Persistence of Antibiotics in Blood of Patients with Acute Tubular Necrosis and Uremia. CALVIN M. KUNIN and SEARLE REES, Boston, Mass. (Introduced by Maxwell Finland).

The "half-life" of antibiotics in serum was determined after single intravenous infusions, or after cessation of previous antibiotic therapy by assaying the serum concentrations of the antibiotics at intervals. Values were determined in normal subjects and in patients with acute tubular necrosis during oliguric or diuretic phases. The antibiