE group compared with controls. When the glucose-C^{14} was injected 20, 44, and 68 hours after PTE treatment, a stimulated matrix synthesis was manifested by a progressive increase in the peak specific activity of the matrix hexosamine (38, 53 and 69 per cent, respectively, above the controls) and by a return of the matrix hexosamine concentration to normal by 68 hours. *In vitro* incubation with glucose-C^{14} of minced femur from similarly pretreated rats also indicated increased incorporation of isotope into the matrix hexosamine of the PTH-treated group. These findings indicate an active turnover of bone matrix hexosamine. They also demonstrate a prompt suppression by PTH of matrix hexosamine synthesis accompanying calcium mobilization, followed by an intense compensatory resynthesis of organic matrix.

Dicumarol-induced Depression of a Thrombogenic Coagulation Activity in Man. DANIEL DEYKIN, STANFORD WESSLER,* STANLEY M. REIMER AND V. JANE CRANNEY, Boston, Mass.

We have recently described, in normal human blood, a thrombogenic coagulation activity (STA) that arises from an inactive precursor early in the elaboration of

"intrinsic" thromboplastin, is separable from all known procoagulants, and is measured by a quantitative bioassay. In the present study, assays of the STA activity of human sera, obtained from patients treated with bishydroxycoumarin (Dicumarol), were utilized as a reflection of the interference of this drug with *in vivo* thrombosis. In 7 patients on short-term and 13 patients on long-term therapy, the *in vitro* anticoagulant effects of Dicumarol were measured both by a modified Quick procedure and by the "thrombotest," whereas the *in vivo* antithrombotic effect was determined by the bioassay of STA activity. In addition, in the short-term subjects, serial *in vitro* assays of clotting factors II (prothrombin), VII (proconvertin), IX (PTC), and X (Stuart), and *in vivo* assays of STA activity were followed in all 7 patients together with appropriate controls. The sera of 6 of the 7 short-term patients demonstrated an antithrombotic effect that was observed in only 3 of the 13 long-term patients. In the short-term group the antithrombotic effect was independent of the depression of factors II, VII, IX, and X, singly or in combination, and, in several instances, did not appear until several days after the onset of the anticoagulant effect. We considered these data to represent the first experimental demonstration in human blood of an *in vivo* antithrombotic action of Dicumarol mediated through the intrinsic clotting system; they also indicate that Dicumarol depresses in human blood a hitherto unrecognized thrombogenic coagulation activity.

Left Ventricular Pressure-volume Loops in Man. HAROLD T. DODGE, HAROLD SANDLER AND ROBERT E. HAY, Seattle, Wash. (introduced by Robert S. Evans).

A method for determining left ventricular (LV) pressure-volume (P-V) loops in man has been developed and applied to study left ventricular hemodynamics in 12 subjects with mitral and/or aortic valvular disease. LV volumes (LVV) were calculated from biplane angiocardiograms taken at 6 per second and timed with respect to the cardiac cycle. Single composite volume curves were constructed from volume observations over several heart beats. P-V loops were constructed by relating LVV to LV pressure (LVP) recorded during (6 subjects) or just prior to angiocardiography. Individual LV P-V loops showed marked differences in magnitude and configuration, which were determined primarily by the type and severity of the valvular lesion. LV stroke work was calculated from the enclosed area, $\oint$ PdV, of the individual P-V loops and is the only method for calculating LV work in man with aortic and/or mitral valvular insufficiency. Stroke work varied from 55.9 to 457 g-m per beat. The location of individual P-V loops on the P-V axes varied widely and reflected the state of myocardial compensation. The area lying beneath the P-V loops, $\int_{V_S}^{V_D} P_D\,dV$, P_D = diastolic LVP, represented work done in distending the diastolic LV and shifts in the static level of pressure in the vascular system. With valvular insufficiency and heart failure, this area became large and was equivalent to work values as high as 79 g-m per beat. Under these conditions, a large error is introduced by methods which include this area in calculating LV work. LV P-V loops provide a previously unavailable method for calculating LV work in man with valvular insufficiency or mixed valvular lesions. This method does not require calculation of kinetic work in the aorta and permits differentiation of work done on the LV in diastole from work done by the ventricle in systole.

Malabsorption of Labeled Vitamin B_{12} in Rats with Intestinal Diverticula. R. M. DONALDSON, JR., Boston, Mass. (introduced by F. J. Ingelfinger).

Malabsorption of vitamin B_{12} by patients with the "blind-loop syndrome" is attributed to abnormally located intestinal bacteria, but exact mechanisms are unknown. E. W. Strauss and F. H. Gardner demonstrated malabsorption of labeled vitamin B_{12} in rats with mid-intestinal diverticula and showed that malabsorption was corrected by oral neomycin. Fecal excretion of radioactivity was used to measure Co^{60}-labeled vitamin B_{12} ($Co^{60}B_{12}$) absorption, and the results of Strauss and Gardner were confirmed. Absorption by rats with diverticula remained abnormal when $Co^{60}B_{12}$ was bound to neutralized rat gastric juice (RGJ). After surgical removal of intestinal pouches, $Co^{60}B_{12}$ absorption promptly increased to normal. A similar increase was noted when pouches were left in place but were surgically "by-passed" so that oral $Co^{60}B_{12}$ no longer entered the sacs. When isolated segments of ileum were perfused *in vivo* with $Co^{60}B_{12}$ bound to RGJ, intestinal uptake of radioactivity was the same in rats with diverticula as in control animals. Radioactivity was recovered from sac contents for 18 hours after oral $Co^{60}B_{12}$, and at 5 hours this amounted to 60 per cent of the dose. $Co^{60}B_{12}$ bound to RGJ was injected into pouches of untreated rats and rats fed neomycin. The precipitate of sac contents, thought to represent primarily bacteria and their products, was markedly reduced in amount in neomycin-fed rats. Furthermore, radioactivity recovered from precipitated contents was 76 to 94 per cent of the injected dose in untreated rats but never exceeded 25 per cent in neomycin-fed animals, and in treated rats the proportion of injected radioactivity found in pouch walls was greatly increased. These results suggest that malabsorption of labeled vitamin B_{12} in rats with diverticula is not due to bacterial elaboration of toxins or lack of intrinsic factor, but is more likely a consequence of uptake of $Co^{60}B_{12}$ by bacteria within the intestinal sac.

The Effect of Acetazolamide on the Secretion of Sodium and Potassium by the Human Stomach. D. A. DREILING, A. LINDER AND N. COHEN, New York, N. Y. (introduced by H. D. Janowitz).

The secretion of HCl by the mammalian stomach is dependent in part on the presence of carbonic anhydrase activity, as we have previously reported to this Society.

To obtain further evidence on the role of this enzyme in the gastric mucosa we have investigated the effects of the inhibitor acetazolamide on the secretion of Na and K by the human stomach. Gastric secretions were obtained by constant nasogastric suction in 20 subjects with a wide range of secretory activity. In 10, secretion was stimulated by histamine before and after the i.v. administration of acetazolamide (75 to 100 mg per kg) and analyzed for Na, K, Cl and H (titratable acidity to pH 3.5 and 7.0). Histamine augmented the rate of secretion of Na and K as well as demonstrating its expected increase in HCl secretion. Acetazolamide inhibited the output of Na and K as well as confirming the previously demonstrated reduction in acid output. The reduction of K and Cl was directly correlated with the reduction in rate of secretion. The reduction in H output was greater than could be accounted for by flow rates, and that of Na less; the well known inverse relation between Na and H concentrations was again observed. These results suggest that some Na enters the gastric juice in exchange for H, and that some K enters along with H. The effects of acetazolamide appear to be explicable in terms of the primary process of secretion and not necessarily on any subsequent process of exchange.

Early Effects of Vitamin A on Calcium, Citrate and Phosphorus Metabolism in Man. THEODORE DULL, PETER F. MAURICE, DOROTHY H. HENNEMAN AND PHILIP H. HENNEMAN,* Jersey City, N. J.

Mellanby, Wohlbach and others have demonstrated that vitamin A accelerates bone remodeling. Recently Clark has reported that vitamin A alters radiocalcium metabolism in the rat. Hypervitaminosis A in children produces painful periosteal new bone formation; in adults only acute toxic effects have been reported. We have administered vitamin A, 200,000 U daily for 3 to 4 weeks, during complete balance studies in 2 patients with hypoparathyroidism, 2 with sarcoid and hypercalcemia, 1 with presumed hyperparathyroidism and one normal subject. Vitamin A serum levels rose from 35 to 106 and from 65 to 176 μg per 100 ml in 2 subjects. Vitamin A markedly depressed serum and urinary citrate in all 6 subjects. Citrate changes preceded, were more marked than and persisted longer than the calcium and phosphorus changes. Serum calcium decreased from 10.9 to 9.1 mg per 100 ml in a patient with presumed hyperparathyroidism and decreased slightly in two other subjects. Urinary calcium fell significantly in 5 subjects; fecal calcium was unaltered. Urinary phosphorus decreased in 4 subjects; serum and fecal phosphorus did not change. Serum alkaline phosphatase and creatinine clearances were unchanged during vitamin A. Earliest effects were seen by 3 days, were maximal after 3 weeks and persisted for 1 to 3 weeks after discontinuation of vitamin A. Two subjects showing responses were hypoparathyroid. The phosphaturic effect of parathyroid extract and vitamin D enhancement of calcium absorption were unimpaired by vitamin A. Thus vitamin A in large doses in man appears to favor calcium and phosphorus retention by bone. These data do not indicate whether vitamin A stimulates osteogenesis or inhibits osteoclasis.

Demonstration of Tissue Antigens in the Blood Platelets. SHIRLEY EBBE AND MARIO BALDINI, Boston, Mass. (introduced by William Dameshek).

The previous finding in humans that repeated infusions of platelets from blood group-compatible donors reduced the viability of subsequently infused platelets raised the possibility that platelet antigenicity might be partially or totally dependent on transplantation antigens. To study this problem, 1) the survival time of skin homotransplants in rabbits previously sensitized to homologous platelets was determined, and 2) the viability of homologous platelets infused in rabbits sensitized by skin homografts was measured using platelets labeled *in vivo* with P^{32}. Skin homografts in rabbits sensitized by repeated intradermal injections of homologous platelets were rejected more rapidly than were homografts in nontreated controls. Skin homografts from the specific platelet donors had shorter survival than nonspecific homografts from other donors. Because platelet preparations used were not absolutely free of leukocytes, the reverse approach was then adopted. Viability of infused homologous platelets was measured in rabbits in which one to three consecutive skin homografts had previously been applied. After three grafts, viability of platelets from both specific and nonspecific donors was markedly reduced. Animals sensitized with one skin homotransplant followed by one platelet infusion showed strikingly reduced viability of subsequently infused homologous platelets, while a single skin homotransplant or a single platelet infusion did not appreciably change the viability of infused homologous platelets. This phenomenon had the characteristics of a secondary immunologic response to the applied skin homograft. Conclusions are that: 1) blood platelets and skin cells have antigens in common; 2) the common antigens are not strictly individual-specific; 3) it is possible that these platelet "tissue antigens" are transplantation antigens, and that measurement of platelet viability may become useful in the study of transplantation immunity; 4) these results imply that in thrombocytopenic bleeding repeated transfusions of blood platelets will have continued hemostatic effectiveness only in immunologically tolerant recipients.

Metabolism During Potassium Transport in Human Red Cells. ROBERT E. ECKEL* AND HARVEY LODISH, Cleveland, Ohio.

Cold-stored human blood accumulates fructose diphosphate (FDP) at the expense of inorganic phosphate (IP) and glucose. The red cells, washed in a saline-Tris buffer, and incubated with K (0.005 M), transport K in and Na out in the absence of added substrate for over 1 hour (transporting cells). In the absence of added K, the K concentration in the medium is about 0.001 M and no net transport occurs (resting cells). Intracellular

K concentrations are the same under the two conditions. Usually, but not always, transporting cells use more FDP. In all cases, transporting cells produce lactate and IP approximately equal to the FDP used. Resting cells produce about 20 per cent less of each. The fate of the remaining carbon and P in resting cells is not yet known. Several intermediates including pyruvate and lipid fractions have been excluded. Ouabain exaggerates the metabolic effects of hypokalemia and, in the presence of K, mimics the effects of hypokalemia. Nucleotide levels are low and little affected by experimental conditions. The following working hypotheses are suggested: 1) close coupling between glycolysis and ion transport exists in these experiments; 2) the increment in lactate and IP production in transporting cells represents a significant fraction of the metabolic cost of transport; 3) the diversion of carbon and P from these end-products demonstrates a control of intracellular metabolism by a mechanism (ion transport) involving extracellular ion concentration. That this change in metabolism is due to the arrest of transport and not to the hypokalemia per se is presumed from the similar metabolic effects of hypokalemia and ouabain and the assumption that ouabain is a specific inhibitor of transport.

Responses of the Peripheral Veins in Man to Continuous Positive Pressure Breathing. JOHN W. ECKSTEIN, A. W. HORSLEY AND WILLIAM K. HAMILTON, Iowa City, Iowa (introduced by E. L. DeGowin).

Venous pressure-volume curves were obtained from the right forearm of 8 healthy men, with a plethysmographic method. Venous pressure was measured in the left arm. Airway pressure was monitored through a needle inserted into a face mask. Observations were made with subjects breathing against ambient pressure and repeated when they inspired from and expired into a rubber reservoir bag. Outflow from the bag was restricted so that mask pressure remained positive during all respiratory phases. The flow of compressed air into the bag was sufficient to prevent serious elevation of CO_2 in the inspired air. Observations were made at a lower (average, 8.2 mm Hg) and higher (average, 15.0 mm Hg) level of mean mask pressure in each subject and repeated at the higher level 30 to 45 minutes following intravenous injection of morphine sulfate (15 mg). Venous pressure increases were roughly proportional to increases in mask pressure. Venous tone increased in 7 and remained unchanged in 1 of 8 subjects. In 4 the increase was sufficient to overcome the increase in venous pressure and prevent blood from pooling in the forearm. Pooling would have occurred in each case if venous tone had not increased. Forearm venous blood volume averaged 2.7 ml per 100 ml of tissue during control periods, 2.6 ml at the higher, and 2.6 ml at the lower mask pressure. The volume at the higher level would have averaged 3.2 ml if there had been only an increase in venous pressure and no increase in tone. Morphine did not prevent or modify significantly the venous response to breathing at the higher mask pressure. These observations suggest that increased venous tone may aid the return of blood to the heart during pressure breathing.

An Appraisal of the Venous Hematocrit. SEYMOUR EISENBERG, Dallas, Tex. (introduced by Ben Friedman).

The venous hematocrit has been criticized on the grounds that 1) it does not reflect the ratio of cells to plasma throughout the body, and 2) it entails errors due to "trapping" of plasma within the red cell layer and to incomplete packing of cells. To explore the validity of these criticisms, the following studies were performed. Utilizing an improved technic for plasma volume determination, the body hematocrit/venous hematocrit ratio was determined in 20 normal subjects; "plasma trapping" and incomplete packing were investigated with radioiodinated serum albumin-labeled plasma and microscopic examination of the cells. Labeled (I^{131}) polyvinylpyrrolidone (PVP-K90) was employed as a reference substance to measure plasma volume, and the red cell mass was simultaneously determined with Cr^{51}. The range of the body hematocrit/venous hematocrit ratio in 18 subjects was 90 to 105, with a mean of 96. Normal subjects were given radioalbumin (RISA, with <1 per cent free I^{131}). The radioactivity in whole blood and plasma sampled from these subjects was then determined and the ratio of cells to plasma calculated. Although these values agreed fairly well with corresponding Wintrobe hematocrit values, the latter were slightly lower than this calculated ratio (mean = 1 mm packed cell volume). The hematocrit tubes were then cut below the cell-plasma margin and the specific activity in suspensions of the packed cells determined; by this technic the amount of "trapped plasma" was consistently less than 1 per cent. Lastly, these cells were found to be markedly crenated. It is concluded that the venous hematocrit (Wintrobe) is an accurate measure of the ratio of cells to plasma. Previous estimates of the venous hematocrit/body hematocrit ratio have been falsely low. Minor errors due to slight trapping of plasma within the red cell layer are offset by crenation of the red cells.

Iron Uptake of Human Bone Marrow Incubated with Chloramphenicol. A. J. ERSLEV* AND I. IOSSIFIDES, Philadelphia, Pa.

Aplastic anemia is a well substantiated but rare complication of prolonged therapy with chloramphenicol. However, ferrokinetic studies have suggested that chloramphenicol quite commonly induces a reversible suppression of the erythropoietic tissue. In order to study the biochemical mechanism of this suppression and its possible relationship to aplastic anemia, human bone marrow obtained from patients undergoing rib resection was suspended in human plasma, divided into numerous aliquots, and the effect of chloramphenicol on the cellular uptake of Fe^{59} and total iron was determined after 4 and 21 hours' incubation. Chloramphenicol was found to induce a decrease in iron uptake directly

proportional to the concentration of drug with an uptake of 66 per cent of normal at a concentration of 400 μg per ml (about 15 times usual therapeutic levels). This decrease was probably not caused by a change in the rate of cellular multiplication since the difference between colchicinized and noncolchicinized samples was the same at all drug levels. The principle metabolic end-product of chloramphenicol, the biologically inactive glucuronide, was examined in the same system and found to be completely inert. However, three biologically inactive stereoisomers were found to be as "toxic" as chloramphenicol. The action on iron metabolism was related to the dichloroacetamide side chain rather than to the nitrobenzene group, since a naturally occurring hydrolytic product with a hydroxyacetamide side chain was inert. In order to gain a perspective of these findings relative to aplastic anemia, antibiotics not believed to cause aplastic anemia were also tested. Penicillin, streptomycin, kanamycin and vancomycin were found to be inert. However erythromycin was as "toxic" as chloramphenicol and all the tetracyclines were found to be even more "toxic," with iron uptake of 33 per cent of normal at a concentration of 100 μg per ml. The significance of these data will be discussed.

Urine Flow Response to Mannitol Loading in Unilateral Renal Disease. MELVIN H. FARMELANT AND BELTON A. BURROWS,* Boston, Mass.

External monitoring of individual kidney areas following intravenous injection of radioactive Diodrast or Hippuran yields curves whose fall-off rates are primarily a function of the urine flow rate. Dividing the ratio of the radioactivities existing in each kidney area at the time of peak uptake into the ratio present after an interval provides a percentage expression of the relative fall-off rates of the two curves for that interval. Such calculations were made in 27 hypertensive patients who showed a significant difference between the kidneys in urine flow rates by this technique. Comparisons were made between the effects of water loading and mannitol loading on urine flow rate differences (UFRD) in each patient. Four patients were similarly studied during ureteral catheterization. With water loading, 16 patients with renal arterial disease (proven by aortography, catheterization studies and surgical exploration) showed a greater mean UFRD than did the 11 patients with intrinsic or obstructive renal disease (47 vs 29 per cent, $p < 0.01$), with little overlapping of values. With mannitol diuresis, the mean UFRD in patients with arterial disease was reduced by 23 per cent, and in the intrinsic-obstructive group by 4 per cent, with no overlap in the percentage change in UFRD. The results with ureteral catheterization were comparable. Two patients with arterial lesions had a diminished UFRD and one showed no mannitol response during salt depletion, the expected UFRD and mannitol response being restored by salt repletion. These studies indicate that in man, as in the experimental animal, alterations in renal blood flow-pressure relationships result in marked reductions of urine flow, which can be partially restored by mannitol diuresis. Comparable UFRD's and mannitol responses were not observed in parenchymal or obstructive renal disease.

A New Protein Disturbance Associated with Rheumatoid Arthritis. RICHARD S. FARR, GERALD P. RODNAN AND ELLIOTT C. LASSER, Pittsburgh, Pa. (introduced by Jack D. Myers).

All human sera bind I^{131}-labeled sodium acetrizoate (I*Urokon) as measured by equilibrium dialysis performed at room temperature for 18 hours when the outside bath contained 0.8 per cent dextran in saline borate buffer at pH 7.4. When the concentration of unbound Urokon in solution at equilibrium was 73.8 μg per ml, a 0.95 per cent solution of human albumin bound 70.1 μg per ml and 1:5 dilutions of human sera from a variety of disease states, including rheumatoid arthritis, bound between 15.5 and 63.5 μg Urokon per ml. Under these conditions, the amount of Urokon bound was found to be related to albumin concentration. However, when the concentration of unbound I*Urokon at equilibrium was 0.06 μg per ml, 10 of 16 rheumatoid arthritis sera bound 2.7 to 4.3 times more Urokon than could be accounted for on the basis of albumin concentration. In contrast, the highest value obtained with 20 normal sera was 1.2×, with 15 scleroderma sera 2.5×, with 10 lupus erythematosus sera 1.9×, and with 38 sera from other acutely or chronically ill patients 2.4×. Human γ-globulin does not bind Urokon and the increased binding by rheumatoid sera did not correlate with globulin concentration or latex fixation reaction. Urokon binding to sera is highly specific in the sense that the addition of an acetylamino group at the fifth position in the benzene ring of Urokon prevents the interaction. Some other substituted benzoic molecules compete for Urokon binding sites, but increased binding by rheumatoid serum does not appear to be due to antisalicylate. These findings suggest that Urokon and related substances compete for specific receptor groups on normal albumin. Studies are in progress to determine whether increased Urokon binding associated with rheumatoid arthritis is due to an unusual albumin or to other peculiar proteins associated with the disease.

Experimental Reactivation of Subsiding Rheumatic Fever. ALVAN R. FEINSTEIN AND MARIO SPAGNUOLO, Irvington-on-Hudson, N. Y. (introduced by Currier McEwen).

The present work demonstrates the experimental provocation of rheumatic activity in asymptomatic patients and provides an explanation for the mechanism of the post-therapeutic rebound phenomenon in rheumatic fever. A 2-week course of treatment with prednisone, aspirin, or nothing was given experimentally to 88 randomly allocated patients convalescing from rheumatic fever. The experimental treatment in each patient was begun exactly 1 month after discontinuation of the initial course of treatment, a time when all patients had no *clinical* evidence of rheumatic activity. During retreatment, the

sedimentation rate and C-reactive protein were invariably lowered by prednisone, but were sometimes raised by aspirin. The mean hematocrit rose with prednisone and fell with aspirin. When the experimental therapy was stopped, a recrudescence of fever, tachycardia, arthralgia, erythema marginatum, or congestive heart failure occurred in 9 of 29 patients treated with prednisone and in 1 of 29 patients treated with aspirin. The 30 controls remained asymptomatic. The experimental rebounds were more frequent in patients with severe cardiac damage and occurred only in patients who had shown clinical or laboratory evidence of rheumatic activity immediately after their initial course of treatment. These results contradict the hypotheses that the rheumatic rebound is due to either 1) polycyclic rheumatic activity, 2) relative hypoadrenalism, or 3) insufficient duration of therapy. They indicate that the rebound represents the appearance of rheumatic inflammation which accumulated under therapeutic suppression. They also indicate that anti-inflammatory therapy can be detrimental for asymptomatic rheumatic patients whose only abnormalities are in laboratory tests.

Granulocyte Response to Endotoxin in Myeloproliferative Disorders. MARTIN E. FINK, PAUL CALABRESI AND STUART C. FINCH,* New Haven, Conn.

Studies were performed to determine the magnitude and characteristics of the leukocyte response to endotoxin in patients with myeloproliferative disorders. Sixteen patients without hematological abnormalities, 4 with chronic granulocytic leukemia (CGL), 4 with myelofibrosis and 2 with polycythemia vera received injections of Pyrexal, 0.1 μg intravenously. Total and differential leukocyte counts were performed before injection and at hourly intervals for 7 hours. The properties of the mobilized granulocytes in the myeloproliferative group were also compared with four controls with respect to hourly changes in 1) enzyme content (alkaline phosphatase), 2) cytoplasmic integrity (lysozyme release), 3) participation in the local inflammatory response (Rebuck technique), and 4) phagocytic capacity (sensitized starch granules). The typical response was a granulocytic leukocytosis reaching its peak in 3 to 5 hours. The average absolute granulocyte increase in the normals was 6,590 cells per mm^3 (range, 2,220 to 12,393) and consisted only of mature cells compared to 21,200 mature granulocytes per mm^3 (range, 12,740 to 29,880) and 2,776 immature forms per mm^3 (range, 1,690 to 4,389) in patients with CGL, although their disease was well controlled with busulfan. In three patients with classical myelofibrosis, increases of only 978 granulocytes per mm^3 (range, 0 to 1,478) were obtained. The fourth patient whose baseline leukocyte count was 62,000 and, unlike the others, had no elevation of granulocyte alkaline phosphatase, manifested a normal granulocyte response, as did the subjects with polycythemia. A slight but consistent elevation of the granulocyte alkaline phosphatase score was observed at 1 to 2 hours. A significant rise in serum lysozyme level was detected only in one patient with CGL following a peak response of 27,990 granulocytes per mm^3. In all cases, mobilized granulocytes manifested no change in their ability to participate in the local inflammatory response or in their capacity to phagocytize sensitized starch granules.

The Course of Urinary Tract Infections Secondary to Catheterizations. JANET J. FISCHER, Chapel Hill, N. C. (introduced by Louis G. Welt).

One hundred ninety-four patients hospitalized on the Gynecology Service were studied and followed to determine the incidence of, and the course of urinary tract infections in this group over a 2 year period. The vast majority of these patients had carcinoma, usually of the cervix, but some had carcinoma of the uterus or of the vagina. Ninety per cent of the patients were catheterized and 40 per cent had an indwelling catheter in place for at least 2 days. At the time of admission to the study, 85 per cent were uninfected. Two to 4 weeks later, 35 per cent (26 patients) of these uninfected patients subjected to one catheterization, 25 per cent (2 patients) of those not catheterized at all, and 60 per cent (34 patients) of those with indwelling catheters were infected. Ninety per cent of the infections were due to gram-negative rods, 45 per cent to *Escherichia coli,* and 25 per cent to Aerobacter-Klebsiella group. At 3 months, 50 per cent of the patients with *E. coli* still were infected. Repeated infections with different organisms were present in 3 per cent of the patients. Contrary to the accepted concept of the course of carcinoma in these patients, analysis of these data support the idea that the occurrence of urinary tract infections in this group of patients is related to catheterizations performed as part of therapy, and that some of these patients manifest uremia as a result of progressive urinary tract infection before urinary tract obstruction has occurred.

The Diagnosis and Quantitative Evaluation of Mitral Regurgitation. CHARLES W. FRANK, FRIDO H. KIEFHABER, ALPHONZO JORDAN, TURHAN AKYOL, BARRY B. GALTON AND YOGESH ARORA, New York, N. Y. (introduced by Howard A. Eder).

It has long been appreciated that mitral regurgitation can be recognized by the appearance of an indicator dye within the left atrium immediately after its introduction into the left ventricle. We have found that regurgitation induces a specific systemic arterial disappearance pattern following ventricular injection. The normal single exponential rate of disappearance is initially accelerated (while atrial dye content is increasing), and finally retarded (while the mixed atrioventricular volumes are washed out together). The volume of the regurgitant flow may be calculated from simultaneous left atrial and systemic arterial dye dilution curves following ventricular injection if certain conditions prevail. One is that the left atrial sample accurately represents the blood-dye mixture which re-enters the left ventricle from the atrium, in which case the rate at which dye disappears from the atrial sampling site will be identical to the final

rate of disappearance from the ventricle. This same rate should also be exhibited after injection into the atrium. Left atrial and systemic arterial dye dilution curves were obtained from 21 patients with rheumatic heart disease following injections of indocyanine green into the left ventricle and left atrium. Ventricular injection was made through a catheter designed to promote an initial even dispersion of dye within the ventricle. Left atrial sampling and injections were performed through a Ross needle. Ventricular disappearance rates were estimated from systemic arterial dilution curves. Mitral regurgitation was detected in 10 patients by atrial sampling. In 8 of these, the disappearance rate from the atrium following ventricular injection was similar to the final rate from the systemic artery, and to the arterial disappearance rate following atrial injection. This indicates that atrial mixing and sampling conditions were adequate to permit quantitative measurement of regurgitant flow.

The Initial Step in Active Sodium Transport by the Toad Bladder. HOWARD S. FRAZIER, ELEANOR F. DEMPSEY AND ALEXANDER LEAF,* Boston, Mass.

Movement of sodium from bladder epithelial cell interior to serosal medium occurs by active transport. Since net sodium transport continues when as little as 0.1 mEq of sodium is present in mucosal medium, studies were undertaken to learn how sodium moved across the mucosal surface. Electrical forces at the mucosal surface were measured with intracellular glass microelectrodes. The potential was measured at 114 or <0.2 mEq sodium per L of mucosal medium. In the spontaneously active state the cell interior was always electrically positive to the mucosal medium. In the short-circuited condition the cell interior was at the same potential or very slightly negative to it. Therefore electrical driving forces do not explain net entry of sodium from low sodium media. The concentration forces were evaluated by altering sodium concentration in the mucosal medium and measuring isotopically the pool of cellular sodium which derived from the sodium in the mucosal medium. The results were consistent with the interpretation that the concentration of sodium in the cell water was always less than that in the mucosal medium and hence that a concentration gradient could account for mucosal entry of sodium. The short-circuit current as a function of mucosal concentration of sodium showed saturation kinetics. This could result from saturation either of mucosal entry or serosal exit of sodium from the cell. Measurements of the cellular sodium pool showed that entry was rate-limiting, and although passive, not by free diffusion. Vasopressin increased the active transport of sodium at any mucosal concentration of sodium by increasing mucosal permeability to sodium and hence the availability of sodium at the serosal pumping site. This site of the hormonal action is the same as that previously demonstrated for urea and water. It is unnecessary to postulate a direct effect of the hormone on the sodium pump.

Response to Repeated Platelet Transfusions from the Same Donor. EMIL J. FREIREICH, ALLAN KLIMAN, LAWRENCE A. GAYDOS AND LESLIE R. SCHROEDER, Bethesda, Md. (introduced by C. Gordon Zubrod).

Development of effective platelet replacement therapy has been hampered by inadequate supplies of fresh platelets and by the anticipation of progressive resistance to repeated transfusions. We have studied the response to repeated platelet transfusions from a single donor by utilizing plasmapheresis, a technique which increases at least 32-fold the quantity of fresh platelets that a donor can supply. Platelet-rich plasma (500 ml) obtained by plasmapheresis of a single donor was administered within 6 hours of collection to 28 children with severe thrombocytopenia secondary to acute leukemia. A significant increase in circulating platelet count with progressive fall to pretreatment levels was observed in 26 patients. Platelets were transfused as often as 3 times per week. Twenty patients received 2 or more treatments at least 7 days apart from a single donor (median—6 treatments per patient, range 2 to 19). Increase in circulating platelet count and rate of decline to pretreatment levels remained comparable throughout courses of repeated platelet transfusions of 8 to 365 days (median 25). Control of gross hemorrhage, correlated with increase of circulating platelets, was repeatedly observed. Thus resistance to repeated platelet transfusions from the same donor could not be demonstrated. Plasmapheresis of blood donors to provide platelet-rich plasma in large quantities proved a repeatedly effective method of platelet replacement therapy and offers a means of controlling donor factors in platelet transfusion studies.

The Origin of Fasting Plasma Triglycerides in Man. SAMUEL J. FRIEDBERG, ROBERT F. KLEIN, DAVID L. TROUT, MORTON D. BOGDONOFF AND E. HARVEY ESTES, JR., Durham, N. C. (introduced by David T. Smith).

Available evidence indicates that a fraction of intravenously administered palmitic acid-1-C^{14} appears as C^{14}-labeled plasma triglycerides (TG). In order to assess the quantitative significance of the conversion of plasma free fatty acids (FFA) to plasma TG in man, C^{14}-labeled palmitic, oleic and stearic acids were administered intravenously to 13 fasting male subjects without known disorders of fat metabolism. Thereafter venous blood was sampled at appropriate and frequent intervals over a period of 24 hours. The following were determined: total plasma FFA and plasma TG; also, activities of plasma TG, cholesterol, cholesterol esters, phospholipids and FFA. Plasma lipids were separated by silicic acid chromatography and by chemical methods. It was found that radioactivity appeared almost exclusively in triglycerides during the first 6 hours and that the disappearance of triglyceride during this period was first-order in type. Later, activity appeared in free cholesterol, cholesterol esters and phospholipids. From these determinations the following data were derived: 1) the individual plasma FFA turnover rates, 2) plasma

TG turnover rates, and 3) the fraction of administered labeled fatty acid initially equilibrating into plasma as labeled TG. From the fractional turnover rate of fasting plasma TG, which ranged in normals from 10 to 35 per cent per hour, and from the other data it was estimated that plasma FFA are the major source of plasma TG and that all three triglyceride fatty acids turn over. It was also found that the fractional turnover rates for triglyceride fatty acid were the same for oleic, stearic, and palmitic acids, but that relatively less stearic acid appeared in TG. The anticipated level of plasma triglycerides derived from the tracer studies agreed well with the observed plasma TG concentration.

Nonspecificity of Human Skin Graft Rejection. ELI A. FRIEDMAN, J. WALDEN RETAN, DAVID C. MARSHALL, LAWRENCE B. HENRY AND JOHN P. MERRILL,* Boston, Mass.

Previous studies of mammalian homograft rejection emphasized the individual specificity of the transplantation antigen(s). Berrian and Jacobs have recently shown that mice preimmunized with homologous spleen cells subsequently reject the skin of the splenic cell donor or a nonspecific donor in a pattern dependent upon the number of shared transplantation antigens. Attempts to immunize humans with leukocytes harvested from peripheral venous blood by a dextran sedimentation technique have been unsuccessful via either intravenous or subcutaneous routes. Sixteen ambulatory adults were "immunized" with 2 to 30×10^7 leukocytes injected intracutaneously at 30 different sites 6 days prior to challenge grafting. Full-thickness skin homografts taken from unrelated individuals and from the leukocyte donor were placed on the recipient's arm. Serial gross observation and microscopic examination of periodic graft biopsies were compared with control unmodified rejections in other patients. Leukocyte donor skin was rejected as a "white graft" (hyperimmune state) in 8 instances, and as an accelerated rejection in 7. Of 14 simultaneously placed grafts from nonleukocyte donors, 4 were rejected as white grafts and 6 as accelerated rejections. The type of rejection encountered was partly contingent upon the number of immunizing leukocytes employed. In this human system transplantation immunity was not entirely individual-specific. The technique suggests a method by which diversity or similarity of transplantation antigens in the prospective human transplant donor and recipient might be explored.

Cystinuria: Amino Acid Clearances and the Occurrence of a Cystine Peptide. GEORGE W. FRIMPTER, AUDREY BASS, EUGENE FURTH, MELVIN HORWITH AND DAVID D. THOMPSON,* New York, N. Y.

Renal excretion of amino acids was studied in 9 patients with cystinuria. Ion exchange chromatography with an automatic apparatus was used for identification and quantitation of amino acids. In 4 patients inulin and endogenous amino acid clearances were measured simultaneously. Reduced reabsorption of cystine, ornithine, lysine and arginine was observed in all patients. In all of the urinary ion exchange chromatograms, a previously undescribed compound was demonstrated. This substance was not found in urine from normal subjects or from patients with pyelonephritis, nephrosis, metabolic conditions with stone formation, cystinosis, or other amino acidurias. The compound was not detected in plasma of normal subjects or patients with cystinuria. It was not found following incubation of normal urine with cystine, cysteine or methionine, or in urine of normal subjects after ingestion of these amino acids, or in urine of a normal dog during massive cystine excretion due to intravenous cysteine loading. The compound *was* present in urine of a dog with cystinuria and cystine stones, but was *not* found in urine of a Kenya genet, a species that uniformly exhibits cystinuria but does not form cystine stones. Traces of the substance were found in stones from patients with cystinuria. The compound was isolated by a series of chromatographic separations using various ion exchange resins. Following these procedures the compound was shown by chromatography to produce a single peak, with an Rf identical to that noted originally. The purified compound gave positive tests for peptide, and upon hydrolysis yielded a large amount of cystine plus other amino acids. A rapid escape through cellophane suggested a low molecular weight. The possible relationship of this compound to cystinuria is considered.

The Effects of Guanethidine on Triiodothyronine-induced Hyperthyroidism in Man. THOMAS E. GAFFNEY AND EUGENE BRAUNWALD, Bethesda, Md. (introduced by Robert W. Berliner).

This investigation, designed to assess the contribution of the sympathetic nervous system to the human hyperthyroid state, was carried out because it has been suspected that many of the manifestations of hyperthyroidism are mediated or potentiated by the sympatho-adrenal system. In 4 normal subjects triiodothyronine was administered in daily doses of 250 to 350 μg and resulted in clear-cut signs and symptoms of hyperthyroidism. The heart rates during sleep rose to levels 35 to 55 per cent above control, basal metabolic rates rose to levels 25 to 50 per cent above control, and serum cholesterol fell by 75 to 105 mg%. Palpitations and tremor characteristic of hyperthroidism also developed in all subjects. Guanethidine, a drug which selectively interrupts adrenergic reflexes and produces tissue catecholamine depletion, was then administered in daily doses of 20 to 50 mg, while triiodothyronine was continued. In all 4 subjects the heart rate returned to control levels, the basal metabolic rate fell to 18 to 23 per cent above control levels; palpitations and tremor disappeared, but serum cholesterol remained depressed. When guanethidine was withdrawn the tachycardia, hypermetabolism, palpitations and tremor returned. These observations indicate that the tachycardia, hypermetabolism, and tremor of hyperthyroidism are intimately related to

activity of the adrenergic nervous system and/or tissue catecholamine content; the cholesterol-depressing effect of triiodothyronine appears to be unrelated to these factors. It is suggested that guanethidine may be of clinical value in rapidly counteracting many of the manifestations of the hyperthyroid state. Pharmacologic inhibition of the adrenergic system may also be useful when thyroid hormones or their analogues are administered in order to depress serum cholesterol.

The Survival of Virus-treated Erythrocytes in Normal and Splenectomized Rabbits. EDWARD GARDNER, JR., CLAUDE-STARR WRIGHT* AND BETTIE Z. WILLIAMS, Augusta, Ga.

The survival of virus-treated erythrocytes in normal and splenectomized rabbits was studied using radiochromium (C^{51}) labeled cells. This investigation is a part of a long-range program attempting to correlate destruction of the damaged or modified red blood cell and the spleen. Erythrocytes were labeled with radiochromium (C^{51}) using *in vitro* and *in vivo* technics. The survival of normal erythrocytes in normal animals was the same by both technics. Normal erythrocytes in normal rabbits showed a mean apparent half-time survival of 12 days. Intersection of the survival curve with the time axis occurred between 55 and 60 days for those studies carried to completion. Normal erythrocytes in splenectomized animals showed a half-time survival at the upper limits of normal. Erythrocytes treated with influenza and Newcastle disease virus showed a shortened survival time in normal rabbits. Newcastle disease virus apparently produced more damage to the red blood cells than influenza virus as judged by the markedly shortened survival of cells treated with this agent. Erythrocytes treated with influenza virus appeared to survive longer in rabbits previously splenectomized. These findings suggest that the removal of this source of reticuloendothelial tissue prevented a more rapid removal of the virus-modified cells from the general circulation.

Stimulatory Effect of Phenobarbital on Steroid Metabolism. LEONARD D. GARREN, A. H. CONNEY AND GORDON M. TOMKINS,* Bethesda, Md. and Tuckahoe, N. Y.

Administration of phenobarbital and other drugs greatly increases the activity of several drug-metabolizing enzymes which are localized in liver microsomes and which require reduced triphosphopyridine nucleotide (TPNH) for activity. Since several steroid-metabolizing enzymes resemble the drug systems in their intracellular distribution and cofactor requirements, the effect of phenobarbital administration on the enzymic metabolism of steroids by rat liver microsomes was investigated. It was observed that microsomes from 40-g rats which had been injected with phenobarbital (75 mg per kg) for several days catalyzed a rapid oxidation of TPNH in the presence of androsterone (or 4-androstene-3,17-dione). Microsomes from untreated controls did not catalyze such a reaction. Partition chromatography of the products of androsterone metabolism revealed a single compound, more polar than the substrate, which possessed an unaltered keto-group at C-17. Its infrared spectrum suggested that it was a hydroxylated derivative of androsterone. The spectrum was not consistent with a 2-hydroxy compound and paper chromatographic studies showed that 11β-hydroxy-androsterone was not the product. Further studies are in progress in order to identify the unknown steroid. Thus, administration of a foreign compound, phenobarbital, influenced the metabolism of normally occurring steroids by apparently stimulating their hydroxylation by a TPNH-dependent microsomal enzyme.

The Role of Activation Product in the Thromboplastin Generation Test. LAMONT W. GASTON AND HELEN DEORSAY, Boston, Mass. (introduced by Kurt J. Isselbacher).

Serum obtained from blood incubated for 24 hours at 37° C supports normal generation of thromboplastin in the thromboplastin generation test (TGT), but there is a delay of 5 to 6 minutes before maximal activity is reached. If a sample of the serum is then kept in a glass tube, there is a progressive increase in the rate but not the amount of thromboplastin formation. If kept in a siliconed tube, however, the TGT curve is unaltered. Using the activation product (AP) assay of Waaler which measures the combined activity of Hageman factor and plasma thromboplastin antecedent (PTA), we observed that the increased rate of thromboplastin formation following exposure of the serum to glass was paralleled by a rising level of AP with no accompanying change in the Stuart factor level. The effect of glass contact (i.e., increase in rate but not quantity of thromboplastin formation) was exactly duplicated by addition to a TGT incubation mixture containing serum from a siliconed tube of a preparation of AP containing no plasma thromboplastin component (PTC) and only a trace of Stuart factor. The rate of thromboplastin formation, as distinct from the amount formed, has been attributed to a "Factor X" or, more recently, to prephase accelerator. It seems likely, however, that rate of formation is controlled by AP. Still another serum factor other than Stuart factor, PTC, and AP may also affect the TGT. The serum of a patient with Hodgkin's lymphoma performed abnormally in the TGT yet contained normal amounts of Stuart factor and PTA and showed the glass contact phenomenon described above.

Asynchronous Thymidine Uptake by Human Chromosomes. JAMES L. GERMAN, III, New York, N. Y. (introduced by Alexander G. Bearn).

Chromosomes of germinal and somatic cells of plants and lower animals may undergo asynchronous replication of deoxyribonucleic acid. Of special interest is the

demonstration by Taylor that in somatic cells of hamster the sex chromosomes and certain other chromosomes of the smallest order may be relatively late in undergoing chromatid reproduction during interkinesis. It is undetermined whether a similar sequence occurs in germinal cells or whether the phenomenon is of importance in relation to the frequency of non-disjunction of chromosomes at meiosis. Human nucleated blood cells of 2 males and 1 female, freshly isolated from the individual using phytohemagglutinin, were placed in suspension tissue cultures. After 3 days *in vitro,* cells undergoing division were accumulated in metaphase by 30-minute exposure to desacetyl-methyl-colchicine. At various intervals preceding this metaphase arrest, different cultures were placed in a medium containing tritium-labeled thymidine for a 10-minute period. Radioactivity of individual chromosomes was determined by radioautography. Tritium uptake, indicating DNA synthesis, was observed in as many as 4 per cent of both large and small round cell nuclei. In 7 experiments examination of 1,369 metaphase figures indicated that thymidine incorporation had occurred in 95 per cent of such cells in the time period of 30 to 4 hours preceding metaphase. Cells reaching metaphase which had been exposed to labeled thymidine shortly before or after this period showed relatively little radioactivity. Essentially no uptake occurred during the 2 hours immediately preceding metaphase. Furthermore, label uptake was asynchronous, some chromosomes of the complement incorporating thymidine when exposed to tritium-labeled thymidine at a given time before mitosis while others remained nonradioactive. Therefore, cells cultured from venous blood 1) display a cycle in excess of 30 hours; 2) some chromosomes are later than others in completing thymidine incorporation.

Release of Glutamic-oxalacetic Transaminase in Virus-infected Tissue Cultures. V. E. Gilbert, R. Pavia and A. I. Braude,* Pittsburgh, Pa.

Elevation of serum glutamic-oxalacetic transaminase (GOT) in viral hepatitis suggests that viruses release this enzyme from cells. In order to determine whether GOT is released from viral infected cells, GOT was assayed in tissue cultures infected with various cytopathogenic viruses. Spectrophotometric measurement disclosed sharp rises of GOT in fluids of stationary cultures of monkey kidney (MK), human embryo kidney (HEK), HeLa and human amnion (FL) upon infection with all enteroviruses examined. Echo 9 (MK, HEK), 7 and 12 (MK) and Coxsackie B3 (MK, HEK, HeLa, FL) and B5 (MK, HeLa, FL) released GOT in proportion to the degree of gross cell destruction. GOT was not released until cytopathogenicity progressed beyond early stages despite early virus release. Peak values of 50 to 169 U per ml per minute were reached coincident with the disappearance of most swollen cells. Complete destruction of MK cell sheets by Echo 12 released only half of total cellular GOT from these infected cells. This suggests that many cells resist infection or lysis. Unlike enteroviruses, adenovirus type 7 failed to release GOT upon infection of MK, HeLa, and FL despite severe cytopathogenicity; and only slight elevations occurred when the sheet left the glass. These findings coincide with the fact that cells infected with adenoviruses characteristically resist lysis and release only few viral particles. In preliminary studies with this adenovirus, total cellular GOT remained below that of uninfected MK cultures. In MK cultures infected with Ca (croup-associated) virus GOT was slowly released in quantities comparable to enteroviruses but without discernible cytolysis. Herpes simplex infection of MK and HEK cultures released only low levels of GOT as marked cytopathogenic changes developed. These studies indicate that GOT is released from cells of diverse origin by certain cytopathogenic viruses but not by other viruses capable of different types of cell injury.

The Antigenicity of Synthetic Polypeptides. Thomas J. Gill, III and Paul Doty, Cambridge, Mass. (introduced by Gustave J. Dammin).

Linear chain synthetic polypeptides were studied for their ability to elicit precipitating antibody formation and skin reactivity in rabbits. The metabolism of a radioiodinated polypeptide was also followed. The polypeptides had a molecular weight range of 100,000 to 200,000 and contained glutamic acid, lysine, tyrosine and phenylalanine in different combinations. The polypeptides containing glutamic acid and lysine (3:2) plus 5.8 per cent tyrosine and 8.7 per cent phenylalanine elicited an average antibody titer of 208 and 80 μg Ab N per ml serum, respectively; these were the highest titers observed. Copoly-glutamic acid-lysine (3:2) elicited an average antibody titer of 70 μg Ab N per ml serum. Rabbits sensitized with each of these three polypeptides developed Arthus reactions in skin test sites. Polyglutamic acid and polylysine were not immunogenic. Thus, copoly-glutamic acid-lysine was immunogenic while the homopolymers were not. Aromatic amino acids are not necessary for immunogenicity, but antigens containing them elicit higher antibody titers. A polypeptide containing glutamic acid and lysine (3:2) plus 1.3 per cent tyrosine elicited an average antibody of only 18 μg Ab N per ml serum and produced a skin reaction with a delayed time course. These synthetic antigen-antibody systems behaved like native protein systems with respect to the effects of dilution, salt concentration and time on the formation of the specific precipitate, but were somewhat more sensitive to temperature and pH variations. The molar antibody:antigen ratios for these synthetic antigens were 3 to 4 times higher than those found with native proteins of the same size. The physicochemical and biological behavior of these synthetic polypeptide antigens showed them to be good protein models in an immunological system. The synthetic control of structure offers many possibilities to study the effect of a single parameter in the antigen on the nature of the immune response.

Auto-immunity in Liver Disease. Serological Investigations and Clinical Correlation. MUHARREM GÖKCEN, Minneapolis, Minn. (introduced by F. W. Hoffbauer).

The presence of circulating autoantibodies against human liver antigens has been sought by means of a modified complement fixation test in 171 patients with various types of liver disease. The liver autoantibodies have been demonstrated in all active cases of primary biliary cirrhosis, "postnecrotic" cirrhosis, "plasma cell hepatitis," "lupoid hepatitis," drug-induced jaundice, and in some cases of subacute lupus. The autoantibodies of primary biliary cirrhosis appeared to be distinct from those of postnecrotic cirrhosis in terms of reacting with different liver antigens. One hundred thirty-two different sera from blood bank donors served as controls and were screened with different liver antigens; none gave a positive titer. The autoantibody titer was well correlated with the severity of the disease process as well as with the amount of serum γ-globulin in which the autoantibodies resided. With spontaneous or steroid-induced remission, the serum autoantibodies either declined significantly or disappeared. The autoantibodies were lacking in a case of agammaglobulinemia with active postnecrotic cirrhosis. A titer of 1:128 was noted in 1 of 15 patients with acute hepatitis; 14 were negative. One case prolonged for 3 months developed a high titer of postnecrotic type antibodies. Cases of Laennec's cirrhosis did not have complement-fixing type antibodies but had the hemagglutinin type in low titer. Immunelectrophoretic studies were done in 20 patients selected from among the above 171 cases. A heavy β_2 M globulin (macroglobulin) band was observed in all 3 cases of Laennec's cirrhosis, 5 postnecrotic cirrhosis, 3 primary biliary cirrhosis, 1 lupoid hepatitis and 1 plasma cell hepatitis. This was but faintly present in 20 normal individuals and in 7 patients with jaundice, including 3 with biliary obstruction, 1 ulcerative colitis, 1 drug (PAS-INH)-induced, 1 recurrent jaundice of pregnancy, and 1 of plasma cell hepatitis in steroid-induced remission.

A Study of the Patterns of Solute Excretion in Acute Renal Failure in Relation to the Duration and Pathogenesis of Oliguria. MARTIN GOLDBERG, LEWIS W. BLUEMLE, JR. AND GEORGE D. WEBSTER, JR., Philadelphia, Pa. (introduced by J. Russell Elkinton).

Forty patients with acute renal failure were studied during oliguria and diuresis. In both periods, the urine flow was linearly related to excretion of sodium and total solute. The rate of increase of solute excretion during oliguria was generally constant and similar from patient to patient when plotted against a percentile time abscissa (days from onset of oliguria/duration of oliguria $\times$ 100). From this relationship a formula may be derived for predicting the approximate duration of oliguria in most patients. With few exceptions the peak of diuresis and the onset of rapid decline of blood urea nitrogen occurred simultaneously after a period of time following onset of diuresis equal to about one-half the duration of oliguria. During oliguria the ratio of urinary sodium to total solute varied from 0.03 to 0.35, the lowest values obtaining in severe trauma, burns, and congestive heart failure. The ratio C_{NA}/C_{Cr} varied considerably in individual patients. The ratio of non-electrolyte solute to total solute ranged from 0.16 to 0.67 and did not vary consistently with changes in composition of plasma solute occurring either spontaneously or during hemodialysis. With the advent of diuresis the urinary nonelectrolyte solute fraction was consistently 0.4 to 0.6. In several overhydrated patients the urine was hypotonic (U/P osmolality < 0.7) early in the oliguric phase. During oliguria, while urine flow is dependent on solute excretion, the lack of close correlation between changes in plasma and urine solute composition suggests that the tubules of a reduced number of nephrons are functioning in a qualitatively normal manner while glomerular filtration rate (GFR) is impaired. Diuresis is associated with an increasing urea osmotic load, an increasing number of participating nephrons and a rising GFR.

Specific Neural Stimulation of Norepinephrine Secretion by the Adrenal Medulla During Anoxia. ALAN GOLDFIEN, WILLIAM F. GANONG AND BERTRAM WISE, San Francisco, Calif. (introduced by Richard J. Havel).

We have previously reported that asphyxia (trachea clamp) and anoxia (100 per cent N_2) are unique stimuli to adrenal medullary secretion in the dog in that they regularly produce a proportionally greater increase in norepinephrine (N) than in epinephrine (E). Studies have been done to distinguish between interference with the conversion of N to E before release, and activation of a specific nervous pathway as the cause of this qualitative alteration in response. These experiments have been performed on dogs anesthetized with pentobarbital. Section of the splanchnic nerve or spinal cord and local or paravertebral procaine markedly depress the secretion of E and N. Infusion of cyanide into the arterial supply of the left adrenal in 5 dogs with bilateral adrenal cannulas caused an increase in the proportion of N on the left but not the right. However, the increase appeared late in contrast to the immediate change observed with intravenous cyanide or anoxia. In 4 dogs, the adrenal was perfused with oxygenated blood during asphyxia of the recipient. In these experiments the typical increase in N occurred. Infusion of anoxic blood into the adrenal of the normal dog did not increase N secretion. These results indicate that the N response results from nervous stimulation. Additional studies in 5 dogs in which chemoreceptor activity was modified by section of cranial nerves 9, 10 and 11 suggest that chemoreceptors may initiate the reflex increase in N since an increase in N secretion comparable with that observed in the normal dog was not found after this procedure.

Reactivity of the Renal Circulation in Essential Hypertension. ERVIN A. GOMBOS, WILLIAM H. HULET, PIERRE BOPP AND DAVID S. BALDWIN,* New York, N. Y.

The cause of arteriolar vasoconstriction in essential hypertension is unknown. Increased vascular reactivity has been proposed as a possible mechanism, and it has been suggested that sodium content of the vessel wall affects such responsiveness. In the present study, the reactivity of the systemic and renal circulations to infused noradrenaline and adrenaline has been compared, using percentile changes, in normotensive (N) and hypertensive (H) individuals on regular and restricted sodium intake. Noradrenaline caused equal increases in mean systemic pressure (Pm) and equal decreases in renal plasma flow (RPF) in N and H, producing comparable increases in renal resistance (RR) in N (+68 per cent) and H (+50 per cent). For given levels of Pm, RPF was lower in N than in H. Adrenaline did not change Pm but reduced RPF equally in N and H and to the same extent as did noradrenaline; RR increased less with adrenaline than with noradrenaline. Sodium restriction reduced Pm and RPF in H but did not affect either in N; Pm response to noradrenaline was exaggerated in H, unaffected in N; reductions in RPF were enhanced in N, unaltered in H. During sodium restriction, then, noradrenaline caused greater increases in RR in both H and N. Sodium restriction did not alter Pm response to adrenaline in either H or N, nor were the responses in RPF or RR affected in a consistent manner. We conclude: 1) Vascular reactivity to vasoconstrictor agents is not increased in either the systemic or renal circulation in essential hypertension. 2) Vascular reactivity is not decreased by sodium restriction in hypertension; indeed, systemic arteriolar reactivity is enhanced. 3) Autoregulation of the renal circulation in both H and N is suggested by our observation that greater increases in RR occur with noradrenaline, where Pm is increased, than with adrenaline, which does not affect Pm.

Potassium Excretion in Renal Disease. HARVEY C. GONICK, MORTON H. MAXWELL, RALPH E. CUTLER, J. THOMAS DOWLING AND CHARLES R. KLEEMAN,* Los Angeles, Calif.

A test was devised in an attempt to quantitate the renal response to an acute K load at various levels of renal function. Following two 1-hour control periods, 0.75 mEq K per kg body weight was administered orally. Urine collections were made for three subsequent 2-hour periods. Bloods were drawn at the midpoint of each period. Samples were analyzed for K and creatinine. During the 6-hour test period, the increment in K excretion above the control was calculated. This was then expressed as a per cent of the administered load ("per cent excretion"). Twelve normal subjects and 16 patients with various types of renal disease were studied. The maximal increase in serum K was approximately 1 mEq per L in both groups. The renal disease patients differed from the normal controls in several important respects: 1) "Per cent excretion" of K was significantly lower, with no overlap (range, 0 to 16 per cent vs 24 to 71 per cent). When this figure was divided by glomerular filtration rate (GFR), some overlap between the two groups was present. A few patients, however, with minimal reductions in GFR, demonstrated a distinct abnormality in K excretion. 2) The elevation in plasma K tended to persist throughout the test period, whereas in the normal subjects the plasma K had returned to control values. 3) The increment in K excretion was invariably slower than in the normal group, in whom almost all of the increment occurred in the first 4 hours. 4) During the control periods, the ratio, K excreted/K filtered, was significantly higher than in the normal subjects (0.57 ± 0.85 vs 0.17 ± 0.08; $p < 0.05$). It appears that under ordinary circumstances the diseased kidney functions at near maximal capacity for K excretion and therefore responds inadequately when challenged with an acute K load. This test may serve as a useful index of tubular function in patients with minimal renal disease.

The Effect of Viral Infection on Cellular Protein Synthesis. IRVING GRAY, JOHN B. TASKER, JR. AND GEORGE R. FRENCH, Fort Detrick, Md. (introduced by William J. Darby).

The virus of Venezuelan equine encephalomyelitis (VEE), Trinidad strain, is a pantropic virus occurring in particularly high concentration for extended periods in the brain. Symptoms of this disease are typical of central nervous system derangement. Chemically, VEE is an RNA-containing virus, presenting the possibility that a mode of action of this microorganism could be through the alteration of cellular protein synthesis known to require cytoplasmic and microsomal RNA. Consequently, it was decided to study the effect of this virus on the protein synthetic ability of brain tissue. Male white mice, Bagg strain, were inoculated with approximately 400 LD_{50}'s of VEE; 50 animals were sacrificed each day for 6 days and the brains pooled in each group. Microsomes, soluble enzymes and RNA were prepared by differential centrifugation. Enzymes were incubated with microsomes in the presence of L-leucine-C^{14} and an ATP regenerating system. The reaction mixture was incubated in air at 37°C, with shaking, for 15 minutes. The microsomal protein was precipitated and uptake of radioleucine determined. The results of six complete assays are reported here. There is an increase in the incorporation of leucine-C^{14} into the microsomal protein, reaching a maximum on the third day following the challenge. There follows a sharp decrease to or below the control values. The increase occurs during a rising virus titer in the brain, while the fall occurs during the time when the virus titer in the brain has reached a maximum and remains relatively constant. The changes appear to be associated with the microsomal rather than the enzymatic fraction. Correlative histology and symptomatology will be discussed.

Experimental Human Trachoma in Six Blind Volunteers. J. THOMAS GRAYSTON,* SAN-PIN WANG, YEN-FEI YANG AND ROBERT L. WOOLRIDGE, Taipei, Taiwan, Formosa.

Six blind volunteers aged 11 to 24 who had no evidence of trachoma were infected in the eye with virus isolated from trachoma patients on Taiwan and grown in the yolk sac of embryonated eggs. Trachoma viruses have only recently been isolated and one purpose of this experiment was to show that the egg-grown virus was capable of reproducing trachoma disease in humans. The acute course of these infections has been reported in J. Amer. Med. Ass. The present report deals with the later clinical course of the infections, effects of a series of injections of a trachoma virus vaccine given to 3 volunteers beginning 2 months after infection, and responses to local and systemic therapy. Pannus, a typical sign of trachoma, developed in the one volunteer whose cornea was not damaged prior to the experiment. Conjunctival scars, diagnostic of trachoma, appeared in each infected volunteer between the seventh and ninth months after inoculation. Each of the 3 volunteers receiving placebo vaccine experienced a spontaneous spread of infection to his uninoculated eye. These spread infections, proven by virus isolation, occurred 1, 5 and 6 months after the original inoculation. Two volunteers who received trachoma virus vaccine experienced no spread of infection. The other one had been inoculated in both eyes. After 9 months all 6 were treated in both eyes with terramycin ointment 4 times per day for 1 month. Following therapy an acute relapse with active conjunctivitis in both eyes occurred in each of 3 volunteers who had received placebo. No recurrent signs of disease were seen in 3 volunteers who had received virus vaccine. Oral sulfa drug therapy was then given for 3 weeks and a complete cure resulted. It is concluded that trachoma was reproduced and that the vaccine probably exerted a favorable influence on the course of the disease.

Effects of Iodine on the Intermediary Metabolism of the Thyroid Gland and their Relation to Iodine-induced Inhibition of Thyroid Hormone Formation. WILLIAM L. GREEN AND SIDNEY H. INGBAR,* Boston, Mass.

Iodide maintains a paradoxical relation with the thyroid gland in that low concentrations are essential for hormonal synthesis, while high concentrations inhibit hormone formation transiently. Persistence of the latter effect is thought to produce iodide myxedema. Studies were performed to determine whether these effects of iodine could be mediated by an action on thyroidal intermediary metabolism, since previous studies indicated that TPNH formed during glucose catabolism is rate-regulating in hormone formation. Sheep thyroid slices were incubated in Krebs-Ringer phosphate medium in which iodide was isosmotically substituted for chloride. Metabolic effects of iodide were correlated with rates of hormonal synthesis determined from the percentile organification of I^{131} and the specific radioactivity of the medium. Iodide induced biphasic effects on hormone formation and glucose metabolism. Progressive increases in iodide concentration to approximately 10^{-4} M induced increasing organification of I^{127}. Concomitantly, there occurred a several-fold increase in the evolution of $C^{14}O_2$ from glucose-1-C^{14} and a less striking increase from glucose-U-C^{14} and glucose-6-C^{14}. Specific activity of evolved CO_2 increased considerably, while oxygen consumption, total CO_2 production, and glucose utilization were moderately increased. These stimulatory effects of iodide were abolished by methimazole and were not evident in liver, kidney, or adrenal. Equivalent concentrations of bromide had no apparent effect. At higher iodide concentrations (up to 5×10^{-2} M), hormonal synthesis declined and stimulatory effects on glucose metabolism were abolished or changed to inhibition. In thyroid homogenates, the increase in concentration of TPNH which followed addition of TPN was less in the presence of added iodide, especially the higher concentrations of iodide which decreased hormonal synthesis. These data indicate that thyroidal glucose metabolism and hormone formation are closely linked. They further suggest that the decrease in hormonal synthesis induced by iodide is related to an inhibition of those processes which generate TPNH.

An Intracellular Protein Intermediate for Hemoglobin Formation. WILLIAM B. GREENOUGH, III AND E. DONNALL THOMAS,* Cooperstown, N. Y.

Normal human and dog marrow were incubated *in vitro* with Fe^{59} and C^{14}-labeled amino acids. Ultracentrifuged lysates of the washed marrow cells were chromatographed on an IRC 50 column. A fraction moving with the solvent front in 0.01 M phosphate buffer at pH 7.0 (fraction I) contained an intermediate for hemoglobin formation. A delay of 5 to 8 minutes occurred before significant C^{14} or Fe^{59} appeared in the IRC 50 main hemoglobin fraction. Fraction I showed labeling within 1 minute and rose to plateau values in 15 to 30 minutes, remaining there until the rate of hemoglobin formation decreased at from 4 to 6 hours. When marrow cells incubated briefly with radioactive substrates were washed and reincubated in unlabeled substrate, a decrease in fraction I activity occurred, while the increase in hemoglobin activity continued. The turnover rate of Fe^{59} in fraction I accounted for all the Fe^{59} appearing in hemoglobin. In the presence of lead acetate, Fe^{59} entered fraction I but hemoglobin synthesis was blocked. Fe^{59} was also incorporated into hemoglobin by a lysate system of marrow with nuclei and stroma removed. In this system added unlabeled fraction I caused a fourfold enhancement of iron incorporated into hemoglobin, and a transfer of Fe^{59} from labeled fraction I to hemoglobin was demonstrated. Fraction I was heterogeneous; however, a single component containing nearly all the Fe^{59} could be demonstrated both by electrophoresis and by chromatography on DEAE cellulose. Heating to 80°C did not alter these properties but did remove much inactive material. The heat-stable component is a nonheme protein having 0.3 to 0.8 per cent iron stable to dialysis against acetate and EDTA at pH 4.6. Fraction I was clearly

distinct from $CdSO_4$-precipitated liver ferritin when compared electrophoretically and by DEAE chromatography. Similar N-terminal amino acid patterns, however, suggest a relationship to ferritin that is currently under investigation.

Studies on Pathogenesis of Typhoid Fever: Endotoxin and Vascular Reactivity. SHELDON E. GREISMAN, RICHARD B. HORNICK AND MERRILL J. SNYDER, Baltimore, Md. (introduced by Theodore E. Woodward).

Resemblances of clinical, pathological, and laboratory manifestations in typhoid fever to those induced by purified endotoxins suggest that the pathophysiologic alterations during *Salmonella typhosa* infection are mediated by endotoxin. This concept was supported in 1950 by demonstration of tolerance to the pyrogenic activity of endotoxin in subjects convalescing from typhoid fever. Such findings, however, lack confirmation and do not parallel observations following human infection with other endotoxin-containing bacteria. The present studies evaluate more critically the evidence for endotoxin activity during typhoid fever. Healthy young male volunteers were studied prior to infection. Responses of systemic arterial blood pressure and of nailfold capillary blood flow to intravenous infusions of norepinephrine were measured under standardized conditions. Subsequently, febrile responses following intravenous injection of 0.5 μg *Sal. typhosa* or 1.0 μg *Escherichia coli* were monitored over 8 hours. Viable *Sal. typhosa* (10^8 to 10^9) were administered orally, and illness was permitted to develop several days prior to chloramphenicol therapy. Febrile reactions to endotoxin and vascular responses to norepinephrine were assayed serially. Significant tolerance to pyrogenic activity of *Sal. typhosa* and *E. coli* endotoxin (fever indices below half the control levels) developed in convalescence, diminishing appreciably by the third week. Moreover, during typhoid fever, norepinephrine infusions at rates only one-half to one-quarter those of the control period induced comparable alterations in arterial pressure and capillary blood flow; fever per se was not responsible. Subjects not exhibiting overt illness did not develop endotoxin tolerance or increased reactivity to norepinephrine. Production of tolerance to pyrogenic activity of endotoxin and vascular hyperreactivity to catecholamines are documented paramters of endotoxin activity. The present findings support the thesis that *Sal. typhosa* endotoxin contributes significantly to the physiologic derangements in human typhoid fever. Resistance to development of overt illness following oral administration of viable *Sal. typhosa,* however, appears based upon mechanisms distinct from endotoxin tolerance.

Volume Regulation and Sodium Homeostasis Independent of Aldosterone. JACOB GROSSMAN, ARTHUR G. GOLDMAN AND MORRIS WOLFMAN, New York, N. Y. (introduced by Louis Leiter).

Vasopressin-induced water retention and increasing body fluid volume are normally accompanied by a natriuresis chronologically associated with a significant fall in urinary aldosterone excretion. Moreover, administration of relatively small amounts of sodium is followed by proportionate degrees of natriuresis, suggesting either that volume regulation is extremely sensitive or that there exists a mechanism for sodium homeostasis independent of fluid volume. An effort was made to separate these two influences and to ascertain their degree of dependence on aldosterone inhibition. Four subjects maintained on unrestricted (170 mEq per day) salt intake were given 9α-fluorocortisol (3 to 4 mg per day) during two separate 6-day periods. During one course of treatment, fluid intake was moderately severely restricted while during the other 6-day period, daily water intake, apart from that in food, was forced to 4 to 5 L. All diets and urinary collections were under metabolic control. The characteristic kaliuresis and sodium retention with progressive hypokalemia were observed in all cases. In 2 subjects, urinary sodium excretion was significantly less on the lower fluid intake despite the higher plasma sodium concentrations, suggesting that volume regulation predominated over that of sodium. In one subject natriuresis was approximately the same during both courses of 9α-fluorocortisol administration. While precedence of sodium homeostasis is suggested, the urine flow during high fluid intake was sufficiently enhanced to prevent a significant increase in volume. In one obese subject on a low (800) calorie diet, sodium excretion was greater during fluid restriction. The progressive increase in natriuresis during treatment indicates that such volume regulation may be independent of aldosterone level. Moreover, the data suggest that volume regulation underlies sodium homeostasis.

Effects of Single Amino Acids on Metabolism of Potassium and Hydrogen Ion. PHILIP W. HALL, III AND GEORGE J. GABUZDA,* Cleveland, Ohio.

The effects of single amino acids on acid-base balance and renal handling of electrolytes were investigated in 8 patients maintained on constant intakes of food and fluid. L-arginine (155 mM as free base) given orally daily for 3 days to each of 6 subjects resulted in increased urinary bicarbonate and potassium excretions, decreased hydrogen ion excretions (titratable acid and ammonia) and increases in urine pH. Blood electrolytes and pH were not altered. Similar changes occurred when L-lysine, but not when L-glutamic or L-aspartic acid was fed in equimolar quantities to some of the patients. Thus the effects noted occurred with basic (cationic) but not with acidic (anionic) amino acids. Subsequent studies were designed to determine whether the cationic amino acids exerted their effects primarily by influencing renal or extra-renal mechanisms. The responses of three patients to 100 mEq hydrochloric acid given orally daily for 3 days, with and without simultaneous L-arginine administration, were determined. When the acid load was given with L-arginine, there was 50 per

cent less reduction in serum bicarbonate concentration, and 40 per cent less hydrogen ion excreted in the urine than when hydrochloric acid was given alone. Urinary potassium excretions resulting from L-arginine given alone or with hydrochloric acid were increased and comparable. A slowly progressive acidosis was induced in a patient with chronic renal disease by administration of hydrochloric acid orally (25 mEq daily). Feeding L-arginine in addition to the acid resulted in stabilization of serum bicarbonate concentration and pH without change in hydrogen ion excretion. When L-arginine was discontinued, serum bicarbonate concentration promptly decreased further. These data are interpreted as follows: basic amino acids 1) behave as cations and probably displace some intracellular potassium, and 2) either decrease the production of hydrogen ions requiring excretion or increase total body buffering capacity.

Effects of P_{CO_2} and pH on the Plasma Binding, Turnover, and Tissue Uptake of Thyroid Hormones. MILTON W. HAMOLSKY* AND MYRON STEIN, Boston, Mass.

Knowledge is limited concerning the factors controlling the distribution of thyroid hormones between the specific binding proteins in plasma and the peripheral tissues. Our studies suggest an important determinant role of carbon dioxide tension (P_{CO_2}) and pH. The *in vitro* uptake by human erythrocytes of I^{131}-thyroxine (T4) and I^{131}-triiodothyronine (T3) from whole blood was strikingly increased in each of 24 euthyroid patients with hypercapnia, returning to normal when P_{CO_2} could be normalized. The *in vivo* turnover rate of infused I^{131}-T4 was similarly increased in 8 of these hypercapneic subjects. "Criss-cross" experiments revealed the alteration to be due to a plasma factor. Experimentally induced hypercapnia and hypocapnia resulted in a marked increase and decrease, respectively, in the *in vitro* erythrocyte uptake of T3 and T4. In dialysis studies, induced hypercapnia (pH stabilized by $NaHCO_3$ addition) caused increased dissociation of T4 and T3 from plasma proteins; conversely, hypocapnia resulted in increased plasma binding, decreased dialysis. A separate controlling role of pH was revealed in dialysis studies of T4 and T3 bound to 1) whole plasma, 2) highly purified inter-alpha globulin, 3) serum albumin, and 4) isolated prealbumin binding protein. Characteristic patterns over a wide pH range were observed for each binding protein, differing for each of the specific binding proteins, and differing for T4 vs T3. *In vivo* alteration of pH in dogs (temporary apnea, varying oxygenation, infusions of Tris or bicarbonate buffer) caused striking changes in T4 and T3 plasma binding—decreased below pH 7.4, increased above 7.4, with a sharp inflection point at 7.4. Comparable results have been observed in patients with altered acid-base balance. The possible physiologic significance of pH and P_{CO_2} changes in over-all thyroidal economy will be considered.

Clinical Applications of I^{125}. PAUL V. HARPER, KATHERINE A. LATHROP, HAROLD L. ENDLICH, WARREN SEIMENS AND ROBERT W. HARRISON, Chicago, Ill. (introduced by Leon O. Jacobson).

While the potential advantages of I^{125} for certain clinical and experimental studies have long been recognized, its high cost and low purity when prepared by cyclotron bombardment of tellurium have limited its use to animal material. Development of production methods using thermal neutron activation of xenon has made available large (0.5 c) amounts of relatively pure (> 99 per cent) isotope. Briefly, its advantages as compared with I^{131} are: 1) The 60 day half-life provides long shelf-life and permits long-term metabolic studies; 2) the low energy dissipation rate greatly reduces radiation exposure of the patient in, e.g., thyroid scanning; 3) the low energy of the principal photon radiation (27.3 Kev) permits greatly reduced crystal size and shielding, and greatly enhanced detector and collimator efficiency. Even apart from these latter advantages, when the same microcurie dosage of I^{125} is used with unmodified equipment, it produces thyroid scans almost indistinguishable from those using I^{131}. In I^{125} Rose Bengal liver scans, metastatic nodules on accessible surfaces of the liver appear with markedly enhanced contrast because the radiation originating in the underlying liver is attenuated in passing through the "cold nodule." Since I^{125} may be counted in an ordinary well crystal with 60 to 70 per cent efficiency, studies involving small *in vitro* samples present no problem. It should be possible to use miniature shielded detector probes applied directly to the great vessels for cardiac output or shunting measurements during cardiac surgery, without undue interference from background radiation. There are, however, serious obstacles to the use of I^{125} where absorption in overlying tissue (HVL $\simeq$ 2 cm) is important, as in quantitative radioiodine uptake studies or brain scanning.

Recovery from Chronic Hypercapnia: The Critical Role of Chloride in Restoration of Normal Acid-base Equilibrium. GORDON D. HAYNIE, RICHARD M. HAYS, ADOLF POLAK AND WILLIAM B. SCHWARTZ,* Boston, Mass.

Balance studies have been carried out in eight dogs during recovery from chronic respiratory acidosis induced by a high CO_2 atmosphere. Four animals received a high salt diet and four others a low salt diet during the period of exposure to CO_2 and throughout the recovery period. At the end of the CO_2 period, plasma bicarbonate concentration ranged from 35 to 38 mEq per L in both groups; plasma chloride concentration was depressed. After being returned to room air, the high-salt dogs showed a prompt reduction in plasma bicarbonate concentration to a normal range of 21 to 24 mEq per L, and chloride concentration rose simultaneously to a normal level. By contrast, the low-salt dogs had a significantly smaller reduction in plasma bicarbonate

concentration, to values of approximately 27 to 30 mEq per L, and became mildly alkalotic; plasma chloride concentration showed little or no change. The fall in plasma bicarbonate in both groups usually took place without loss of bicarbonate in the urine. During the subsequent 6 days, the low-salt dogs stabilized their plasma bicarbonate levels in a range consistently above the normal; the plasma chloride concentration in these animals remained grossly subnormal. The persisent elevation of plasma bicarbonate concentration in the low-salt animals indicates that an elevated rate of hydrogen ion secretion persisted despite a normal plasma carbon dioxide tension. Potassium deficiency did not appear to be a factor in the difference observed between the low- and high-salt groups. When salt was added to the diet of the low-salt dogs, the plasma bicarbonate fell to normal and there was a reciprocal rise in plasma chloride concentration. The reduction in bicarbonate was achieved without urinary bicarbonate loss, but was associated with a marked suppression of acid excretion. Two additional studies of animals given a diet low in sodium but containing a moderate amount of chloride demonstrated that the acid-base disturbance and hypochloremia could be corrected solely by the provision of chloride. A possible mechanism to account for the observed effect of chloride deficiency in impeding the restoration of normal acid-base balance will be considered.

The Influence of Diet on the Excretion of Porphobilinogen in the Allylisopropylacetamide (AIA)-Treated Rat and the Porphyric Human. EMANUEL S. HELLMAN, DONALD P. TSCHUDY, JAMES A. ROSE AND ANNIE R. COLLINS, Bethesda, Md. (introduced by James A. Shannon).

The incidence of porphobilinogenuria induced in starved Sprague-Dawley rats by the injection or intragastric feeding of AIA could be decreased or abolished by progressively increasing the daily intake of carbohydrate (dextrin) or protein (casein hydrolysate) but not by increasing the intake of fat (cottonseed oil). In 3 humans with acute intermittent porphyria an inverse relationship was found between quantitative 24-hour urinary excretion of porphobilinogen and quantitative dietary intake of carbohydrate and protein. The activity of the enzyme that catalyzes the conversion of δ-aminolevulinic acid to porphobilinogen (δ-aminolevulinic acid dehydrase) per unit of liver nitrogen increases during starvation in the rabbit. Incubation of liver slices obtained from a starved rabbit with D-glucose or casein hydrolysate in phosphate buffer at pH 7.0 does not decrease the activity of δ-aminolevulinic acid dehydrase below that observed on incubation of slices in buffer alone. The effect of protein and carbohydrate on the quantitative 24-hour urinary excretion of porphobilinogen in experimental and human acute intermittent porphyria may be mediated through induced changes in the activity of δ-aminolevulinic acid dehydrase in the liver.

Transfer of Autoimmune Nephrosis in Rats by Means of Lymph Node Cells. E. V. HESS AND C. T. ASHWORTH, Dallas, Tex. (introduced by M. Ziff).

Recent studies by others have demonstrated that nephrosis may be induced in rats by the intraperitoneal injection of rat kidney homogenate with Freund's adjuvant. The disease is characterized by proteinuria, hypoproteinemia, hypercholesterolemia and hyperlipemia; histological studies have shown a membranous glomerulonephritis. The method of induction suggests an immunological basis for this condition. This possibility was investigated by attempting transfer of the disease to immunologically tolerant recipients by means of lymph node cells. A total of 44 rats was pretreated neonatally with spleen cells from the prospective donors. When lymph node cells from 12 nephrotic donors were injected intravenously into 17 prepared recipients, histological changes were observed in 12 of 14, major proteinuria in 11 of 14, and an increased serum cholesterol in 8 of 12 animals examined. In control experiments, no histological changes were noted in the kidneys of 11 prepared recipients who received cells for nonimmunized animals or animals injected with Freund's adjuvant alone. There were also no changes in the kidneys of a group of 16 animals consisting of nontolerant recipients of cells from nephrotic rats, recipients tolerant to rats other than the donors, and tolerant recipients who did not receive lymph node cells. Major proteinuria was not observed in any of the 27 animals of the control group and elevation in serum cholesterol occurred in only 2 animals. Immunofluorescent and electron microscopic studies have confirmed the histological observations. The former have shown the presence of γ-globulin in the involved glomeruli, and the latter have demonstrated swelling of the epithelial cytoplasm with obliteration of the foot processes and the presence of hyaline droplets in glomerular and tubular epithelium. The results of these experiments favor an immunological mechanism as the basis for the renal lesion produced in rats by injection of kidney tissue with Freund's adjuvant.

Studies in Man on Gonadotropin-responsive Adrenal Cortical Neoplasia. S. R. HILL, JR., HUGH DEMPSEY, W. R. STARNES, DITSIE PARTLOW AND W. B. NICKELL, Birmingham, Ala. (introduced by Tinsley R. Harrison).

The hormonal dependence and interrelationships of functioning endocrine neoplasia have not been elucidated fully as yet. The opportunity to investigate several aspects of this major medical problem was presented by a patient with a feminizing adrenal cortical tumor. Chromatographic separation and quantitation of individual urinary C_{18}, C_{19} and C_{21} steroids, and serial measurements of urinary total 17-ketosteroids (17-KS), 17-hydroxycorticosteroids (17-OHCS) and estrogens were performed before and after the infusion of corticotropin (ACTH), human chorionic gonadotropin (HCG), containing primarily LH-like activity and pregnant mares' serum gonadotropin (PMS), containing mainly

FSH-like activity, before and after removal of the tumor. There was no appreciable change in C_{18} or C_{19} steroids following ACTH. Elevated control levels of total estrogen (predominantly estriol) and 17-KS (predominantly dehydroepiandrosterone) were observed. Total estrogens doubled following HCG, and increased fivefold after PMS; both changes were due to an increase in the estrone fraction. Total 17-KS were unchanged following HCG, but appeared to increase after PMS. The data imply enzymatic transformations associated with tumor cells and are the first demonstration in man of a gonadotropin-responsive adrenal tumor. Such studies may lead to better understanding and more rational therapy of neoplastic disease.

On the Site of Viral Interference. MONTO HO, Pittsburgh, Pa. (introduced by Maxwell Finland).

The mechanism by which interferon, a mediator of interference and cell resistance, inhibits virus synthesis is unknown, but presumably it acts at an intracellular site. An approximation of this site was made by testing whether or not it blocks the infectivity of viral RNA. Infectious RNA was prepared from poliovirus type III by cold phenol extraction. Monolayers of about 10^6 chick embryo cells, which are genetically immune to the intact virus, were chosen as the substrate for RNA activity in order to restrict the production of intact virus to one cycle, and to eliminate the complicating interactions of intact virus on the host cell. An interferon (S-VIF) was obtained by the action of Sindbis virus on chick cells. Treated monolayers were overlaid with 0.25 ml S-VIF for 3 hours at 36° C and washed before inoculation. Titration of infectivity was by plaque formation on monkey kidney cells. Whereas control chick monolayers inoculated with about 100 plaque-forming units (p.f.u.) of infectious RNA produced from 10^3 to 7×10^3 p.f.u. intact poliovirus, treated cells simultaneously inoculated produced from 0 to 70 p.f.u. To determine whether this inhibition of RNA activity was explained by a difference in the adsorption or inactivation of RNA by cells treated with S-VIF, 1) residual RNA activity was measured after adsorption of RNA on treated and control cell layers, and 2) RNA was incubated with debris of treated and control cells. No difference was found. This inhibition could not be demonstrated when monkey kidney monolayers were substituted for those of chick cells, or if another interferon prepared by the action of a poliovirus type II on monkey kidney cells was used instead of S-VIF on chick cells. These studies together with available information suggest that the site of viral interference is at a point beyond the uncoupling of virus into its nucleic acid and protein components and that this action is mediated by soluble inhibitors that are cell-specific.

Correlation of Culture and Sediment Findings in Urinary Tract Infections. PAUL D. HOEPRICH, Salt Lake City, Utah (introduced by Maxwell M. Wintrobe).

Bacteruria $\geqslant 10^5$ per ml is diagnostic of infection in the genitourinary (GU) tract, but does not define the exact locus of such infection. Pyuria indicates inflammation in the GU tract, but does not reveal the precise site of such inflammation. While intensity of inflammation is reflected in degree of pyuria, only with nephritis does GU tract inflammatory exudate take on morphologically unique form as leukocyte casts (LC). Thus, coexistence of bacteruria and leukocyte casts should enable diagnosis of pyelonephritis. Evaluation of therapy should be aided by serial quantitative examinations of both urinary bacteria and sediment. Three hundred urine specimens from 72 patients were examined by conventional analysis, qualitative and quantitative culture, and enumeration of formed elements of the sediment after staining with: eosin-blue black ink-picric acid; crystal violet-safranin; benzidine-peroxide. Specimens were grouped as: *A*) bacteruria $\geqslant 10^5$ per ml with definite pyelonephritis, 42 specimens from 15 patients; *B*) bacteruria $\geqslant 10^5$ per ml without proved pyelonephritis, 60 specimens from 32 patients; *C*) bacteruria $< 10^4$ per ml with no history of recent antibacterial therapy, 37 specimens from 24 patients; *D*) the remaining 161 specimens were examined in the course of, or immediately following, antibacterial therapy. Pyuria, present in all specimens of *A* and *B*, was most marked in *A;* 84 per cent of *C* specimens had leukocytes, but fewer than *A* and *B* sediments. Pale staining ("viable") leukocytes were seen most often and in largest numbers with *A* and *B* specimens. LC were found in at least 1 specimen from each of the 15 patients of group *A*, in at least 1 specimen from 15 of the 32 patients of *B*, and in 5 group *C* specimens (2 chronic glomerulonephritis, 2 disseminated lupus, 1 acute renal failure). Hematuria was not a marked or frequent finding. Treatment which effected disappearance of bacteruria and LC without cure of pyelonephritis usually did not eliminate pyuria. Both diagnosis and therapy of GU infections are aided by quantitative and qualitative culture and sediment examination.

Renal Sodium Excretion in Man Following Small Increments of Intravascular or Total Extracellular Volume. WALTER HOLLANDER, JR.,* Chapel Hill, N. C.

Much circumstantial evidence suggests that renal sodium excretion is regulated by changes in some component of intravascular volume, and alterations of intravascular volume affect aldosterone excretion. Nonetheless, the question of whether the intravascular or interstitial compartment mediates momentary changes in renal sodium excretion remains unsettled and debated. The current study was undertaken to reexamine this problem utilizing a design suggested by studies of Strauss and co-workers. Subjects were healthy young men with very low basal rates of sodium excretion, having been on a sodium-restricted intake for 1 week. All studies were conducted during the afternoon by the same person in an unvarying manner. A 500 ml positive water balance was maintained throughout. Electrolyte excretion was measured during 30-minute intervals for 2 hours before and 2.5

hours after a 25-minute infusion of either 150 ml of an approximately isoncotic solution of albumin in slightly hypotonic saline (5 studies) or an equal volume of control solution without albumin but otherwise identical to the experimental solution including the albumin vehicle (5 studies). Sodium excretion increased progressively following infusions, probably more after albumin than after control solution (p just less than 0.05). This agrees with studies of Strauss and co-workers in suggesting a highly sensitive "volume receptor" regulating sodium excretion, and it favors an intravascular location. Three additional studies using only 25 ml of control solution and three using a mock design (subjects thought they received an infusion which actually was diverted so as not to enter the vein) revealed increments of sodium excretion comparable to those following 150 ml of control solution; hence, the latter rise, and part of that observed following albumin, presumably resulted from factors other than extracellular expansion. Although not identified in these experiments, such factors obviously represent an important hazard to interpretation of this type of study.

Myocardial Blood Flow as Indicated by the Disappearance of NaI^{131} from the Heart Muscle. WILLIAM HOLLANDER, IRVING M. MADOFF AND ARAM V. CHOBANIAN, Boston, Mass. (introduced by Robert W. Wilkins).

Previous studies have indicated that the removal of an ion injected into a tissue is a function of blood flow. In the present work, the rate of disappearance of NaI^{131} (0.5 ml) injected percutaneously or during open thoracotomy into the heart muscle of dogs and of human subjects was studied as an index of myocardial blood flow. NaI^{131} activity, as determined by external monitoring of the injection site, decreased at an exponential rate with a mean $T_{\frac{1}{2}}$ of 1.5 minutes. In anesthetized dogs the disappearance of NaI^{131} slowed as the coronary artery was narrowed with a clamp and stopped completely following total occlusion of the artery. Following the release of the clamp (and occurrence of reactive hyperemia) the disappearance of NaI^{131} accelerated markedly. In patients with coronary artery disease the exponential removal of NaI^{131} was markedly impaired, being $\frac{1}{4}$ to $\frac{1}{15}$ as rapid as that in "normal" subjects. Sublingual nitroglycerine (0.8 mg) reduced blood pressure but did not increase the rate of removal of NaI^{131} from the myocardium. Exercise in the same patients increased the removal of the isotope without a proportionate increase in blood pressure. Internal "phlebotomy" (by cuffing), when accompanied by hypotension, reduced the removal of NaI^{131}. It is concluded that the disappearance of NaI^{131} from the heart muscle appears to be an index of myocardial blood flow. In coronary artery disease, coronary flow is impaired but appears capable of increasing. The concomitant reduction of blood pressure may account for the failure of nitroglycerin to augment flow.

Mechanism of Endotoxin-induced Coagulation Acceleration. HERBERT I. HOROWITZ, ROGER M. DES PREZ AND EDWARD W. HOOK, New York, N. Y. (introduced by Paul Reznikoff).

A shortening of the clotting time of whole blood or of platelet-rich plasma in the presence of endotoxins of gram-negative bacteria has been reported by McKay and by Robbins and Stetson. The present investigation was undertaken to study the mechanism of endotoxin-induced acceleration of coagulation. The formation of blood thromboplastin depends on an interaction of platelet phospholipid and product I. The phospholipid of intact platelets is not readily available to participate in thromboplastin formation. However, if the platelets are altered, as by thermal injury, phospholipid is more available and an acceleration of clotting can be demonstrated. The availability of platelet phospholipid to participate in coagulation is determined by measuring the clotting time of recalcified platelet-rich plasma after addition of an excess of product I ("product I substrate time"). The product I substrate time of plasma with intact platelets is greater than 20 seconds. In contrast, when platelet phospholipid is fully available the product I substrate time is less than 10 seconds. Incubation of platelet-rich plasma with *Escherichia coli* endotoxin produces a rapid progressive decrease in product I substrate clotting time as compared with controls incubated with saline. The increased availability of phospholipid as determined by this method is apparent by 15 minutes' incubation at 37° C and with optimal dose is maximal by 60 minutes. The release of phospholipid from platelets incubated with endotoxin is accompanied by platelet aggregation and fusion ("viscous metamorphosis"), and roughly parallels transfer of serotonin and a bactericidin for *Bacillus subtilis* from platelets to plasma. Changes in individual clotting activities other than platelet phospholipid have not been found. The present *in vitro* studies utilizing platelet-rich plasma from rabbits suggest that endotoxins increase the availability of phospholipid for the clotting process by an action on platelets.

Mechanism of Action of Thrombin on Platelets. DUDLEY P. JACKSON,* HANS J. SCHMID AND J. RICHARD GAINTNER, Baltimore, Md.

Thrombin, a proteolytic enzyme, initiates certain reactions of platelets including aggregation, clot retraction and release of serotonin. Fibrinogen, a known substrate of thrombin, was investigated for its possible role in these reactions. Concentrates of fresh, washed, human platelets were frozen and thawed repeatedly. The supernatant fluid clotted upon addition of thrombin, indicating the presence of "platelet fibrinogen" as described by others. Fibrinogen could not be obtained from platelets which previously had been incubated with trypsin, although trypsinized platelets appeared morphologically intact. Fibrinogen, therefore, appears to be present on the surface of platelets and can be removed without destruction of the cells. Fresh human platelets suspended in

serum were grossly aggregated by addition of thrombin. Platelets treated with trypsin and subsequently incubated with serum and thrombin did not show significant aggregation. Platelets treated with trypsin and resuspended in purified bovine fibrinogen in a buffered solution of glucose induced retraction of clots formed after addition of thrombin. Platelets incubated with serum and thrombin remained intact until the addition of fibrinogen permitted the occurrence of clot formation and retraction and the irreversible changes of platelets associated with these phenomena. These results suggest that fibrinogen is the substrate of thrombin in the production of platelet aggregation and in the initiation of clot retraction. Platelets treated with trypsin or thrombin released more than 70 per cent of their serotonin. Platelets so treated retained their ability to take up serotonin again. Thrombin blocked the uptake of serotonin by trypsinized platelets, suggesting that fibrinogen is not involved in this reaction.

Reovirus Type I as an Etiologic Agent of the Common Cold. GEORGE GEE JACKSON,* ROBERT L. MULDOON AND GLORIA S. COOPER, Chicago, Ill.

Reovirus, prototype I (ECHO 10) was first recognized in 1954 when it was recovered from the stool of a healthy subject. Later, antigenically related types 2 and 3 were isolated from the respiratory tract as the presumptive cause of acute coryzal illness. We have isolated Reovirus type I from a nasal secretion collected in 1954, from a subject with a common cold. The strain was retained by a 50 mμ filter, ether-resistant, hemagglutinated human group O erythrocytes using a neuraminic acid receptor, and had negligible neuraminidase activity. The filtered nasal secretion was infectious in volunteers and caused a cold-like illness in 37 per cent of 185 subjects after the initial challenge and in 2 of 11 upon rechallenge. The monkey kidney tissue culture harvest was given to volunteers and no illness was produced. However, among 8 subjects given a dose of 10,000 TCD_{50}, all showed a response in neutralizing antibody. Volunteers who received the nasal secretion showed no relationship between the initial level of neutralizing antibody against Reovirus type I and the development of clinical illness. Following the challenge, 9 of 20 subjects who developed a clinical infection and 6 of 18 volunteers who did not, showed an antibody rise to Reovirus type I. Among volunteers given other infectious nasal secretions and who developed respiratory illness a rise was seen in only 1 of 7 tests involving 35 subjects. The data clearly establish the presence of Reovirus type I in the respiratory secretion. Volunteers were infected but there is much doubt as to the etiologic role of reovirus in the causation of respiratory illness. The tissue culture virus infected the nasal mucosa without producing symptoms. It seems likely that the clinical infections produced by the common cold secretion were caused by some other undetected virus.

N^{15}-Glycine Labeling of Stercobilin in Refractory Anemia. G. WATSON JAMES, III* AND LYNN D. ABBOTT, JR., Richmond, Va.

Stercobilin excretion has been reported as being elevated without a concomitant accelerated peripheral erythrocyte destruction in dyserythropoiesis (James and Abbott). Three additional patients with refractory normocytic or macrocytic anemia, hyperplastic bone marrow, and leukopenia, but without thrombocytopenia, have now been studied. Two 500-mg oral doses of N^{15}-glycine (31 atom per cent excess N^{15}) were given at a 4-hour interval. Stercobilin was crystallized from stools collected from five 72- to 96-hour periods. Venous blood was taken at 96-hour intervals for hemin crystallization. N^{15} analyses were performed in a Neir-type mass spectrometer. Serial hematologic studies, serum bilirubin and quantitative fecal urobilinogen were performed. Two normal control subjects showed N^{15}-stercobilin concentrations of 0.022 and 0.021 atom per cent excess N^{15} in the first period, with rapid decline to "normal" (0.013, 0.012) during the next 96 hours. The study patients had N^{15}-stercobilin concentrations of 0.065, 0.058, and 0.023 in the first period. These increased to 0.100, 0.120, and 0.074 atom per cent excess N^{15} in the second 96-hour period and "normal" values were not reached in the 20 days of study. Hemin-N^{15} in the control patients was 0.018 and 0.014. Two study patients had 0.020 maximum hemin N^{15} concentration and the other had a hemin-N^{15} label of 0.044. Active peripheral hemolysis and shortened erythrocyte life span were not evident during the study period. One of the three patients eventually developed acute granulocytic leukemia, and one of the previously reported patients died with this disorder. These data of stercobilin excretion are similar to those previously described and to those observed in pernicious anemia and folic acid deficiency. The data support our previous hypothesis that anabolic stercobilin, reflecting bile pigment synthesis, is markedly stimulated in refractory anemia.

Isolation of Inclusion Conjunctivitis Virus in the United States. ERNEST JAWETZ,* LAVELLE HANNA, PHILLIPS THYGESON, CARL MORDHORST AND CHANDLER DAWSON, San Francisco, Calif.

Inclusion conjunctivitis is an acute nonbacterial eye disease most commonly seen in newborn children, occasionally in adults. It begins with redness, edema, and infiltration of the conjunctiva and a mucopurulent exudate. It does not involve the cornea and, in contrast to trachoma, all lesions heal spontaneously in weeks or months. In epithelial cells from infected conjunctiva inclusion bodies are seen microscopically which closely resemble those of trachoma. Similar inclusions occur sometimes in epithelial cells from the mother's cervix and it is evident that the newborn acquires the infection from the mother's genital tract. From three infants with typical acute inclusion conjunctivitis, viruses were isolated for the first time in America by yolk sac inoculation

of embryonated eggs. Sixteen similar patients yielded no virus. Four to 5 blind passages were required until the strains were lethal for eggs and showed abundant virus microscopically in yolk sac smears. In morphology, stability, growth in eggs, and susceptibility to antimicrobial drugs, these agents resembled trachoma viruses isolated in this laboratory. Inclusion conjunctivitis viruses reached titers of 10^6 to 10^7 egg LD_{50} per ml. About 20 egg LD_{50} constituted a minimal infective dose for cynomolgus monkey eyes, resulting in clinical disease similar to that of man and in the development of inclusion bodies. From experimental infection of the monkey eye, virus was re-isolated for at least 3 weeks. We are searching for laboratory characteristics which would differentiate the viruses of trachoma and inclusion conjunctivitis, to parallel the evident difference in pathogenicity for man.

The Metabolism of Tritium-labeled Folic Acid in Man. D. G. JOHNS AND A. S. V. BURGEN, Montreal, Canada (introduced by Douglas G. Cameron).

After intravenous injection of 15 μg per kg of tritium-labeled folic acid, the plasma concentration falls rapidly so that after 2 hours only 3 to 4 per cent of the dose remains in the plasma and extracellular space. Since only 20 per cent of the dose had been excreted in the urine by this time, the remainder had entered the intracellular space. With smaller doses the excretion is smaller and the rate of tissue uptake accelerated. If 30 mg of unlabeled folic acid is injected 1 or 2 hours after the tritiated material it will displace radioactivity from the cells and raise the blood level of radioactive folic acid. Most of the injected radioactivity is then excreted in the urine in the succeeding few hours; 60 to 80 per cent is recovered as apparently unchanged folic acid, the remainder is due to folinic acid, *p*-aminobenzoylglutamate, a pteridine and two unidentified compounds. Folic acid is evidently stored in the cells largely unchanged. However if there is a delay of 72 hours before injecting the nonradioactive folic acid, much less of the dose is recovered, indicating sequestration of slow metabolic transformation of the stored folic acid. The rate of uptake of folic acid into the cells can be depressed by amethopterin.

A Study of Respiratory Syncytial Virus Infection in Adult Human Volunteers. K. M. JOHNSON, R. M. CHANOCK,* H. M. KRAVETZ, D. RIFKIND AND V. KNIGHT,* Bethesda, Md.

Recent studies have shown that respiratory syncytial virus (RS) is associated with acute respiratory disease in children. Illnesses in children were often characterized by fever and signs of pulmonary involvement including pnuemonia and bronchiolitis. Vouunteer studies were undertaken to determine pathogenic properties of this agent in adults. Forty-one men were given RS virus intranasally. Thirty-three individuals were successfully infected, and 20 developed respiratory illnesses. None of the 20 control volunteers showed evidence of RS infection. Illnesses were mild, afebrile and consistent with descriptions of the "common cold." The observed association between virus challenge and subsequent illness was strengthened by the finding that illness never preceded initial RS virus isolation and the fact that illness occurred for the most part in individuals who shed detectable virus for an interval of more than 2 days. Initial virus isolation from volunteers subsequently developing illness generally occurred on the third or fourth postinoculation day. Circumstances of several initial isolations made after the fifth postinoculation day suggested that these might have represented secondary infection. Neutralizing and complement-fixing antibody responses to RS virus occurred predominantly in subjects who developed clinical illness. This finding together with the correlation of illness with duration of virus shedding suggested that the induced respiratory disease reflected the extent of RS viral infection. RS virus infection in these volunteers represented reinfection, since all men had detectable RS neutralizing antibody prior to challenge. Pre-existing levels of antibody, however, did not influence subsequent RS infection or susceptibility to respiratory illness.

Increased Turnover of Phosphoribosylpyrophosphate, a Purine Nucleotide Precursor, in Certain Gouty Subjects. O. W. JONES, D. M. ASHTON AND J. B. WYNGAARDEN,* Durham, N. C.

This study in gouty subjects was designed to evaluate the turnover of phosphoribosylpyrophosphate (PRPP), a compound intimately involved in the primary regulatory mechanism controlling purine synthesis *de novo*. The reaction in which PRPP and glutamine form phosphoribosylamine (PRA) is the site of feedback control mediated by ATP. A defect of this control might be reflected in a disturbance of PRPP turnover. PRPP is also involved in other reactions, one of which, conversion of imidazoleacetic acid (IAA) to its ribonucleotide, is the basis of this study. Glucose-C^{14} was administered orally to label the ribose moiety of PRPP, and IAA orally to permit sampling of PRPP by analysis of urinary IAA-riboside (IAAR). In 4 control subjects 0.01 to 0.06 per cent of C^{14} appeared as ribose-C^{14} in IARR in 10 hours. (Hiatt found 0.05 to 0.06 per cent in 3 controls.) In 3 gouty subjects who excreted urate excessively, 0.16, 0.20, and 0.31 per cent of C^{14} appeared as IAAR. Time courses of specific activities showed early maxima, three times greater than normal. Relative specific activity (RSA) of ribose ranged from 1.4 to 4.8 in 7 controls, and was 10.9, 14.0, and 16.6 in gouty subjects. C^{14} incorporation and RSA values correlated with urinary urate excretion. In contrast, conversion of IAA to IAAR was not increased in these subjects. One additional gouty subject with normal 24-hour urate excretion, low urate/inulin clearance ratios, and normal glycine-1-C^{14} incorporation values on two occasions, showed normal glucose-C^{14} incorporation into IAAR, and normal RSA values. These results suggest *1*) that gouty subjects who

clearly overproduce urate may have an ineffective feedback control at the PRPP to PRA step, and *2*) that gouty subjects with normal urate outputs may, at least in some cases, have hyperuricemia on the basis of some other mechanism.

Acute Defibrination Syndrome in Metastatic Carcinoma: Role of Tumor Emboli. MUSTAFA KARACA, MARIO STEFANINI* AND BASIL KEKIS, Boston, Mass.

A 72 year old male with bronchiogenic carcinoma developed petechiae and ecchymoses and later died of uncontrollable hematuria. Early in the disease, laboratory studies showed: fibrinogen 61 mg% [tyrosine and $(NH_4)_2SO_4$ precipitation method]; platelet count, 76,000 per mm^3; bleeding time, over 10 minutes; tourniquet test, positive; concentration of AHG, prothrombin and factor V, 33, 60 and 20 per cent, respectively. The "serum factors" were unaffected. No general anticoagulants, antithromboplastin, antithrombin III and VI could be demonstrated. Esterolytic and fibrinolytic activity of plasma was normal; activity of proactivator, activator, plasminogen and antiplasmin was also normal. Survival of fibrinogen and platelets, studied by radioactive and by transfusions technics, showed a much shortened life span. All results remained unchanged in the course of the disease. Autopsy findings showed massive infiltration of lungs, adrenals and liver by tumor tissue. No material stained by PAS or for collagen was seen in tissues or deposited on the intimal wall of organs. *In vitro,* tumor as well as normal tissue showed no proactivator or activator activity. Fresh and acetone-dried normal and tumor tissue showed comparable thromboplastic activity. Numerous tumor emboli were seen within the lung vessels, surrounded by thrombi, composed of fibrin and presumably platelets. Defibrination syndrome of disseminated tumors has been attributed to many mechanisms: failure of fibrinogen production, fibrinolysis, intravascular deposition of fibrin on the intimal wall complicated by fibrinolysis and depolymerization of fibrinogen by proteolysis. The present case demonstrates an additional mechanism—namely, the role of tumor emboli in causing massive intravascular defibrination in a fashion similar to that of amniotic squamae during accidents of pregnancy.

Relationship of Bacteriuria to Hypertension: An Epidemiological Study. EDWARD H. KASS,* WILLIAM E. MIALL AND KENNETH L. STUART, Boston, Mass, Penarth, Wales, and Jamaica, West Indies.

The role of pyelonephritis in the pathogenesis of hypertension is unclear. Asymptomatic bacteriuria has been linked to pyelonephritis and occurs frequently in hospital populations, but its prevalence in nonhospital populations is unknown. The prevalence of bacteriuria and its relationship to blood pressure were investigated in defined rural and urban populations in Jamaica, and in defined mining and agricultural populations in Wales. Over 95 per cent cooperation within the selected populations was obtained. Voided urines for bacterial counts (repeated as needed to establish 96 per cent confidence limits at the level of 10^5 colonies per ml), blood pressures by standardized technique, and brief medical history were obtained. In 3,057 females, the prevalence of bacteriuria was 4.4 per cent (3.4 to 7 per cent in the different groups), with slightly higher prevalence in Wales than in Jamaica. In males, the prevalence was < 0.5 per cent. Bacteriuric females had significantly higher blood pressures than did nonbacteriurics. The prevalence of bacteriuria in females with diastolic pressures > 90 mm was 7.1 per cent and with diastolics < 90 mm was 3.4 per cent ($p < 0.01$). Bacteriuria was more prevalent among married than single women, rose with age and parity, was not familial in occurrence, was not related to diabetes mellitus or to glycosuria, and was not related to albuminuria. Bacteriurics were separable from nonbacteriurics on several grounds, such as the differences in sex incidence of bacteriuria, and the finding that in bacteriurics increased blood pressures are related to increased parity whereas in nonbacteriurics there is an inverse relationship between parity and the height of the blood pressures. The data favor the hypothesis that bacteriuria is etiologically related to hypertension rather than that it is secondary to hypertension and suggest preventive possibilities. Population studies of hypertension are subject to error unless asymptomatic bacteriuria is considered.

Iron and Protein Kinetics Studied by Means of Doubly-labeled Crystalline Transferrin. JAY H. KATZ, Boston, Mass. (introduced by Charles P. Emerson).

Crystalline transferrin (Tr) was labeled with I^{131} to yield a product which contained 0.5 to 0.8 equivalents of iodine per molecule of protein. Yields were 90 to 95 per cent and chromatographic analysis indicated that the iodine was incorporated chiefly as monoiodotyrosine. The iron binding capacity and other physical and immunologic properties appeared to be unaltered. Behavior with reticulocytes in an *in vitro* system was similar to that of uniodinated Tr. Iodinated Tr was administered intravenously to 7 normal adults as the Fe^{59} complex, the level of saturation varying from 35 to 100 per cent. The disappearance of both tracers from the plasma was followed for the first 3 hours and daily thereafter for 17 to 23 days. The clearance and RBC utilization of iron were similar to that found using whole plasma, although the clearance appeared to be somewhat more rapid in the 3 subjects given 100 per cent saturated Tr. Initial diffusion of Tr from the vascular space did not exceed 7 per cent per hour, roughly paralleling albumin in this regard. Hence, if Tr is bound on surface sites of erythropoietic cells, as was suggested by *in vitro* studies, such binding must be of brief duration and, having yielded its Fe, the Tr must be released directly back into the vascular space. Equilibration with the extravascular compartment was achieved in 4 to 5 days. The total exchangeable pool was 0.23 to 0.30 g per kg, 55 to 62 per cent of which was extravascular. After equilibration the Tr disappeared at a single exponential

rate, with a half-life of 6.7 to 8.4 days. The results indicate Tr lightly labeled with iodine maintains its integrity as a transport protein, has a reproducible half-life in normal subjects, and should be of value in the study of aberrations in iron and transferrin metabolism observed in many disease states.

The Production of Fever in Rabbits with Extracts of Tissue Culture Cells Infected with Coxsackie Virus. M. KENTON KING, St. Louis, Mo. (introduced by Carl G. Harford).

Extracts of polymorphonuclear leukocytes have produced fever in animals following intravenous injection. Influenza viruses contained in amniotic fluid from infected embryonated eggs are also pyrogenic. However, viruses grown in tissue culture which is free of the products of inflammation present in the infected chick embryo have not previously produced fever. The following experiments describe the characteristics of fever produced in rabbits by Coxsackie virus grown in monkey kidney cells, a system in which leukocytic pyrogen is excluded. Bottles containing monolayers of monkey kidney cells grown in nutrient media were inoculated with Coxsackie B_1 virus. When the cytopathic effect became marked the cells were collected, pooled, and washed free of nutrient media. Subsequently the cells were suspended in 0.85 per cent saline and disrupted by alternate freezing and thawing before testing. Monkey kidney cells, either whole or disrupted, which had not been inoculated with virus failed to produce fever following injection into rabbits. Consistent fevers were produced, however, by aliquots of disrupted cells containing Coxsackie B_1 virus. The fever regularly followed a latent period of 45 minutes and produced two peaks, one at 90 minutes and the second at 3 to 4 hours. Identical fevers were produced in rabbits previously made tolerant to the fever-producing effect of bacterial endotoxin. Rabbits injected on consecutive days with Coxsackie virus pyrogen exhibited tolerance by Day 3. The pyrogenic activity was not diminished even though aliquots were heated to 70° C for 20 minutes, indicating that the fever is not due to active infection. These findings indicate that extracts of monkey kidney cells infected with Coxsackie virus in the absence of leukocytes produce a characteristic fever following intravenous injection into rabbits. Attempts to obtain similar results with adenovirus and vaccinia virus grown in HeLa cells were not successful.

Studies of Intracellular Amino Acid Transport in Man as an Index of Protein Metabolism: Effect of Growth Hormone. DAVID M. KIPNIS,* MONROE GROSS AND WILLIAM H. DAUGHADAY,* St. Louis, Mo.

Protein synthesis and intracellular amino acid transport are so coordinated in intact cellular systems that changes of rate in one are mirrored by similar changes in the other. A technique has been developed for examining amino acid transport in man using the nonmetabolizable amino acid, α-aminoisobutyric acid (AIB). The results in 9 normal subjects (5 male, 4 female) and in 2 hypophysectomized patients before and after receiving human growth hormone (HGH) form the basis of this report. AIB-1-C^{14} (94 μg per kg body weight, sp. act., 1.6 mc per mmole) was injected intravenously in the nonfasting state. Blood levels and urine recoveries were recorded for 5 to 10 days. AIB disappearance from blood followed a multi-exponential function representing initial distribution in extracellular space, transport into intracellular water and renal clearance. AIB distribution between intracellular water and plasma reached equilibrium after 10 to 12 hours, remaining relatively constant thereafter. AIB was rapidly excreted; 50 per cent of the administered dose appeared in urine in 0.3 to 0.7 day in females and in 1.0 to 1.5 days in males. Over 98 per cent of the total dose was recovered in urine. Renal clearance was 60 to 75 ml per minute in normal and 40 to 50 ml per minute in hypophysectomized subjects, the latter rate increasing to normal levels, as did creatinine clearance, after HGH treatment. The apparent volume of distribution of AIB (V_{AIB}), normalized to 70 kg body weight, was 220 to 280 L in males and 134 to 157 L in females; indicating marked intracellular concentration. In two hypophysectomized patients, HGH treatment increased the low control V_{AIB} levels from 67 to 123 L to 138 and 331 L, respectively, and resulted in marked nitrogen retention. This technique affords a rapid and sensitive measure of growth hormone action and may be applicable for assessing the effects of other hormones and dietary factors on protein metabolism.

Studies on Factors Which May Contribute to the Pathogenesis of Staphylococcal Infection. M. GLENN KOENIG AND DAVID E. ROGERS,* Nashville, Tenn.

The factors which render certain staphylococci capable of producing infection are not clearly understood. While considerable information is available regarding the microbial factors which determine virulence of other gram-positive cocci, the features which characterize "pathogenicity" among strains of staphylococci have not been defined. A single strain of staphylococcus has been shown to form two distinct colonial variants which can be readily identified in special media. These two variants can be derived from a single clone. While both variants produce pigment and coagulase, only one variant consistently produces fatal intraperitoneal disease in mice. The other variant is promptly eradicated *in vivo*. Studies of these variants and the experimental infection they produce have brought to light factors which may act to initiate progressive staphylococcal disease. While both strains are able to grow in the presence of serum, only the virulent variant possesses components which cause it to resist intraperitoneal phagocytosis. These components are antigenic and may be located at the cell surface. Immunization with vaccines prepared from the virulent variant promote intraperitoneal phagocytosis and protect against fatal disease. Vaccines prepared from the avirulent variant do not. The behavior of these two staphylococcal variants, the relationship of certain biologic properties to virulence, and the charac-

teristics of staphylococcal-host cell interactions in an experimental mouse infection will be considered.

Study of "Basket Cell" Metabolic Activity in Human Leukemia by Tritiated Thymidine Radioautography. DONALD R. KORST, EUGENE P. FRENKEL AND JOAN E. WILHELM, Ann Arbor, Mich. (introduced by Sibley W. Hoobler).

The term "basket cell," also known as smudge cell, degenerating cell, naked nuclei, fragmented cell, ghost cell, or nuclear shadows of Gumprecht, covers a group of cell fragments seen in blood and bone marrow. Their origin is generally thought to be from mechanical pressure in the preparation of smears or films. Recent studies with radioautographs utilizing tritiated thymidine (H^3T) reveal that many basket cell forms take up the isotopically-labeled material in high concentration and are perhaps part of an active metabolic pool or primitive proliferating pool of Cronkite. The marrow and peripheral blood from 12 patients with acute and chronic leukemia have been studied *in vivo* and *in vitro* with H^3T. Patients studied *in vivo* received 200 μc H^3T. (specific activity 5.2 mc per mmole), and blood was obtained serially from 15 minutes to 72 hours. Bone marrow was obtained by aspiration at 48 or 72 hours. Five μc H^3T was added *in vitro* to 3-ml aliquots of blood and incubated 5 minutes to 2 hours prior to preparing radioautographs. Slides were then dipped in methyl alcohol to fix and remove thymidine not incorporated into DNA, eliminating a possible artifact. High resolution radioautographs were prepared with Kodak dipping emulsion and stained with Wright's stain. The basket cell concentrates H^3T within 15 to 30 minutes following *in vitro* introduction of the isotope. The *in vivo* label was recognized within 2 hours. This cell is different from the usual fragmented granulocyte or lymphocyte and occurs primarily in leukemia or lymphomas. Mechanical injury to cells *in vitro* does not produce basket cells that incorporate H^3T. It is proposed that this fragmented or disintegrating cell retains some DNA-synthesizing ability and may be a reflection of general proliferative activity. The concentration of H^3T in these cells appears early in the marrow and blood indicating active DNA synthesis. The basket cell is a metabolically active cell or cell fragment in contrast to denegerating granulocytes and lymphocytes. This cell is morphologically identifiable and perhaps is another means of determining proliferative activity in leukemia and lymphoma.

Evidence for Deficiency of Vitamin B_6 in Non-tropical Sprue. O. DHODANAND KOWLESSAR, LORRAINE J. HAEFFNER, GORDON BENSON AND MARVIN H. SLEISENGER,* New York, N. Y.

The urinary metabolites of tryptophan—5 hydroxyindoleacetic acid (5OHIAA), indole-3-acetic acid (IAA), kynurenine (Kyn), xanthurenic acid (XA), and indican—were studied in 10 normal subjects and in 21 patients with non-tropical sprue; 5 received no therapy and 16 were treated with a gluten-free diet. All of the latter were in remission, but only 3 had normal fat absorption. Measurements were made on an 8-hour collection of urine for the following periods: control; after loading with 4.0 g L-tryptophan; after 90 mg. pyridoxine (vitamin B_6) intramuscularly; and after reloading with 4.0 g L-tryptophan plus 90 mg pyridoxine. Reported results (mg) are means and standard deviations; normal values before and after 4.0 g L-tryptophan are: 5OHIAA, 1.6 ± 0.6 and 3.4 ± 1.2; IAA, 3.6 ± 3.0 and 21.8 ± 11.8; Kyn, 0.4 ± 0.15 and 29.2 ± 18.4; XA, 5.4 ± 3.3 and 10.3 ± 3.9; indican, 30.3 ± 16 and 40.6 ± 14.3, respectively. In 5 untreated patients the excretion of 5OHIAA, IAA, Kyn, XA and indican was increased (3.2 ± 0.6; 7.6 ± 4.2; 0.8 ± 0.4; 6.8 ± 3.7; and 55.9 ± 25.3) as well as in the 13 patients on dietotherapy with steatorrhea (3.7 ± 0.9; 6.4 ± 2.4; 0.5 ± 0.2; 9.7 ± 4.7; and 38.1 ± 20.2). Values in the 3 treated patients without steatorrhea were normal except for XA (17.6 ± 4.8). Tryptophan loading significantly increased IAA, Kyn, XA and indican in the untreated group (33.2 ± 14.2; 78.8 ± 48.1; 38.9 ± 30.8; and 73.0 ± 24.3), and in the treated patients with steatorrhea (47.2 ± 21.7; 82.1 ± 36.4; 25.2 ± 14.6; and 40.9 ± 27.0). In the 3 treated patients without steatorrhea, Kyn and XA also rose significantly (134.8 ± 23.1; and 52.2 ± 42.4), but IAA and indican did not increase abnormally. Administration of pyridoxine corrected the abnormal excretion of Kyn in all patients and reduced XA, but not to normal. These results demonstrate a deficiency of vitamin B_6 in non-tropical sprue which is clearly accentuated by loading with L-tryptophan.

The Relative Abundance of Viral Receptors: An Explanation of the Differential Susceptibility of Suckling and Adult Mice to Coxsackie B_1 Infection. CALVIN M. KUNIN AND NORMA E. HALMAGYI, Charlottesville, Va. (introduced by William S. Jordan, Jr.)

The marked difference between infants and adults in susceptibility to various virus infections has been the subject of considerable speculation. One explanation for this difference may be found in quantitative variations in the tissue distribution of those specific virus receptor sites which govern the first step of cellular infection. Adsorption technics and plaque count assays were employed in a study of the distribution of Coxsackie B_1 (Conn. 5 strain) receptor site in various rodent tissues. A heat-, ether-, and chloroform-labile sedimentable virus-adsorbing substance was found in noncultured minces and homogenates of brain of suckling and adult mice and rats; it was not found in any guinea pig or rabbit tissues. Suckling mouse brain was much more active in adsorption than adult brain as indicated by dilution and kinetic studies; cortisone treatment did not enhance receptor activity of adult tissues. Multiplication of virus was demonstrated in adult mouse brain in the absence of overt disease. Slight adsorptive activity was demonstrated in mouse squamous cell carcinoma and

teratoma minces, but not in 8 other mouse tumors or in mouse muscle, liver, gut, blood, fetal or maternal tissues. The marked neurotropism of this Coxsackie B_1 strain in suckling mice was thus correlated with the relative abundance of receptor site in CNS tissue. The enhanced susceptibility of cortisonized adult mice to Coxsackie B_1 infections reported in the literature appears to be due to mechanisms other than increased receptor site activity.

Uric Acid Excretion in Gout: Evidence of an Abnormality in Renal Transport. WILLOUGHBY LATHEM* AND GERALD P. RODNAN, Pittsburgh, Pa.

The mechanism of hyperuricemia in gout remains in dispute. Recent evidence has suggested that uric acid transport by the kidney may be altered in gout and that this may contribute to the hyperuricemia. In the present study the renal transport and excretion of uric acid by gouty patients were examined under conditions of acute intravenous urate loading and compared with a group of nongouty individuals studied under similar conditions. Sodium or lithium urate was infused intravenously (16 to 32 mg per minute) over a 2 to 3 hour period while measurements of plasma and urine urate levels were made and the glomerular filtration rate (inulin clearance) determined. Only subjects with normal filtration rates were included. As the plasma urate level increased during the infusion the filtered load and urinary excretion also increased. A distinct impairment in urate excretion was apparent in all of the gouty subjects studied. At comparable plasma levels (8 to 30 mg%) and filtered loads, the gouty subjects excreted a consistently and significantly smaller proportion of the filtered urate load (from ½ to ⅘ less) than the nongouty controls. This was also true of one of two subjects with essential hyperuricemia studied. In ⅔ of the control subjects the difference between the filtered load and the excretory rate (minimum reabsorptive rate) became constant (average, 10 mg per minute) at high plasma levels. In all of the gouty subjects the minimum reabsorptive rate increased progressively and did not become constant, attaining values of 20 or more mg per minute. These results establish that an abnormality in renal urate transport—either increased reabsorption or decreased secretion—exists in gout. This abnormality may be a basic feature of the metabolic disorder of gout or may be acquired or appear as an adaptative response during the course of the disease.

"Complete" and "Incomplete" Pathologic "Cold" Antibody Activities: One or Multiple Antibody Species? JOHN P. LEDDY, NORMA TRABOLD, SCOTT N. SWISHER* AND JOHN H. VAUGHAN,* Rochester, N. Y.

Human sera containing pathologic "cold" antibodies are recognized by two different serological activities: direct agglutination of most human red cells, and, in the presence of complement, sensitization of erythrocytes to reagglutination when the cells are subsequently tested with "non-gamma" antiglobulin sera. The sensitization of erythrocytes for the antiglobulin test has been called "incomplete" cold agglutinin and has been thought possibly to depend upon an antibody different from the direct agglutinin, as in the case of complete (19S) and incomplete (7S) Rh antibody. Four pathologic cold agglutinating sera were studied by a variety of technics designed to separate, if possible, these two activities; separation was not achieved. Both activities were found to reside in protein fractions having the characteristics of 19S γ-globulins. They sedimented together in sucrose density ultracentrifugation, migrated together in starch block electrophoresis, and eluted together from DEAE columns. Both activities were eluted at 37° C from erythrocytes sensitized at 4° C in the absence of complement; incomplete activity was demonstrable only when complement was subsequently added to the eluate. Both activities were susceptible to destruction by mercaptans. Neither activity could be differentially absorbed from the sera. Neither activity was demonstrable when I-negative erythrocytes were employed. The data, therefore, are consistent with the concept that the two agglutinin activities are attributable to the same antibody molecule; the difference is ascribable to the presence or absence of complement when the antibody combines with the erythrocytes. It has been suggested that the interaction of pathological cold agglutinins with complement is important in the pathogenesis of certain acquired hemolytic disorders. Since this "incomplete" antibody activity is not dependent upon a separate, 7S, mercaptan-resistant globulin, these observations provide impetus to the theoretical possibility that treatment of such patients with agents such as penicillamine might be beneficial.

The Simultaneous Measurement of Pressure and Flow in the Pulmonary Arterial System of Man. G. DE J. LEE, R. BOSMAN, A. J. HONOUR, R. M. MARSHALL AND F. D. STOTT, Oxford, England (introduced by A. B. duBois).

A pneumatic flowmeter has been devised for use with the body plethysmograph. It consists essentially of a servo system maintaining a constant pressure within the plethysmograph, and has a linear response from 0 to 200 ml per second, and a uniform frequency response up to 12 cycles per second. Gas mixtures of differing solubility and concentration were inspired by subjects in the plethysmograph to determine the relative importance of mechanical events within the thorax, and pulmonary blood flow itself, on the pattern of pulsatile gas movement within the lungs during each heart cycle. Small, biphasic, fluctuations in gas volume occurred with each heart beat, irrespective of the gas breathed. These were compression effects by the heart on neighboring viscera and were trivial compared with the fluctuations superimposed by the effects of pulmonary blood flow, shown when soluble gases were breathed. The uptake rate of a soluble gas, such as nitrous oxide, was proportional to its alveolar concentration and to the pulmonary

capillary blood flow. The pulmonary capillary blood flow was pulsatile with each heart beat, and the right ventricular stroke volume could be calculated from the N_2O uptake curves and expired N_2O concentration measured simultaneously. Comparison between this and the Fick cardiac output estimation was within ±8 per cent. The pulmonary capillary flow pattern conformed closely to the pulmonary artery pressure obtained simultaneously during cardiac catheterization. In the normal subject, peak pulmonary blood flow followed approximately 0.2 second after the peak pulmonary arterial pressure. The effect of pulsatile pulmonary capillary blood flow on oxygen uptake was also studied by continuously recording gas uptake when subjects breathed mixed venous concentrations of carbon dioxide in oxygen. The oxygen uptake was pulsatile and was similar to the N_2O uptake pattern.

Continuous Inulin and PAH Clearance Studies During Prolonged Administration of Deoxycorticosterone (DOC) in Normal Man. JAMES B. LEE AND DAVID P. LAULER, Boston, Mass. (introduced by George W. Thorn).

Prolonged administration of mineralocorticoid to normal man results in retention of sodium, chloride and water followed by an "escape mechanism" whereby urinary sodium progressively rises to equal or even exceed intake. Previous studies have suggested an increase in glomerular filtration rate (GFR) associated with this escape; however, the definitive study of continuously monitored renal hemodynamics through a control period followed by the phase of DOC-induced sodium retention and ultimately through the phase of escape has been lacking. In this study, simultaneous evaluation of renal and metabolic events which occur during adaptation to DOC was made upon a normal subject receiving a constant weighed diet (267 mEq sodium). By means of a constant intravenous infusion the GFR (C_{In}) and renal plasma flow (RPF) (C_{PAH}) were continuously monitored for 3 control days and then, during DOC administration, for 7 consecutive days using six 4-hour collection periods each day. Renal clearances of total solute (C_{Osm}) and solute-free water (C_{H_2O}) were determined every 4 hours together with measurements of urinary electrolytes, acidification parameters, creatinine, urea and catecholamines. The experimental design provided for normal activity of the subject, enabling ambulation within a 40-foot radius. During the control period a marked diurnal variation of both GFR and RPF occurred. Within 24 hours after DOC administration, there was a rapid elevation in mean 24-hour renal plasma flow (744 to 911 ml per minute) followed by a more gradual rise in mean 24-hour GFR (123 to 152 ml per minute). A surprising reduction in RPF 24 hours prior to the "escape" was associated with an increase in C_{H_2O} and an elevation in serum sodium. On the day of "escape," maximal natriuresis was associated with a pronounced rise in renal blood flow with no change in mean 24-hour GFR.

A Metabolic Substitute for Vitamin B_{12}. CARROLL M. LEEVY, HERMAN BAKER, OSCAR FRANK AND HERMAN ZIFFER, Jersey City, N. J. (introduced by Harold Jeghers).

Studies of cyanocobalamin (B_{12}) demonstrate that administered B_{12} partly displaces hepatic stores of this vitamin. The present study was undertaken to determine whether it also displaces other substances from the liver. Under conditions of right hepatic vein catheterization, 100 μg of crystalline B_{12} was given to 8 healthy subjects and 7 patients with inactive cirrhosis. It caused release of B_{12} and a previously undescribed "Factor X" in both groups of patients; no alteration in splanchnic blood flow or oxygen consumption was noted. Unlike B_{12}, Factor X is stable to alkaline hydrolysis at pH 11 to 12, 121° C, 16 pounds per square inch for 30 minutes. It is not naturally present in normal serum or serum from patients with elevated B_{12} due to hepatic necrosis. Thirty minutes after intravenous B_{12}, 1 ml of serum from hepatic venous blood yields an average of 14 mg of lyophilized protein-free alkali-thermostable material; 2 hours after its administration, B_{12} activity remains elevated while Factor X activity disappears. Factor X possesses four times the activity of B_{12} for *Lactobacillus leichmannii, Escherichia coli* 113-3, *Euglena gracilis,* and *Ochromonas malhamensis,* the latter organism being the most critical indicator of B_{12} hematopoietic activity. This substance produced two ultraviolet-absorbing spots on ascending chromatography with butanol : acetic acid : water (12 : 3 : 5) which did not correspond to any known purine or pyrimidine moieties. It is concluded that intravenous B_{12} in man triggers hepatic release of an alkali-thermostable factor which permits growth of B_{12}-requiring microorganisms. This factor appears to bypass and completely fulfill the metabolic requirement and function of B_{12}.

Abolition of Digitalis-induced Ventricular Tachycardia by Ryanodine. EDWARD LEONARD AND STEPHEN HAJDU, Bethesda, Md. (introduced by Donald S. Fredrickson).

Ryanodine is a complex water-soluble alkaloid which first evoked interest as the substance responsible for the insecticidal properties of extracts from the plant *Ryania speciosa.* Ryanodine is extremely toxic for mammals. It induces skeletal muscle contracture, and in much smaller concentrations depresses myocardial contractility. In this laboratory it has been observed that ryanodine abolishes the action of cardiac glycosides on contractility of cardiac muscle. In isolated frog heart and rat ventricle strips the effect of cardiac glycosides on contractility can be eliminated or prevented by ryanodine. Because of this digitalis-ryanodine antagonism, studies were undertaken to determine whether ryanodine could reverse digitalis toxicity in intact mammals. In anesthetized cats, otherwise fatal ventricular tachycardia induced by intravenous digitoxin was abolished by ryanodine in all instances within 5 to 15 minutes. The

resultant heart rate after ryanodine tended to be in the normal range, and the cardiac cycle was initiated by the sinus or by another focus which was always supraventricular. The intravenous injection of ryanodine in the amount required to abolish ventricular tachycardia had no effect on the ECG of normal cats. Thus far, similar results have been obtained in the dog. Despite the suppression of ventricular arrhythmias, preliminary experiments suggest that ryanodine may not reverse digitalis-induced conduction defects. Thus the AV block caused by digitalis in a dog was not reversed by ryanodine; and administration of digitoxin to a ryanodine-treated cat caused appearance of AV block without ventricular arrhythmia. Ryanodine, with its capacity to reverse the action of digitalis on ventricular muscle, merits investigation as a specific agent for the reversal of digitalis-induced ventricular arrhythmias.

Direct Measurement of Human Cardiac Excitability. DAVID H. LEWIS, HOWARD F. WARNER AND MARY B. ALLAN, Philadelphia, Pa. (introduced by Truman G. Schnabel, Jr.).

During routine cardiac catheterization opportunity has been taken to quantitate the excitability of the human heart. A wire electrode, insulated except at the tip, was passed through the catheter and advanced until the tip lay within the myocardium. The transition from an intracavitary position to an intramyocardial one was recognized by the change in the recording from the electrode from an intracavitary potential to a form resembling an action potential. Square wave stimuli were delivered to the heart, of varying amplitude (0.1 to 25.0 v) and duration (1 to 15 msec) either as single shocks with a known delay from the R wave or as continuous stimuli applied at varying times in every fourth cycle. In 25 patients, records have been obtained 13 times from the right atrium, 3 from the left atrium, 8 from the right ventricle, and once from the left ventricle. Results from the atrium and ventricle were similar. Threshold was of the order of 0.5 to 4.0 v and rose as the duration was decreased below 7 msec. Absolute refractoriness was noted in the range of 250 to 300 msec after the onset of electrical activity at the point of stimulation. Relative refractoriness was seen but the curve of amplitude vs interval became perpendicular at 1 to 2 v above threshold. Responses were obtained during the recovery phase of the action potential. Prolonged latency in either the absolute or relative refractory period was not seen. Supernormality, with threshold stimuli, was observed, but was the exception rather than the rule. Multiple responses following a single stimulus (vulnerability) were seen in cases with spontaneous premature contractions, but were rare. It was quite pronounced in one instance following a large dose of intravenous digitalis. In both the atrium and ventricle repetitive stimulation reproduced the phenomenon of parasystole.

Porphyrin Synthesis in Thalassemia and Sickle Cell Anemia. HERBERT C. LICHTMAN AND FELIX FELDMAN, New York, N. Y. (introduced by Ludwig W. Eichna).

Normal porphyrin metabolism is a prerequisite for optimal heme synthesis. Hemoglobin production is known to be reduced in sickle cell anemia and thalassemia. The present study was designed to determine whether a particular portion of the pathway for heme synthesis is intact in these hereditary anemias. Measurements were made of the amount of uroporphyrin, coproporphyrin and protoporphyrin synthesized, *in vitro,* from Δ-amino levulinic acid using the circulating erythrocytes of patients with thalassemia and sickle cell anemia as a source of the necessary enzymes. Comparisons were made with normal erythrocytes. Uroporphyrin production was entirely comparable in the three groups. Coproporphyrin production was increased twofold in the thalassemic group compared with the normal or sickle cell anemia groups. Protoporphyrin synthesis was elevated 40- to 45-fold in both anemia groups. An increased number of identifiable immature cells (reticulocytes and nucleated red blood cells) was present in the incubation mixtures derived from the anemia groups. The increase in total porphyrins and especially of protoporphyrins synthesized in the system in the anemia groups may very well be due to the increased numbers of immature cells present. Conclusions: 1) No deficit in porphyrin synthesis, *in vitro,* from Δ-amino levulinic acid could be demonstrated using thalassemia or sickle cell anemia erythrocytes as enzyme source. 2) A marked increase in total porphyrin synthesis was found, especially of protoporphyrin, in the incubation mixtures containing erythrocytes of thalassemia and sickle cell anemia patients.

Removal of Damaged Cells from Intestinal Mucosa. MARTIN LIPKIN, H. OLIVER BROWN AND MILTON L. LEVINE, New York, N. Y. (introduced by Thomas P. Almy).

Nuclei of rapidly proliferating jejunal epithelial cells of mice were labeled by injection of C^{14}-thymidine. Animals were sacrificed at varying intervals thereafter, and specific activity determined on mucosal precipitates containing DNA. In parallel experiments utilizing H^3-thymidine, tissues were prepared for study by microradioautography. In control animals, the specific activity of the incorporated thymidine began to decline 35 to 40 hours after injection. Microradioautographs demonstrated that this interval was required for the normal column of labeled epithelial cells to migrate from the crypts to villus tips and begin extrusion. In the experimental group, animals were injected simultaneously with colchicine and labeled thymidine. Thus, crypt cells were labeled and then damaged as they underwent mitosis. The decline in specific activity in this group began in 6 hours; 30 hours after injection, one-quarter of the label had been removed from the mucosa

of the experimental group, while there was no loss of label from the controls. Microradioautographs revealed that labeled cells with colchicine-induced damage were removed from the crypt column beginning within 6 hours after injection. Progressive loss of these cells was noted thereafter, and the cells appeared to be extruded directly from the crypt lumen, preventing migration onto the villi. Following removal of the damaged cells, the villi and remaining crypt and villus epithelial cells appeared normal. The study demonstrates a method of response of intestinal mucosa to damage induced in a limited number of regenerating cells. The possible role of this response in the production of ulceration of the gastrointestinal tract will be further evaluated.

Eosinophilia as a Consequence of Antigen-antibody Interaction. MORTIMER LITT, Boston, Mass. (introduced by B. D. Davis).

Eosinophilia occurs in such a wide variety of pathological situations that it has seemed as if no common mechanism underlies the phenomenon. The peritoneal cavity of guinea pigs was used as a site for testing the eosinophilotactic potency of various materials, and the results indicate that eosinophilia is related to a particular phase of the immune response. Exudates were obtained by lavage through an abdominal trocar and the total population of cells characterized by standard hematologic techniques. After active sensitization, by repeated weekly injections of foreign protein, eosinophilia appeared within 24 hours after injection of the antigen. Passive transfer of tissue (peritoneal lining or lung), tissue extracts, or serum from sensitized animals produced eosinophilia, provided that the sensitizing antigen accompanied the material transferred, and that the recipients had been actively sensitized with a noncross-reacting protein. Washed immune precipitates were equally effective; these consisted of either bovine serum albumin with rabbit antibody, or hemocyanin with guinea pig or rabbit antibody. Such mixtures were effective in antigen excess, equivalence and antibody excess. The activity of the antisera disappeared after absorption with specific antigen. Thus, the active transfer factor in serum seems to be antibody and the active material *in vivo* appears to be an immune complex. The present findings appear to account for the common association of eosinophilia with hypersensitive states, since immune complexes also initiate various hypersensitive reactions. In addition, it appears likely that during the recuperative phase of an infectious disease, the eosinophilia, which is so frequently seen, signals the beginning production of antibody.

A New Method Suitable for Clinical Measurement of Cerebral Circulation Time. W. D. LOVE, L. P. O'MEALLIE AND W. W. LEMMON, New Orleans, La. (introduced by G. E. Burch).

When a bolus of radioisotope circulates through the brain, the time course of cerebral radioactivity resembles that in arterial blood. However, because of the time required for isotope to traverse vessels within the area monitored, the cerebral curve has a lower, later peak and a more gradual downslope. The differences between these curves have been used as the basis for calculating mean cerebral circulation time (MCCT). The clinical advantages of this "wave distortion" method of analysis are 1) MCCT can be measured without carotid or jugular puncture, and 2) differences in cerebral circulation to separate areas can be determined. In a model, radioactivity was recorded by virtually lag-free counters which monitored inflowing and outflowing fluid and the "brain" during passage of a radioactive bolus. True MCCT was equal to the difference between circulation times to the inflow and outflow points. MCCT was estimated from inflow and organ curves by two methods. One, an empirical procedure, was based on the difference in location of the median point in the two curves. The second was a curve-fitting technique. In a range of circulation times from 4.1 to 23.4 seconds there was a mean ± 0.2 second, or ± 1.6 per cent difference between MCCT calculated by these methods and the actual value. Similar studies were done in 13 anesthetized dogs with the cranium exposed surgically for monitoring. Radioactivity in the infraorbital artery and torcular Herophili was continuously recorded by aspirating blood through tubing under monitors. One carotid and both vertebral arteries were ligated. I^{131}-albumin was injected proximal to a mixing chamber in the other carotid. MCCT values in 53 determinations were manipulated to range from 0.9 to 30.0 seconds. Mean difference between the inflow-outflow and inflow-organ techniques was ± 1.4 seconds, or ± 16.6 per cent.

Relationship of Sulfhydryl Inhibitors and Reductive Conditions to the Action of Insulin on Adipose Tissue. W. S. LYNN, JR.,* EUGENIA EARNHARDT AND ROSE BROWN, Durham, N. C.

Recent studies by Langdon have implied that reduced insulin may be the active form of the hormone. Schwartz and co-workers also have hypothesized that antidiuretic hormone probably exerts its effects through a disulfide exchange reaction. We, therefore, have studied the effects of -SH inhibitors as well as reducing conditions on rat adipose tissue, in the presence and absence of insulin. Both iodoacetate and parachloromercuribenzoate (PCMB) (2×10^{-5} M) mimic the effects of insulin on this tissue *in vitro;* i.e., more glucose 6-C^{14} (5-fold) is converted to fatty acids and more glucose 1-C^{14} to CO_2. O_2 uptake and lactic acid production are slightly reduced, tissue content of ATP and G6P increases, and the tissue content of glucose and water decreases in the presence of these -SH inhibitors, as well as insulin. The effect of insulin, along with -SH inhibitors, is additive. Furthermore, under strong reductive conditions (i.e., N_2 and HCN or N_2 and H_2S) insulin, PCMB, or iodoacetate potentiates the effects of these reductive conditions on lactate production and fatty acid synthesis from glucose or butyric acid. Thus, sulfhydryl inhibitors produce the

same effects as insulin. Insulin potentiates the effects of -SH inhibition, and insulin and -SH inhibitors potentiate the effects of strong reducing agents. None of these agents or conditions is lipolytic. The primary action of insulin thus appears to be related to the easily altered sulfhydryl content of this tissue, and may act, like PCMB, by reacting with tissue -SH groups.

Protein-Bound Sulfur (PBS); Its Role in Inhibiting Thyroid Protein-bound Iodine. F. MALOOF AND M. SOODAK, Waltham and Boston, Mass. (introduced by J. Lerman).

A cytoplasmic particulate system which transfers the sulfur of thiourea to protein (PBS) in the presence of thiocyanate ion (10^{-3}M) and DPNH (10^{-3}M) has been isolated from animal or human thyroid tissue. The inhibition of the *in vitro* formation of protein-bound iodine (PBI) by thyroid particulate protein requires a 20-fold greater concentration of cysteine (10^{-4}M) than thiourea (5×10^{-6}M), although the reduction potential (Eo′) of cysteine, −0.15, equals that of thiourea, Eo′ = −0.21. Incubation of S^{35}-labeled cysteine (10^{-4}M) with the thyroid particulate protein produces only one-tenth as much PBS as thiourea even with thiocyanate and DPNH. This suggests that PBS formation is important in inhibiting PBI. The stimulation of *in vitro* PBI formation by various peroxide-generating systems suggests that a peroxidase is involved in iodination. This has led to the assumption that thiourea acts by inhibiting peroxidase. Thiourea (5×10^{-5}M) or iodide was incubated with thyroid protein plus flavin mononucleotide (FMN), in the light, as a peroxide-generating system. FMN (10^{-3}M) leads to the formation of PBS (10 mμmoles) equal to that of the thiocyanate-DPNH system. However, the FMN and the thiocyanate-DPNH system differ, since the former is inhibited by dark, by thiocyanate (10^{-3}M), and is only partly (60 per cent) inhibited by heat (100° × 3 minutes). PBI formation in the presence of FMN is about 1 mμmole. Preincubation of the particulate protein with sulfite (0.01 M), a compound known to cleave disulfide bonds specifically, inhibits the subsequent formation of PBS or PBI by FMN. These data lead to the following conclusions. A disulfide bond in thyroid tissue is essential for the formation of both PBI and PBS by FMN. Thiourea cleaves this disulfide bond to form PBS. The action of thiourea in inhibiting PBI formation in the presence of a peroxide-generating system may be due to the formation of PBS.

Active Transport of Iron In Vitro by Duodenal Segments and the "Mucosal Block." JAMES G. MANIS AND DAVID SCHACHTER,* New York, N. Y.

Everted segments of rat duodenum transport iron *in vitro* against concentration gradients from fluid bathing the mucosa to fluid bathing the serosa. The active transfer is observed only in the proximal small intestine, is dependent on oxidative metabolism and is limited in capacity. Two steps are involved in the net transport across the gut. 1) The initial and more rapid step is uptake at the mucosal surface of either Fe^{++} or Fe^{+++}; 2) the subsequent transfer to the serosal surface is less rapid, rate-limiting, and relatively specific for Fe^{++}. The active transport mechanism in the duodenum is markedly influenced by pregnancy, by inflammation, and by prior doses of iron. Duodenal segments from rats in the third week of pregnancy transfer iron much more readily than do segments from nonpregnant female controls. Intramuscular injection of turpentine into a hind limb is followed in 2 to 4 days by depression of the iron transport. Administration by gastric tube of 0.4 to 4.0 mg of iron (as $FeSO_4$ or $FeCl_3$) decreases markedly the net transport of iron across the gut *in vitro*. The inhibition can be observed 1.5 hours after the dose and persists for approximately 24 hours. Prior doses of iron salts decrease both steps of the active transport mechanism *in vitro*. The inhibition of mucosal uptake corresponds to the "mucosal block" suggested by experiments *in vivo*, whereas the inhibition of serosal efflux is a new observation which suggests the more appropriate term "transport block." The "transport block" is apparently a direct effect of iron salts on the duodenum. Prior incubation of duodenal segments in media containing either Fe^{++} or Fe^{+++} decreases the net transport of iron observed subsequently.

Evidence for Heterogeneity Among Subjects with Glucose-6-phosphate Dehydrogenase Deficiency. PAUL A. MARKS,* RUTH T. GROSS AND JULIA BANKS, New York, N. Y.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency has been detected, in varying incidence, in many population groups around the world. The trait appears to be due to a sex-linked gene of intermediate dominance. Previous studies in this laboratory indicated that mutant subjects vary in the severity of the enzyme deficiency and in the thermostability of the G6PD. A comparison has now been made of the electrophoretic mobility and the properties of the catalytic site of G6PD purified 2,000-fold from red cells of 6 normal and of 12 mutant subjects. The mutant subjects varied in expression of this trait as measured by the red cell G6PD activity (RBC-G6PD). The following results were obtained. 1) G6PD of 1 female and 2 males of an Italian family differed from normal G6PD in having a faster electrophoretic mobility and a decreased affinity for TPN. These male mutants were distinguished from other mutant Caucasian males studied in that RBC-G6PD was decreased only 50 per cent. 2) G6PD of a male of Negro-Caucasian parentage (RBC-G6PD decreased 90 per cent) had a lower affinity for TPN, but was similar to normal G6PD in the other properties studied. 3) G6PD of 4 Negro males (RBC-G6PD decreased 85 per cent), 1 Negro female (RBC-G6PD decreased 50 per cent), 1 Caucasion male and 1 female (RBC-G6PD decreased 95 to 98 per cent) all without anemia, and 1 Caucasian male with congenital nonspherocytic hemolytic anemia (RBC-G6PD decreased 98 per cent) were similar to normal G6PD in the prop-

erties of the catalytic site and in electrophoretic mobility. These data indicate that an altered enzyme is present in certain mutant subjects. In addition, G6PD deficiency in man appears to be genetically, as well as phenotypically, heterogeneous.

Effect of Proteolytic Enzymes on Red Cell Rh_0 (D) Content. S. P. MASOUREDIS,* San Francisco, Calif.

The effect of proteolytic enzymes on the Rh_0 (D) red cell content was studied with incomplete I^{131} anti-Rh_0 (D). Aliquots of an R^2R^2 RBC suspension were treated at 37° C with 0.3 per cent trypsin for 10 minutes and with 0.01 per cent papain for 30 minutes. Controls consisted of the same untreated R^2R^2 cell and an rr cell subjected to the same enzymatic treatment. Papain-treated RBC took up 23 per cent more I^{131} than the untreated cells and the trypsin-treated RBC bound 15 per cent more I^{131}. This difference between the enzyme-treated Rh_0 (D) positive cells and the untreated cells was significant at the 5 per cent level, whereas the difference between the two enzymes was not significant at this level. The Rh_0 (D) negative cell took up less than 10 per cent of the I^{131} bound to the R^2R^2 cell and there was no difference between the I^{131} bound to the enzyme-treated and untreated RBC. To test the specificity of the additional I^{131} bound to the enzyme-treated Rh_0 (D) positive cell, the cells were hemolyzed with distilled water and the stroma bound I^{131} was determined after washing the stroma with distilled water. The stroma of the untreated Rh_0 (D) positive cell contained 90 per cent of the total cell bound I^{131} and the stroma of the enzyme-treated cells contained 97 per cent of the original I^{131} bound to the intact cell. Only 45 per cent of the I^{131} was recovered in the stroma of the Rh_0 (D) negative cell after hemolysis. These results indicate that enzymatic treatment of Rh_0 (D) positive red cells with papain or trypsin exposes additional Rh_0 (D) antigen sites on the red cell stroma that are not available in the untreated cell. It appears that the agglutinability of these enzymatically treated cells is due to an increase in antigen sites which in some fashion yields an adequate structure for agglutination.

Dominant Role of Pulmonary Vascular Engorgement as a Determinant of Dyspnea. H. PAGE MAUCK, JR. AND WILLIAM SHAPIRO, Richmond, Va. (introduced by John L. Patterson).

The present observations explored the pathogenesis of dyspnea in patients with and without the rapid induction of pulmonary engorgement. Pulmonary wedge pressure and arterial gas tensions were measured in 13 subjects, 11 with primary systemic hypertension and left ventricular enlargement, one with mitral stenosis, and one with obstructive pulmonary emphysema. Dyspnea was induced in the supine position by breath-holding or exercise on a recording ergometer. Precise correlations were obtained by patient signals at the onset of discomfort (later described). Four patients exercised without developing dyspnea, wedge pressure rise or gas tension abnormality. Dyspnea during breath-holding was experienced by 7 patients without wedge pressure rise but after a 13 mm Hg fall in P_{O_2} and a 6 mm Hg rise in P_{CO_2}. In contrast, 5 hypersensitive patients who exercised maximally (532 kg-m per minute for 2 minutes) experienced dyspnea after a mean rise in wedge pressure of 20 mm Hg, even though P_{O_2} had risen 10 mm Hg and P_{CO_2} fallen 2 mm Hg. The actual wedge pressures at the onset of dyspnea were in the narrow range of 27 to 34 mm Hg, and directional changes in gas tensions were consistent. The patient with emphysema exercised for 4.5 minutes at 493 kg-m per minute and became dyspneic when the wedge pressure had risen from 5 to 15 mm Hg, the arterial P_{O_2} had fallen from 60 to 46 mm Hg, and the P_{CO_2} had risen from 70 to 79 mm Hg. Arterial pH changes were small in all experiments. The similarity of the descriptions of dyspnea by the different patients was notable. These observations are consistent with the hypothesis that the onset of dyspnea is related to the summated effects of multiple respiratory stimuli. When present, pulmonary vascular engorgement plays a dominant role, with 30 mm Hg as the apparent critical level of a rising wedge pressure during exercise.

Tolerance to Bacterial Endotoxin Induced by Experimental Pyelonephritis. WILLIAM R. MCCABE, Chicago, Ill. (introduced by Harry F. Dowling).

Tolerance to the toxic effects of the endotoxins of gram-negative bacteria is a thoroughly documented phenomenon that is readily produced by repeated injections of endotoxin. It has not been observed to occur during clinical or experimental infections. Comparison of the clinical courses of 173 patients with gram-negative septicemia suggested that the relatively mild course exhibited by patients who had concomitant chronic pyelonephritis might be a manifestation of tolerance to endotoxin and prompted the present investigation of the pyrogenic effect of bacterial endotoxin in pyelonephritic rabbits. Pyelonephritis was produced by temporary ureteral ligation followed by the intravenous injection of *E. coli* (0:111,B:4) while weight-matched controls received a similar injection of heat-killed bacteria. Two weeks after the induction of infection, both groups of animals were challenged with 0.5, 1.0, and 2.0 μg of *S. enteritidis* endotoxin (Difco) and fever curves were determined. Rabbits with active pyelonephritis not only exhibited a significant diminution ($t_{24} = 7.91$; $p < 0.001$) in the total magnitude of fever (mean 12.6 ± 6.6) as compared with controls (mean 43.7 ± 10.2) but also demonstrated the classical tolerant fever curve in which a primary temperature rise without a subsequent secondary elevation was observed. Prior administration of thorium dioxide (Thorotrast) abolished the tolerant state and induced a febrile response analogous to that observed in the control animals. These results indicate that proliferation of gram-negative bacteria in the kidney is capable of inducing tolerance to heterologous endotoxin.

Immunization of Man with Globulin-modified Measles Vaccine. FRED R. MCCRUMB, JR., SCHELDON KRESS, RICHARD B. HORNICK, MERRILL J. SNYDER AND ANN E. SCHLUEDERBERG, Baltimore, Md. (introduced by Charles L. Wisseman, Jr.).

Attenuated measles-virus vaccines propagated in canine renal or chick embryo cell culture were used to imunize several hundred susceptible children. Study of routes of immunization revealed that amounts of virus minimally infective for tissue culture were highly immunogenic in man upon parenteral administration. Aerosolized virus also resulted in a high take rate as evidenced by appearance of antibody following vaccination. Intranasal vaccination was associated with widely variable take rates without apparent explanation. Clinically overt reactions to parenterally inoculated vaccines were not invariably benign; 20 to 30 per cent of vaccinees developed fever in excess of 103° F. Respiratory manifestations and constitutional symptoms were uncommon and cutaneous eruptions were those of a modified infection. Less reaction was observed following respiratory administration of vaccine. Despite this reduction in reaction rate, the authors concluded that attenuated measles-virus vaccines were not sufficiently innocuous for large-scale use. Virus did not spread spontaneously to those susceptible children in intimate contact with vaccinees. However, viremia and pharyngeal shedding of the vaccine strain were demonstrated in a few instances. Specific neutralizing and complement-fixing antibody appeared in high titer after administration of attenuated viruses. The slow degradation of these antibodies suggested that immunity would be of long duration. Immunized children were resistant to naturally occurring measles 10 and 15 months after administration of vaccine. The reaction to administered vaccines was reduced by modifying the induced infection with human γ-globulin. Marked decrease in the incidence of significant reaction without alteration of immune response suggests that this method is applicable to large-scale immunization. The paradox of virus and antibody interacting to the advantage of the host without impairment of antigenicity is of some interest. Factors presumably operative under these conditions are discussed.

Differentiation of Nervous and Hormonal Control of Renal and Limb Vascular Beds Utilizing Bretylium and Guanethidine. JOHN C. MCGIFF, Philadelphia, Pa. (introduced by Calvin F. Kay).

Simultaneous and continuous measurements of renal and limb (femoral) blood flows (venous outflow rotameter) of the anesthetized (chloralose) dog during increased intracranial pressure permit observation of enhanced sympathetic vasoconstrictor activity and the alteration of this activity by agents blocking sympathetic nerves (bretylium and guanethidine). In 9 dogs increased intracranial pressure unmodified by these drugs is followed by a striking pressor response (105 per cent of control), a reduction of limb (mean, 40 per cent) and renal (mean, 53 per cent) blood flows, the latter occasionally falling to zero. Renal vascular resistance is augmented (mean, 360 per cent) more than femoral vascular resistance (mean, 240 per cent). The pressor response is reduced within 20 to 40 seconds by bretylium (2 to 7 mg per kg). Concomitantly, renal and femoral flows are always increased. Correspondingly both femoral and renal resistances are reduced, the former much more than the latter. This difference in regional resistance changes is presumably due to continued action of catecholamines (unopposed by bretylium) to which the renal bed is demonstrably more sensitive. Guanethidine has effects similar to bretylium in reducing sympathetic vasoconstrictor activity. In the absence of increased intracranial pressure, the resting anesthetized dog responds to intravenous administration of bretylium or guanethidine with hypotension within 1 minute, which may be preceded by a transient hypertension. Femoral flow increases sharply (from 21 to 330 per cent), whereas renal flow is reduced, although less so than blood pressure; thus, renal resistance is reduced. The opposite effect on regional blood flows is attributed to the drug's unmasking a greater degree of sympathetic tone involving the vessels of skeletal muscle than the vessels of the kidney in the "resting" state. Renal resistance, but no femoral resistance, is increased if an initial hypertensive phase is elicited. This latter effect is presumably related to the liberation of levarterenol to which the kidney is more sensitive.

The Effects of Squatting on the Circulation. MALCOLM B. MCILROY* AND THOMAS V. O'DONNELL, San Francisco, Calif.

The effects of assuming the squatting position on the arterial oxygen saturation, pulse rate and arterial blood pressure have been studied in normal subjects and patients with congenital heart disease. The changes in saturation were followed with an ear oximeter and the arterial pressure usually measured directly in the brachial artery. The following explanation is proposed for the symptomatic relief often seen on squatting in patients with Fallot's tetralogy. 1) Squatting raises the peripheral systemic resistance and increases the systemic venous return by removing the distending force of gravity upon the arteries and veins below the heart. As a result, more of the right ventricular output is directed to the lungs, and the volume of blood shunted into the aorta is reduced. 2) The increase in cardiac output and blood pressure seen on squatting is inversely proportional to the volume of blood in the central reservoirs of the heart and lungs. 3) Squatting produces a marked increase in the arterial oxygen saturation only in patients with a small "central blood volume." 4) The anatomy of the heart in Fallot's tetralogy, with a right ventricular-to-aortic shunt, provides a situation in which small changes in the parallel resistances of the pulmonary stenosis and the systemic arterial bed have a great effect on the arterial oxygen saturation.

Studies on the Effect of Inosine on the Metabolism of Blood Cells During Storage at 4° C. THOMAS J. MCMANUS, Boston, Mass. (introduced by John G. Gibson, 2nd).

The effect of two different anticoagulant solutions, with and without the addition of inosine prior to collection, on the carbohydrate and phosphate metabolism of blood cells during 35 to 42 days of storage at 4° C has been studied. The solutions were acid-citrate-dextrose (ACD), citrate-phosphate-dextrose (CPD), and ACD and CPD with inosine added (ACDI and CPDI) in amounts sufficient to give a final concentration of 2 g per 500 ml of blood. In the presence of inosine, glucose utilization in both solutions was significantly inhibited, whereas lactate production did not vary from the controls. Phosphate ester hydrolysis is initially retarded in CPD compared to ACD, not reaching its maximal rate until the second week of storage. In ACDI and CPDI, this ester pool is much more stable and does not begin to break down to any large extent until after the third week of storage. After 5 to 6 weeks of storage, the gross intracellular phosphate partition in all the solutions appeared similar whether inosine was present at the time of collection or not. CPD remained less acid than ACD throughout storage with an essentially parallel fall in pH. However, CPDI became acid more rapidly than ACDI, resulting in the same pH in both bloods by the fourth week. Pentose and hypoxanthine concentrations leveled out after 35 days, suggesting that the contribution of the nucleoside to carbohydrate and phosphate metabolism had ceased, due to its essentially complete utilization. We conclude that the addition of inosine to the anticoagulant solution prior to collection is probably not the optimal procedure for utilizing this metabolic adjuvant. The amount of purine that is shown to accumulate in inosine-treated blood, however, represents a serious hazard to patients with impaired renal function.

Simultaneous Change in Hepatic Glucose Utilization, Hepatic Glucose Production and Hepatic Glucose Balance During Glucose Loading. DAVID MEBANE, FRANK LECOCQ, BURTON COMBES AND LEONARD L. MADISON,* Dallas, Tex.

Since net hepatic glucose output equals hepatic glucose production minus hepatic glucose utilization, changes in one or both of these parameters may account for the decrease in hepatic glucose output that attends glucose loading. In these studies changes in hepatic glucose balance, hepatic glucose production and hepatic glucose utilization were measured simultaneously during glucose loading in 5 dogs with portacaval shunts, a preparation permitting measurement of hepatic rather than splanchnic glucose balance. Hepatic glucose balance was determined every 15 minutes for 160 minutes from measurements of hepatic blood flow (Bradley's technic) and hepatic venous-arterial glucose concentration differences. Hepatic glucose production was determined from changes *across the liver* in the specific activity of blood glucose labeled by a constant infusion of glucose-U-C^{14}. Hepatic venous and arterial glucose specific activities were determined by the gluconate method of Blair and Segal. Hepatic glucose utilization is the difference between hepatic glucose and hepatic glucose balance. During control periods, hepatic glucose production and hepatic glucose output were identical (72.7 and 72.1 mg per minute), thereby indicating the absence of hepatic glucose utilization during fasting. Glucose infusions (3 to 12 mg per kg per minute) resulted in a decrease in hepatic glucose output consequent to both a decrease in hepatic glucose production and an increase in hepatic glucose utilization. The 72 per cent decrease in hepatic glucose output was the result of a 51 per cent decrease in hepatic glucose production from 72.7 to 35.3 mg per minute and a simultaneous increase in hepatic glucose utilization from 0 to 15 mg per minute. These data indicate for the first time that the decrease in hepatic glucose output which follows glucose loading is the consequence of both an increase in hepatic glucose utilization and a decrease in hepatic glucose production.

Alterations in Steroid Hormone Secretion Produced by Inhibition of Cholesterol Biosynthesis. JAMES C. MELBY, MARLYN ST. CYR AND S. L. DALE, Little Rock, Ark. (introduced by Richard V. Ebert).

Interference in the formation of cholesterol, a precursor of the steroid hormones, might be expected to result in reduced steroid hormone production and/or emergence of noncholesterol pathways of steroid formation. To study this effect triparanol, a triphenylethane derivative which inhibits desmosterol conversion to cholesterol, was given laboratory animals and human subjects in dosage sufficient to reduce net cholesterol synthesis. Steroidogenesis *in vitro* was assessed by incubation of adrenal slices obtained from rats pretreated with triparanol and from controls. Acetate-1-C^{14} or mevalonate-2-C^{14} was added to all and corticotropin to half of the incubates. Corticosterone, deoxycorticosterone and aldosterone were separated by paper chromatography. Eluates were divided for quantitation and scintillation counting. Pretreatment with triparanol reduced corticosteroid production by 50 to 75 per cent. Specific activities of individual corticosteroids, however, demonstrated a 2- to 10-fold increase over controls. Alterations of steroid specific activity in corticotropin-stimulated preparations suggest the existence of a noncholesterol pathway of steroid synthesis. Triparanol in a dose of 1,000 mg per day for 10 days significantly reduced cortisol and aldosterone production of healthy human subjects. Impairment of adrenal secretory responsiveness to corticotropin stimulation and to pyrogen stress was demonstrated. Remission of features of primary aldosteronism, Cushing's syndrome and adrenal virilism was associated with a curtailment of corticosteroid and androgen production in 4 patients receiving triparanol. Testicular androgen production was unaffected by triparanol administration in a male with Addison's disease

and in healthy adult males during exogenous corticosteroid-induced adrenal suppression.

Physiological Implications of Changes in Oxyhemoglobin Dissociation. W. F. MILLER, R. L. JOHNSON, JR. AND J. D. O'KEEFE, Dallas, Tex. (introduced by Carleton B. Chapman).

A re-evaluation of previous studies from this laboratory showed large shifts to the right of the O_2-Hb dissociation curve in normal subjects and patients with mitral stenosis during severe exercise, and both at rest and during exercise in severely anemic patients. Only part of these shifts can be explained by pH changes. The magnitude of these shifts at the 50 per cent saturation level are: normal 15 mm (12 mm pH-dependent), anemia 17 mm (10 mm pH-dependent), mitral stenosis 10 mm (7 mm pH-dependent) $p < 0.02$. It is often stated that these shifts aid diffusion of O_2 to the tissues, but only Keys and co-workers have attempted to quantify such effects. If tissue diffusion is a limiting factor in O_2 exchange, the rate of exchange would be a function of the mean capillary O_2 tension, a factor not considered by Keys' group. With our data obtained at maximal O_2 intake and employing a dynamic mathematical analysis, estimates of maximal resistance to diffusion in exercising muscle mass (mm Hg per L per minute) were obtained: normal 14, anemia 25, mitral stenosis 28. With these, a graphic analysis yields estimates of the maximal increase in O_2 intake resulting from observed shifts: normals 9 per cent, anemia 10 per cent, mitral stenosis 3 per cent. According to the method of Keys' group, the increase in our anemic patients at maximal O_2 intake would be 21 per cent. Since, in the present analysis, O_2 tension of mixed venous blood from the exercising extremity was assumed to be the same as end-capillary O_2 tension in the exercising muscle, the actual resistance to diffusion in muscle is probably less than our estimates. Thus, the importance of shifts in the O_2-Hb dissociation, during heavy exercise, in facilitating O_2 uptake is small.

Studies on the Blood-cerebrospinal Fluid Barrier in Hepatic Failure. EDWARD W. MOORE, GEORG W. STROHMEYER AND THOMAS C. CHALMERS,* Boston, Mass.

Animal studies indicate that the distribution of ammonia across membranes is pH-dependent, according to non-ionic diffusion theory. In hepatic failure, however, correlations between ammonia concentrations, pH, and neurological disturbances are often lacking, suggesting abnormality in the blood-CSF barrier. Functional integrity of the barrier was studied by comparing blood and CSF concentrations (after intravenous infusion) of antipyrine, sufadiazine and *p*-aminohippurate (PAH)—compounds distributed according to non-ionic diffusion theory. Plasma drug concentration was maintained constant after priming, and CSF obtained at intervals for 8 hours through an indwelling spinal catheter. CSF/plasma equilibrium ratios for antipyrine and PAH (12 patients) and sulfadiazine (7 patients) were predictable from existing pH gradients and were similar to those previously reported in subjects without liver disease. In sharp contrast, steady state CSF/arterial ammonia ratios were independent of pH gradients, and CSF ammonia was linearly related to arterial ammonia over a wide range of concentrations. Because this linear function did not intercept zero, the CSF/arterial ammonia ratio rose with increasing arterial concentrations, being significantly greater in patients with cirrhosis. The difference between expected CSF/arterial ratios (calculated from existing pH gradients) and observed ratios was inversely related to arterial ammonia concentration. Although steady state CSF/arterial ammonia ratios showed no correlation with pH gradients, alteration of gradients by drug infusion qualitatively changed these ratios in the expected direction. In conclusion, the functional integrity of the blood-CSF barrier for partially ionized compounds is intact in cirrhosis. That CSF ammonia is linearly dependent on arterial concentration irrespective of pH suggests artifact in the methodology (Nathan-Rodkey and Conway) or binding sufficient to obscure the pH gradient effect. The proportion of artifact appears to decrease at higher arterial concentrations, probably accounting for the closer correlations noted in animal studies and also for the fact that only qualitative changes in distribution ratios occur in response to pH change.

Extreme Sensitivity of Parathyroid Hormone Secretion and of Renal Response to Phosphate Infusion. PETER A. F. MORRIN, ERIC REISS AND W. BURTON GEDNEY, St. Louis, Mo. (introduced by Neal S. Bricker).

In studies of renal phosphate excretion it is commonly assumed that alterations in parathyroid hormone secretion induced by changing plasma phosphate concentrations require no special consideration. This assumption has been examined critically in the dog. In euparathyroid dogs, increasing the plasma phosphate only 0.5 to 1.0 mg% by slow phosphate infusion resulted in doubling to tripling of the fraction of filtered phosphate excreted (C_{PO_4}/GFR) while the apparent phosphate reabsorption rate remained unchanged. A constant phosphate reabsorption is expected only at T_m whereas these experiments were performed at plasma phosphate levels (4.6 to 6.0 mg%) of one-half to one-third that required to saturate tubular reabsorptive capacity. When a high C_{PO_4}/GFR has been attained by a slow phosphate infusion, administration of calcium intravenously promptly restored this ratio to or below baseline values. Calcium exerted this effect even though its infusion caused further elevation of plasma phosphate. When the procedure was reversed and calcium infused from the beginning, phosphate infusion resulted in a constant C_{PO_4}/GFR and an increasing rate of apparent phosphate reabsorption. The specific effect of calcium on parathyroid secretion was demonstrated by a parathyroidectomized dog given parathryroid extract intravenously at a constant rate. In this experimental preparation calcium had little effect on C_{PO_4}/GFR. Most studies

were performed in dogs with unilateral renal disease. As in previous studies with this experimental model, the pattern of phosphate excretion in disease and control kidneys was closely comparable under all conditions examined. These experiments demonstrate unusual sensitivity of the mechanisms responsible for parathyroid hormone release. Distinction cannot be made between a direct stimulatory effect of minimal hyperphosphatemia on parathyroid secretion and an effect mediated by slight depression of ionized calcium. The evidence presented also indicates that parathyroid hormone regulates C_{PO_4}/GFR at all levels of filtered phosphate.

Refined Antihypertensive Medullorenal Extract and the Protective Action of the Kidney Against Hypertension. E. E. MUIRHEAD,* J. W. HINMAN, E. G. DANIELS, M. KOSINSKI and B. BROOKS, Detroit and Kalamazoo, Mich.

Renoprival hypertension of the dog occurs consistently when the intake includes sodium and dietary protein. This hypertension is ameliorated by a renal homotransplant. Uretorocaval anastomosis, under identical circumstances, is not conducive to hypertension. Finely divided whole kidney and renal medulla explanted to the peritoneum and lungs, respectively, prevent the hypertension. A nontoxic crude extract of renal medulla prevents this hypertension. The extract decelerates the evolution of renoprival hypertension when massive sodium loads are interposed (Na intake 7.5 to 15 mEq per kg per day), thus yielding results similar to those of ureterocaval anastomosis. Based on the above, it has been suggested that the protective action of the kidney against hypertension resides in the medulla and that this function may be extractable as a principle. The medullorenal extract fails to prevent the renin pressor effect but prevents renoprival hypertension when daily renin pressor peaks are superimposed on nephrectomy plus protein and sodium (slope Δ BP vs zero, p 0.65). Nonrenal tissue extracts under these conditions are not protective against hypertension (slope Δ BP vs zero, $p < 0.001$). The medullorenal extract yields results similar to those of intact kidneys. Utilizing ethanol fractionation with and without prior dialysis, the medullorenal extract was separated into four fractions: 1) The 50 per cent ethanol insoluble fraction was ineffective (BP vs renoprival state, p 0.9; vs medullorenal extract, $p < 0.02$). 2) The 10 per cent ethanol insoluble fraction yielded partial protection against renoprival hypertension (BP vs renoprival state, $p \backsim 0.05$; vs extract, p 0.15). 3) The dialysate was inconclusive, seemingly effective and ineffective lots being derived. 4) The supernatant solution after 50 and 55 per cent ethanol precipitation was consistent in preventing renoprival hypertension (vs renoprival state, $p < 0.001$; vs extract, $p > 0.9$). The latter fraction contains 2 to 3 mg organic solids per ml. The crude extract and fractions are not pyrogenic to rabbits. It would seem that the antihypertensive factor of the renal medulla toward renoprival hypertension has been considerably refined and concentrated.

The Mechanism of Aldosterone Stimulation Following Hemorrhage in the Hypophysectomized Dog. PATRICK J. MULROW AND WILLIAM F. GANONG, New Haven, Conn. and San Francisco, Calif. (introduced by J. W. Hollingsworth).

In order to study how hemorrhage stimulates mainly aldosterone secretion by the adrenal cortex of hypophysectomized dogs, 10 hypophysectomized dogs were nephrectomized and then acutely bled. Control and posthemorrhage samples of adrenal vein blood were collected in consecutive periods. Aldosterone, corticosterone and 17-hydroxycorticoids were measured. In a previous study, in all 10 hypophysectomized dogs, hemorrhage had increased aldosterone secretion (mean secretory rates: control, 10; posthemorrhage 17 mμg per minute; $p < 0.01$). In 8 of 10 hypophysectomized, nephrectomized dogs, hemorrhage failed to stimulate aldosterone secretion (mean secretory rates: control, 3.4; posthemorrhage, 5 mμg per minute). Glucocorticoid secretion was unaltered. Intravenous administration of 1 IU of ACTH to 4 of the dogs markedly stimulated glucocorticoid and aldosterone secretion, indicating a responsive adrenal. Intravenous administration of crude saline extracts of normal dog kidneys and partially purified renin extracts of normal and hypophysectomized dog kidneys caused a marked increase in aldosterone secretion ($p < 0.01$) within 20 minutes, and a slight to moderate increase in glucocorticoid secretion. A marked pressor response also occurred. Following the "renin" extracts, the high aldosterone secretory rates persisted through several collection periods, despite a decreasing blood pressure and a return of 17-hydroxycorticoid secretion to normal. Infusions of synthetic angiotensin II at several different rates (0.08 to 1.7 μg per minute) stimulated aldosterone secretion within 20 minutes. The smaller doses increased chiefly aldosterone secretion, while larger doses increased 17-hydroxycorticoid secretion as well. In contrast, 2 to 10 mU of ACTH stimulated 17-hydroxycorticoid secretion to equal or greater levels than the larger angiotensin doses, "renin" or crude kidney extracts, but did not stimulate aldosterone secretion. It is concluded that the kidney via the renin-angiotensin system is the mechanism by which hemorrhage stimulates aldosterone secretion in the hypophysectomized dog.

The Adrenal Glands and Calcium Metabolism. W. P. LAIRD MYERS AND WALTER LAWRENCE, JR., New York, N. Y. (introduced by Rulon W. Rawson).

In studies of antitumor agents in patients with hypercalcemia secondary to cancer, it was noted that cortisone was often effective in restoring normocalcemia. Although tumor regression seemed a reasonable explanation, patients frequently failed to show other indications of this. Therefore, the possibility of a nonspecific effect

on calcium metabolism was considered. Balance studies in 3 such hypercalcemic patients revealed no evidence that excessive absorption of calcium was responsible for the hypercalcemia. Cortisone in 1 of these patients led to an expected increase of urinary and fecal calcium without alteration of the serum calcium suggesting that the effect of cortisone on the serum calcium is independent of its effect on calcium balance. Cortisone was then found to produce reversible hypocalcemia when given to a patient with hypoparathyroidism maintained on parathyroid extract and, when given in high doses to 1 of 2 patients with functioning parathyroid carcinomas, partial reversal of hypercalcemia occurred. These data suggest a possible antagonism between cortisone and parathyroid hormone. During adrenal insufficiency in adrenalectomized dogs, hypercalcemia occurred in 12 of 19 dogs and 8 survived to show restoration of normocalcemia when cortisone was again administered. Parathyroidectomy failed to abolish this hypercalcemia of adrenal insufficiency in 7 studies in 4 dogs. Thus although an antagonism between cortisone and parathyroid hormone may exist, the hypercalcemia of adrenal insufficiency does not appear to depend upon intact parathyroids. Preliminary data in 2 dogs revealed no significant changes in serum citric acid and ultrafilterable calcium during adrenal insufficiency and hypercalcemia did not appear to be mediated through changes in serum electrolytes or plasma volume. The data thus far indicate that the adrenal glands exert a dual effect on calcium metabolism, namely, on calcium balance and on the equilibrium existing between skeletal and extracellular calcium.

Respiratory Gas Tensions and Gastric Acid Secretion. A. NAITOVE AND S. M. TENNEY,* Hanover, N. H.

The high incidence of peptic ulceration reported to be associated with chronic pulmonary emphysema has stimulated interest in the possible role that *in vivo* levels of oxygen and carbon dioxide tensions may play in modifying gastric acid secretion. Most recently, reports on emphysematous patients would seem to indicate no significant correlation between the absolute levels of either basal or histalog-stimulated acid secretion and the degree of respiratory acidosis. On normal subjects we have found that postprandial gastric acid secretion was in general directly related to the alveolar carbon dioxide tension, but for any given carbon dioxide value, secretion was inversely related to the alveolar oxygen tension. However, fasting secretion in man was not affected by altered respiratory gas tensions, and in the unanesthetized dog 7.5 per cent CO_2 caused a decrease in the level of acid secretion in a Heidenhain pouch stimulated with histalog. Clearly, oxygen and carbon dioxide can significantly modify an existing state of acid secretion, but the nature of that alteration depends on the stimulus employed for the background secretion. The interacting effects of oxygen and carbon dioxide and the possible mechanisms through which their action is exerted will be discussed.

Heme and Globin Synthesis in Maturing Human Erythroid Cells. DAVID G. NATHAN AND FRANK H. GARDNER,* Boston, Mass.

The synthesis of heme and globin during maturation of human erythroid cells has been assessed by measurement of the daily incremental specific activity of glycine in peripheral blood hemin and globin following a single intravenous injection of glycine-2-C^{14}. These studies together with measurements of the rate of incorporation of Fe^{59} into peripheral red blood cells and hemin have been done both in adult males, patients with pernicious anemia, and a patient who received vitamin B_{12} simultaneously with the precursor radioisotopes. The results indicate that in the normal individual globin is maximally synthesized early in maturation. A linear decline in its synthetic rate occurs as the erythroid cell matures. Heme appears to be maximally synthesized midway in maturation with lower synthetic rates both early and late in erythrocyte development. In the megaloblastic maturation of pernicious anemia, heme and globin synthetic rates are closely linked, but the maturation time for many of the erythroid cells is distinctly prolonged. Vitamin B_{12} shortens the maturation time to normal and may increase the rate of globin synthesis.

Persistent Myocardial Hyperemia and Increased Oxygen Consumption During Recovery from Exercise in Man. W. A. NEILL, R. J. WAGMAN, J. V. MESSER, H. J. LEVINE, N. KRASNOW AND R. GORLIN,* Boston, Mass.

Work performed in excess of O_2 consumption (qO_2) by exercising skeletal muscle results in increased O_2 uptake during recovery. Left ventricular (LV) work also may rise more than qO_2 during effort. LV recovery metabolism, however, has not been studied. LV coronary flow (CF), qO_2, work and tension time index (TTI) were determined in 28 patients at rest, during 6 to 8 minute exercise and during a 4 minute recovery period starting either at the end of exercise or 1.5 minutes later. Exercise work and TTI increased more than CF and qO_2, and efficiency rose significantly in 50 per cent of patients. Although LV hemodynamics had returned to near rest levels, recovery CF remained significantly elevated in 71 per cent. The recovery CF level appears to be related to the degree of exercise LV stress. In 10 patients, recovery CF was much greater than at rest (+50 to +200 per cent), without high concurrent recovery LV hemodynamics. In 8 of the 10, exercise CF was comparably increased (+41 to +136 per cent); all of these had abnormally elevated LV work due to heart disease. In the remaining 2, exercise TTI was excessively increased but CF did not rise until recovery, with corresponding reciprocal changes in exercise-recovery efficiency. In 10 with smaller increases in recovery CF (average +30 per cent) exercise CF rose comparably (average +31 per cent). Recovery CF was near resting in the 8 remaining patients. Exercise CF and LV stress caused by disease or exercise were lower than in the others. Coronary O_2 extraction was constant throughout in 20 patients. In all 8 with increased extraction during

exercise, extraction remained high through recovery, suggesting persistent O_2 needs out of proportion to CF. Average efficiency was higher during exercise (+28 per cent) and lower during recovery (−16 per cent). At least part of the apparent increase in exercise efficiency may represent utilization of anaerobic or stored energy, requiring O_2 for postexercise restoration. No excess lactate production during exercise has been demonstrated.

Effect of Plasma Iodide Concentration on the Accumulation of Stable Iodide by the Human Thyroid. WIL B. NELP, HENRY N. WAGNER, JR. AND JAMES H. DOWLING, Baltimore and Bethesda, Md. (introduced by Palmer H. Futcher).

Conventional methods of measuring radioiodine uptake do not permit one to measure the rate of accumulation of stable iodide by the thyroid gland. A technique of neutron activation analysis was developed to measure quantitatively the iodine content of biological fluids. With this technique, it was possible to measure thyroidal uptake of iodide in micrograms per unit time, and to relate it to plasma inorganic iodide concentration. In 34 euthyroid subjects, the uptake of stable iodide was 6.2 μg per 2 hours (range, 1.4 to 20 μg per 2 hours). Plasma concentrations ranged from 1 to 10 μg per L. Iodide uptake was greatest when plasma concentrations were highest. To study the effect of increased plasma iodide concentrations, euthyroid subjects were given increasing amounts of oral iodide for periods as long as 47 days. The thyroid accumulated iodide at an increasing rate until the plasma iodide level reached 75 μg per L. At greater plasma concentrations, the iodide accumulation fell, but remained several times higher than normal. In euthyroid subjects subsequently given increasing amounts of dietary iodide, the radioiodine uptake (per cent dose per 2 hours) was initially 7.2 per cent when corrected for neck extrathyroidal radioactivity by the perchlorate block method. Administration of iodide sufficient to increase plasma concentrations from 5 to 50 μg per L decreased radioiodine uptake 0.1 per cent per μg per L increase in plasma concentration. At serum concentrations greater than 100 μg per L, the per cent dose uptake decreased at a slower rate. Despite the decrease in radioiodine uptake, the thyroid continued to accumulate stable iodine at a rate six times the normal value, even after oral administration of iodine for 1 month.

Levels of Plasma ACTH in Surgical and other Acutely Ill Patients. DON H. NELSON,* COLLIN E. COOPER, ERNEST M. GOLD AND E. RAY RUTHERFORD, Los Angeles, Calif.

Plasma ACTH has been measured in patients during periods of acute medical and surgical illness using the method of Lipscomb and Nelson for assay of ACTH in plasma. These were compared with simultaneous measurements of plasma 17-hydroxycorticosteroids. Levels of ACTH preoperatively have varied from nondetectable to 0.7 mu per 100 ml of plasma. One-half hour following a surgical procedure the levels have generally been between 1 and 5 mU per 100 ml. Levels at 4 hours postoperatively are usually approaching and at 24 hours have reached preoperative values. Patients who have a number of acute medical diseases have also been demonstrated to have elevated plasma ACTH. These include patients with hypoglycemia, diabetic coma, cerebral vascular thrombosis, and acute myocardial infarction. Although the very high plasma corticosteroids in terminal patients have generally been attributed to decreased metabolism of the steroid, it is of interest that such patients may also have elevated levels of plasma ACTH.

Intracellular Buffer Systems in Muscle and Brain. GEORGE NICHOLS, JR.,* Boston, Mass.

The existence of buffer systems outside the extracellular space is well known. However, little information is available concerning buffer capacities of individual tissues and the relative importance of different buffers in maintaining pH in different cells, although it seems likely from differences in function and composition that these might vary widely. To examine these possibilities, titrations of homogenate of muscle and brain freshly prepared in isotonic KCl have been performed in a closed system under a stream of nitrogen at 35° C. After measuring initial pH, aliquots of HCl were added, recording pH after each addition, until a pH of 4 or less was reached when it could be presumed that all the HCO_3^- and $CO_3^=$ initially present had been converted to CO_2 and removed by diffusion. The homogenate was then titrated back with NaOH to about pH 9. By comparing the slope of the titration curve, using acid, with the subsequent back titration, using alkali, the relative importance of the "CO_2" and the sum of the other buffer systems in each tissue could be estimated. Comparing the acid titration curves at pH 7, the total buffer capacity of brain (0.140 mEq per pH per g dry weight) was found to be slightly greater than that of muscle (0.135). However, on back titration the two values were quite different—brain buffer capacity was now only 0.081 while muscle was 0.130. It appears therefore that the CO_2 system is an important buffer in brain accounting for 42 per cent of the total buffer capacity, while in muscle this system supplies only 4 per cent and other buffers such as proteins are the important ones. The implications of these findings in terms of cell function, tissue buffering during acid-base disturbances, and the susceptibility of different organs to acidosis and alkalosis of different types will be discussed.

Micropuncture Studies of Mercurial Effect on Na Fluxes in Proximal Tubule of Necturus. DONALD E. OKEN, WILLIAM J. FLANIGAN AND RAJA N. KHURI, Boston, Mass. (introduced by C. Sidney Burwell).

Stopped flow microperfusion experiments were performed on adult Necturi anesthetized with tricaine methanesulfonate and weighing approximately 200 g. The animals received 2 mg inorganic mercury equivalent subcutaneously 24 hours prior to micropuncture. The perfusing fluid used was isosmotic with Necturus plasma,

containing 100 mmoles NaCl, 2.2 mmoles glucose, and tracer amounts of $Na^{24}Cl$ and C^{14}-inulin. Sodium efflux (tubule fluid to plasma) in 9 control experiments on 5 animals was 249 ± 27 pmoles per cm^2 second, influx 167 ± 29 pmoles per cm^2 second, and net flux 82 ± 9 pmoles per cm^2 second, values comparable with those reported previously. In 18 experiments on 11 animals receiving mercaptomerin, sodium efflux was 168 ± 11 pmoles per cm^2 second, influx 113 ± 9 pmoles per cm^2 second, and net flux 60 ± 8 pmoles per cm^2 second. Statistical analysis of the data revealed that both unidirectional fluxes and net fluxes were reduced significantly ($p < 0.01$ for influx and net flux, $p < 0.05$ for efflux). Since sodium influx into the proximal tubule is a passive process, diminution of sodium influx suggests, in the absence of a reduced transtubular electrochemical potential gradient, a decreased permeability of the tubule to sodium. Although intratubular electrical negativity is reduced after mercurials (Giebisch), this alone cannot account for the decreased sodium influx since efflux is similarly decreased. The decrease in net sodium flux observed in these experiments also is compatible with decreased tubular permeability to sodium without necessarily invoking change in the mechanisms for active sodium transport. Aldosterone, substituted for mercury at a dose of 10 μg intraperitoneally 4 to 8 hours prior to 15 similar experiments on 7 animals, failed to produce any significant change in efflux, influx, or net flux of sodium in the proximal tubule of Necturus kidney.

Metabolism of Pyruvate-2-C^{14} in Normal and Diabetic Humans. ROBERT E. OLSON,* I. ROSABELLE MCMANUS AND PAUL SWEENEY, Pittsburgh, Pa.

It has been postulated by some workers that a defect exists in the metabolism of acetyl-CoA via the Krebs tricarboxylic acid cycle in diabetes mellitus. In order to test this hypothesis, trace doses of pyruvate-2-C^{14} (as the sodium salt) were given intravenously to 4 fasting normal subjects and to 4 ketogenic diabetics withdrawn from insulin for 24 hours. The time course of change in total and specific radioactivities of blood pyruvate, α-ketoglutarate, lactate, glucose and respiratory CO_2 were followed for 12 hours. Pyruvate and α-ketoglutarate were isolated as their 2,4-dinitrophenylhydrazones and purified by silicic acid chromatography. The C^{14} in blood glucose was measured as CO_2 after periodate oxidation of potassium gluconate followed by Hg^{++} oxidation of carbons 2 to 5. After permanganate oxidation of the blood lactate, C^{14} in C-1 was measured as CO_2 and C^{14} in carbons 2 to 3 as acetaldehyde dimedon. The specific activity of the respiratory CO_2 reached maximal specific activity in 20 to 30 minutes in both normals and diabetics. In normals the recovery of $C^{14}O_2$ over 12 hours was 43 to 58 per cent of the administered dose, while in diabetics it was 26 to 46 per cent. The biological half-life of pyruvate in both groups was 1.2 ± 0.4 minutes. Lactate and α-ketoglutarate reached maximal specific activity equally rapidly in both groups and declined to low levels over a 2 hour period. The total C^{14} in the blood glucose was significantly elevated in the diabetic group. These findings are consistent with a diversion of pyruvate toward gluconeogenesis in the diabetic without a significant alteration in its metabolism via the tricarboxylic acid cycle.

Antidiuretic Hormone-like Effects of Adenosine-3',5'-phosphate (Cyclic AMP) and Theophylline in the Toad Bladder. JACK ORLOFF * AND JOSEPH HANDLER, Bethesda, Md.

The response of toad bladder (*Bufo marinus*) to antidiuretic hormone (ADH) is characterized by an increase in the permeability of the membrane to water, a rise in membrane potential (P.D.) and an increase in sodium transport (short-circuit current). The effects are considered specific for neurohypophyseal principles. ADH also promotes the release of cortisol from the adrenal and glucose from liver, effects analogous to those of ACTH and epinephrine, respectively. Since the latter hormones increase cyclic AMP formation and phosphorylase activity in the specific tissues sensitive to their action, it was considered that the ADH effect in toad bladder might also involve the intermediacy of cyclic AMP. In consonance with this view, addition of 10^{-3} M cyclic AMP, but not 5' AMP, to the medium bathing the serosal surface of the toad bladder sac increases the osmotic flow of water across the membrane. In addition, P.D. and short-circuit current also rise. The effects are reversible and qualitatively indistinguishable from those produced by vasopressin (Pitressin). Theophylline (10^{-2} M), which inhibits cyclic AMP degradation in other tissues, also mimics the response to vasopressin in toad bladder. The effect of theophylline on water movement is additive to that of either a submaximal dose of vasopressin or cyclic AMP. N-ethylmaleimide prevents the changes in water movement produced by vasopressin, cyclic AMP and theophylline. Acidification of the bathing medium (pH 6.5) which inhibits the vasopressin effect, does not eliminate the response to cyclic AMP. In view of the similarity of action of vasopressin and the nonhormonal preparations studied, it is suggested that ADH may induce its effect on membrane permeability in both toad bladder and renal tubule by stimulating the formation and accumulation of cyclic AMP.

The Kinetics of Bilirubin Formation In Vivo. J. DONALD OSTROW, JAMES H. JANDL* AND RUDI SCHMID,* Boston, Mass.

The rate and efficiency with which hemoglobin is converted to bilirubin was studied in rats, with both hemoglobin solutions and intact erythrocytes. Small amounts of antibody-coated rat erythrocytes or free hemoglobin, labeled with C^{14} and Fe^{59}, were injected intravenously into bile fistula rats. The rate of clearance of Fe^{59} from the circulation was compared with the rate of excretion of C^{14} in bilirubin isolated in crystalline form from serial bile samples. Although biliary excretion of bilirubin-C^{14} began within 30 minutes following the injection of either

labeled erythrocytes or hemoglobin, there was a delay of 3 hours between the half-disappearance of labeled erythrocytes or hemoglobin from the circulation and half the excretion of bilirubin-C^{14}. Injection of either labeled erythrocytes or hemoglobin resulted in a temporary increase in biliary excretion of nonlabeled endogenous bilirubin. The efficiency of conversion of the injected heme-C^{14} to the excreted bilirubin-C^{14} ranged from 43 to 65 per cent. By contrast, over 90 per cent of bilirubin-C^{14} injected intravenously was recovered in the bile. These studies demonstrate that, *in vivo,* degradation of heme to bilirubin requires on the average 3 hours, although some conversion takes place within 30 minutes of sequestration of heme pigment from the circulation. The rate of conversion is similar, whether this occurs predominantly in the liver, as with free hemoglobin, or in the spleen, as with sensitized erythrocytes. In either instance, from 35 to 57 per cent of the heme moiety of hemoglobin is apparently degraded to metabolites other than bilirubin.

Bile Acid Fever and Inflammation in Man. ROBERT H. PALMER, PAUL B. GLICKMAN AND ATTALLAH KAPPAS, Chicago, Ill. (introduced by Richard L. Landau).

The pyrogenic action of neutral steroid metabolites of the 5β-H series in man has been reported previously. This study demonstrates extension of pyrogenicity to related C_{24} bile acid steroids and describes the structural and species specificity of this action. Nineteen free and conjugated bile acids were examined for thermogenic properties. Of 13 free compounds, only the 3α-monohydroxysteroid, lithocholic acid, had consistent and intense pyrogenic action in man. Hydroxylation at the 6α, 7α, 7β, and 12α positions, singly or in combination, or introduction of a ketone at carbon-7 or 7 and 12 suppressed the pyrogenicity of lithocholic acid. Derivatives of this steroid produced by C_3 acetylation, or C_{24} methylation, and the physiological glycine conjugate all had significant thermogenic and inflammatory activity. In contrast, taurolithocholic acid, another physiological conjugate, had little or no pyrogenic action but caused intense inflammation. Fever produced by lithocholic acid, like that produced by etiocholanolone and other neutral steroid pyrogens, was species specific for man. The results of this study indicate: 1) The endogenous biliary steroid, lithocholic acid, has significant inflammatory and pyrogenic action in man. Aberrations in the degradation of cholesterol to bile acids or enteric microbial dehydroxylation of these compounds may result in excessive production of this extremely active steroid pyrogen which may, by inference, then participate in febrile and inflammatory clinical disorders. 2) The potent pharmacological properties of chemical and physiological esters of lithocholic acid demonstrate that *in vivo* conjugation processes do not necessarily terminate the biological activity of steroids. 3) Production of intense inflammation without significant fever by taurine-conjugated lithocholic acid strongly suggests that nonspecific inflammation per se does not explain the mechanism of steroid fever in man.

The Mechanism of Hormonal Regulation of Glucose Oxidation in the Thyroid. IRA PASTAN AND JAMES B. FIELD, Bethesda, Md. (introduced by J. E. Rall).

Thyroid-stimulating hormone (TSH) at a concentration of 50 μU per ml stimulates oxidation of glucose-1-C^{14} to $C^{14}O_2$ in thyroid slices. Stimulation is observed 5 minutes after addition. These observations suggest TSH acts directly on glucose oxidation in thyroid. Since the yield of CO_2 from the first carbon of glucose is greater than that from the sixth carbon, TSH appears to stimulate preferentially the hexose monophosphate pathway (HMP). During stimulation with TSH *in vitro,* there is a progressive rise in thyroidal TPN and an equivalent fall in DPN. Since TPNH and DPNH levels are unaffected, and since TPN is rate-limiting for glucose oxidation via the HMP, it is postulated that the mechanism of action of TSH is by stimulation of the conversion of DPN to TPN, a reaction carried out by DPN kinase. Pyridine nucleotide levels were measured fluorometrically and also by a new assay for TPN. In this assay which utilizes 6-phosphogluconate-1-C^{14} and 6-phosphogluconic dehydrogenase, the $C^{14}O_2$ produced is proportional to TPN. Epinephrine, adrenochrome (produced during incubation of thyroid with epinephrine), monoiodotyrosine and diiodotyrosine all increase glucose-1-C^{14} oxidation by thyroid slices. Catalytic amounts of epinephrine and adrenochrome stimulate TPNH oxidation by thyroid homogenates. Monoiodotyrosine and diiodotyrosine are deiodinated in the thyroid by a TPNH-dependent enzyme. Therefore, since a major factor in the control of glucose oxidation in the thyroid is the level of TPN, substances which alter this level alter the pathway of glucose oxidation of the thyroid.

Acquired Resistance to Allergic Encephalomyelitis and the Role of a Serum Factor. PHILIP Y. PATERSON, S. MARTIN HARWIN AND NORMAN C. DIDAKOW, New York, N. Y. (introduced by Lewis Thomas).

Allergic encephalomyelitis (AE), induced in rats by injection of nervous tissue combined with Freund's adjuvant, is under study as a model for elucidating the cellular and humoral factors implicated in autoimmune disease. Although transfer of AE was accomplished in our hands by means of lymph node cells, unsuccessful attempts to transfer it using serum leaves the role of circulating antibrain antibodies still unclear. Our recent studies indicate that rats sensitized to spinal cord and adjuvant recover promptly from AE and later exhibit a striking resistance to the disease. For example, 31 rats were permitted to recover from the paralysis induced by a single injection of spinal cord-adjuvant and then challenged 4 to 6 weeks later. Following challenge with spinal cord-adjuvant, only 1 of the 31 rats became paralyzed. Histological evidence of AE was found in this animal and in 6 others; the remaining 24 rats had no demonstrable encephalomyelopathy. As expected, 14 of 18 control rats developed AE. Serum transfer experiments indicate that a circulating factor is impli-

cated in AE resistance. Fifteen recipients were sensitized to spinal cord-adjuvant and treated on alternate days with pooled serum from donors similarly sensitized 3 weeks previously. Only 1 of these 15 recipients became paralyzed. Many AE lesions were subsequently found in this recipient as well as in 3 others. Of the remaining 11 recipients, however, 6 showed only minimal AE lesions and 5 had no evidence of AE at all. In contrast, typical AE occurred in 12 of 14 control recipients sensitized to spinal cord-adjuvant but treated with pooled serum from normal rats or donors previously sensitized to kidney-adjuvant. It is conceivable that the circulating factor which protects against the development of AE may be antibrain antibody. Studies to settle this point are in progress.

Biochemical Detection and Histological Study of Muscular Dystrophy in the Preclinical Stage. CARL M. PEARSON, SUDHIR R. CHOWDHURY AND WILLIAM M. FOWLER, Los Angeles, Calif. (introduced by William N. Valentine).

Serum enzymes are known to be elevated during the active clinically progressive stages of muscular dystrophy. Moreover, several brief reports have indicated that levels are highest early in the clinical disease and taper toward normal as weakness becomes extreme. Enzyme elevations do not occur in secondary (neuropathic) muscular weakness; hence, when present, they are indicative of a primary myopathy, if other organ disease can be excluded. We have conducted a detailed clinical and serum enzyme study of several families in which dystrophy is known to exist and have found elevated enzyme levels (glutamic oxalacetic transaminase, glutamic pyruvic transaminase, aldolase, lactic dehydrogenase, pyruvic dehydrogenase) in several young boys who were at the time without demonstrable muscular weakness. It appears that comparably high levels are present in the preclinical and early clinical stages of the disease. The youngest child was 4 months old when first tested; his enzyme levels were all very high. Four of his brothers had developed clinical dystrophy at age 3 years and it is likely that he also will. In another family, early muscular weakness developed in 3 fourth-generation offspring of a dystrophic family, 3 years *after* finding elevated serum enzymes in these children. Muscle biopsy obtained from several persons in the preclinical stage, when compared with biopsies taken in other family members with successive degrees of weakness, has shown "early" muscle fiber alterations and connective tissue increase even in the earliest patient studied (aged 4 months). Furthermore, it is apparent that much histological change, including fiber swelling, necrosis and active regeneration as well as interstitial fibrosis, occurs before clinical weakness appears, at which time the usual muscle biopsy is obtained. It is probable that both repeated muscle fiber necrosis and increased sarcolemmal membrane permeability contribute to the continuous elevation of serum enzymes. The basic biochemical defect in this disease remains to be elucidated. It seems likely that further studies of this type will provide clues as to when the dystrophic process actually begins. This will probably be in the neonatal period in some cases.

Studies with Purified Bovine Parathormone: Effects in Normal, Hypoparathyroid and Pseudohypoparathyroid Subjects. MAURICE M. PECHET,* HOWARD RASMUSSEN, EVELYN L. CARROLL AND IRMA GRAMER, Boston, Mass.

Complete metabolic balance studies were carried out with Rasmussen purified parathormone (PTH). During the first day of administration of PTH, there was a phosphorus diuresis in all subjects, especially pronounced in the normal and hypoparathyroid subjects. The phosphaturic response continued in the normal and hypoparathyroid subjects, although at a reduced level, throughout the period of PTH administration, but in the pseudohypoparathyroid subjects, the phosphorus diuresis ceased after the second to fourth day. In the normal subject after the fourth day there was a further increase in phosphorus excretion. Upon withdrawal of PTH there was a prompt, marked decrease in phosphorus excretion. In the normal and hypoparathyroid subjects the changes in calcium excretion lagged behind those in phosphorus excretion, and in the pseudohypoparathyroid subjects the changes in calcium excretion were negligible. Serum phosphorus fell and serum calcium rose during PTH administration, the changes being most pronounced in the normal and hypoparathyroid subjects. On starting PTH administration there was a prompt potassium loss for the first day or two, and on stopping PTH there was a prompt potassium retention for the first 2 days. These changes occurred *pari passu* with changes in phosphorus excretion on starting and stopping the administration of PTH. The potassium and phosphorus "on and off" effects occurred in all instances and may indicate changes in intracellular fluid. Urinary pH rose during PTH administration and then dropped sharply on the first day following withdrawal of PTH. The data can be interpreted as follows: 1) PTH induces a slow bone response; 2) PTH induces a prompt renal tubular phosphaturic response, the phosphorus coming predominantly from the intracellular fluid. The pseudohypoparathyroid subject does show a renal phosphaturic response albeit a sluggish one.

Serum Enzyme Levels Following Acute Arterial Occlusion of Dog Limbs. RAYMOND PENNEYS, UGO MANZOLI, SAMUEL BELLET AND LEONARD FEINBERG, Philadelphia, Pa. (introduced by Hugh Montgomery).

These experiments were done to relate levels of the serum enzymes, glutamic oxalacetic transaminase (GOT), isocitric dehydrogenase (ICD), and lactic dehydrogenase (LDH), to ischemia of the animal limb, in the same manner that they have been related to ischemia of the myocardium. In the first group of experiments, embolism of a hind limb was produced in 16 dogs by the injection of

a lycopodium suspension into the femoral artery. This resulted in severe, ischemic damage to the limb, progressing to gangrene in 13 of the 16 animals. All three enzymes increased within 4 hours after embolization, much before the appearance of gangrene, and remained so during the 96 to 144 hour observation period. The greatest increase was in GOT, the average value of which rose from approximately 40 U before embolization to 1,000 U, at 24 to 48 hours after embolization; the next greatest increase was in ICD, which rose from 100 to 600 U, at 12 to 24 hours; and the least was in LDH, from 100 to 300 U, at 12 to 24 hours. Sham operations on 4 dogs produced no significant rise in enzyme levels, demonstrating that embolization, per se, was responsible for the increase in serum enzymes. A second set of experiments was done to study the effect of milder degrees of ischemia of the limb. The femoral artery was occluded, by clamping, for 1 hour and for 3 hours in two groups of 6 animals each; in neither group was there any evidence of serious damage to the limb. Levels of GOT and ICD remained essentially unchanged during the 24 hour observation period after occlusion. Levels of these serum enzymes, then, are related to the degree of ischemic damage of the limb. Such enzyme changes probably reflect the degree of damage of the deeper tissues of the limb, something not provided by the routine clinical examinations, and should therefore be of value in the management of patients having acute, severe peripheral arterial obstructions.

The Nature of the Heterogeneity of Normal Adult Human Myoglobin. GERALD T. PERKOFF,* ROBERT L. HILL AND FRANK H. TYLER,* Salt Lake City, Utah.

Myoglobin has been prepared from normal human muscle obtained at autopsy. Such preparations can be separated into three major and two minor components when chromatographed at 6° C on diethylaminoethylcellulose which has been equilibrated previously with 0.005 M Tris-(hydroxymethyl) aminomethane buffer, pH 7.85, and can be separated into three components by electrophoresis on starch gels at pH 8.6. Each major chromatographic fraction migrates electrophoretically like one of the components observed on starch gels. The percentage of each major fraction (F1, F2 and F3) in whole myoglobin varies among different preparations. Generally, F1, F2 and F3 account for 55 to 65, 15 to 20 and 15 to 30 per cent, respectively, of the total myoglobin. Spectral analysis indicates that F1 is acid metmyoglobin and that F2 and F3 are alkaline metmyoglobins. F1 and F2 have identical visible and ultraviolet spectra when converted to ferromyoglobin, oxymyoglobin and cyanmetmyoglobin. The electrophoretic and chromatographic mobilities of F1 and F2 are identical when these are converted to cyanmetmyoglobin. F1 and F2 show identical peptide patterns when tryptic digests are compared by two-dimensional electrophoresis-chromatography ("fingerprinting"). Analyses of whole myoglobin and F1 by the fluorodinitrobenzene and phenylisothiocyanate end-group techniques show glycine to be the single amino terminal residue. The electrophoretic mobility of F3 cyanmetmyoglobin differs from that of F1 and F2. However, the significance of this observation is uncertain because traces of nonheme proteins present in F3 have prevented definitive characterization of this fraction. It can be concluded that F1 and F2 are identical proteins but behave differently when isolated from muscle as the result of differences in the state of the heme prosthetic group. These results must be considered in the interpretation of studies of myoglobin from patients with muscle disease.

Serum Antinuclear Factors in Patients and Relatives: Observations in SLE and Liver Disease. VICTOR E. POLLACK, ENNO MANDEMA AND MYRON GARSENSTEIN, Chicago, Ill. (introduced by Robert M. Kark).

A semiquantitative technique was developed to study antinuclear factors (ANF) in sera of patients with systemic lupus erythematosus (SLE). Fresh, unfixed smears of human buccal mucosal cells were incubated for 90 minutes with serum undiluted and in serial dilutions. After thorough washing they were incubated for 30 minutes with antihuman globulin serum conjugated with fluorescein isothiocyanate and examined microscopically with ultraviolet light. The "titer" of ANF was the highest serum dilution with which specific nuclear fluorescence was demonstrated. Appropriate immunologic controls were done, and reproducibility was established. The demonstration of specific nuclear fluorescence with serum diluted 1:4 distinguished clearly between SLE patients and healthy subjects, and the demonstration of specific nuclear fluorescence with serum diluted $\geq 1:4$ was called a positive test. Negative tests were found in all 40 healthy subjects, and in 71 of 75 patients with diseases "unrelated to SLE." By contrast, 75 of 81 SLE patients had one or more positive tests; in all, positive tests were found with 225 of 248 sera from SLE patients and ANF titers up to 1:4,096 were recorded. ANF were found in sera of 18 of 82 patients with disorders "related to SLE"; e.g., scleroderma, rheumatoid arthritis. ANF were studied in sera of 125 relatives of 21 SLE patients; most were asymptomatic, but three new cases of SLE were found. Positive tests were found, many in high titer, in 44 of 85 first degree and in 9 of 40 second degree relatives, indicating that in SLE a tendency to develop antinuclear factors is inherited. Preliminary control studies on families of healthy subjects were negative. ANF were also demonstrated in one acute and 4 chronic cases of hepatic disease with hypergammaglobulinemia. To date two families have been studied; ANF were demonstrated in 6 of 9 relatives, suggesting a relationship between SLE and "lupoid hepatitis."

Presence of an Erythropoietic Labile Iron Pool. MYRON POLLYCOVE, Berkeley, Calif. (introduced by John H. Lawrence).

The concept of an erythropoietic labile iron pool is central to models previously developed and employed to

calculate hemoglobin synthesis, mean erythron life span, storage iron deposition and miscible storage iron in over 480 human subjects. This pool is much more labile than miscible storage iron which corresponds in amount to ferritin and is calculated to be 85 mg in normal subjects (heme iron in immature erythrons, approximately 30 mg). To investigate the presence of a corresponding morphologic or biochemical entity, human and canine marrow and circulating immature erythrons were analyzed directly. Within 2 hours after intravenous injection of tracer amounts of transferrin-bound radioiron, Fe^{59} was concentrated chiefly in marrow cells. Doubly crystallized heme was prepared from marrow and circulating immature erythrons. Soon after injection, radioiron occurs chiefly in nonheme form which decreases exponentially as heme radioiron increases. Approximately half of the radioiron was present in nonheme form at 36 hours in man and 7 hours in dog, in close agreement with rates calculated from simultaneous iron kinetic studies. Erythron lysis and isolation of membranes demonstrated that nonheme radioiron was on the membrane, while heme radioiron was present almost entirely in the interior. Fe^{59}-labeled reticulocytes were obtained from patients 30 to 60 minutes after intravenous injection of Fe^{59}. After incubation for 1 hour in plasma, 12 to 18 per cent of the reticulocyte radioiron transferred to plasma transferrin in proportion to plasma iron-binding capacity; less than 2 per cent transferred to the control ACD solution. In conclusion: 1) A labile iron pool is present in immature erythrons. 2) This pool is predominantly on the cell membrane, derives iron from plasma transferrin, transfers iron back to plasma transferrin, and furnishes iron to the interior for heme synthesis. 3) Iron in excess of heme iron is removed by feedback to plasma.

Renal Effects and Site of Action of Organomercurials in Hydropenic Man. JEROME G. PORUSH, MARVIN H. GOLDSTEIN, GILBERT M. EISNER AND MARVIN F. LEVITT,* New York, N. Y.

Previous studies in this laboratory demonstrating that free water clearance remained constant during a mercurial diuresis in hydrated man suggested that the major site of organomercurial action is distal to the water-clearing segment. To test this hypothesis in hydropenic man, the characteristics of the urine (V, C_{osm}, and $T^c_{H_2O}$) were studied during comparable diureses produced by nonspecific solute diuretics (aminophylline, hypertonic salt and mannitol) and organomercurials (meralluride, mersalyl and mercaptomerin). With the nonspecific agents, as C_{osm} increased from 1.5 to 15 ml per minute, a progressive rise in $T^c_{H_2O}$ was observed to peak values of 6.6, 6.9, and 5.4 ml per minute for aminophylline, salt, and mannitol, respectively. During comparable organomercurial diureses, $T^c_{H_2O}$ remained constant at those levels which prevailed prior to the onset of the saluresis, despite a wide range in control $T^c_{H_2O}$ values (1.0 to 7.0 ml per minute). In 10 of 12 experiments in which a nonspecific agent was superimposed during a mercurial diuresis, $T^c_{H_2O}$ increased an average of 2 ml per minute. In other studies, blood pressure was abruptly reduced during comparable diureses produced by organomercurials or hypertonic salt infusions. The subsequent fall in glomerular filtration rate was associated with a comparable fall in C_{osm} in both groups, but only those subjects receiving salt displayed an appreciable increase in urine osmolality. Urine osmolality was virtually unchanged in the mercurialized subjects during the hypotension. These findings suggest a disparity in solute transport by the loop of Henle, at every level of solute excretion, between a mercurial and a nonspecific solute diuresis. The capacity to maintain or increase $T^c_{H_2O}$ during a mercurial diuresis argues against a block at this site. The relatively diminished rate of loop transport in the mercurial experiments therefore supports the hypothesis that the major effect of organomercurials occurs distal to the loop of Henle.

Dependence of Bile Formation on Bile Salt Secretion in the Dog. RUDOLF PREISIG, HENRY O. WHEELER * AND HERBERT L. COOPER, New York, N. Y.

Bile flow and electrolyte excretion were measured during rates of bile salt secretion ranging from 6 to 125 μmoles per minute (maintained by intravenous infusion of 1.3 per cent sodium taurocholate) in 40 studies on 4 cholecystectomized, unanesthetized dogs (20 to 25 kg) with Thomas duodenal cannulae. Bile flow (0.04 to 1.02 ml per minute)—during cholinergic blockade with intravenous pipenzolate methylbromide, 0.4 mg per minute—was directly proportional to taurocholate secretion rate in each dog. The proportionality factors were 0.007, 0.007, 0.009 and 0.010 ml bile per μmole taurocholate secreted. Excretion rates of chloride and bicarbonate were also approximately proportional to taurocholate secretion rate over the entire range in two dogs and at secretion rates above 20 μmoles per minute in the other two. Proportionality factors were 0.37, 0.33, 0.58 and 0.62 μEq chloride and 0.15, 0.11, 0.27 and 0.20 μEq bicarbonate per μmole taurocholate secreted. During intravenous secretin (2.5 U per minute) sufficient to produce maximal choleretic response, bile output was augmented in each dog by an approximately constant amount (0.28 0.29, 0.22 and 0.33 ml per minute) regardless of taurocholate secretion rate. Secretin-induced increments in chloride (28, 31, 29 and 47 μEq per minute) and bicarbonate (24, 33, 18 and 26 μEq per minute) were also approximately constant and independent of taurocholate secretion. Pipenzolate methylbromide did not depress the choleretic response to intravenous secretin, or to endogenous secretin elaborated during intraduodenal 0.05 N HCl administration. Thus the excretion of biliary water, chloride and bicarbonate apparently depends upon secretion of bile salts. This phenomenon is consistent with the view that water and inorganic electrolytes enter the bile along electrochemical gradients created by active bile salt secretion. Secretin-induced choleresis appears to involve an additional and altogether independent mechanism.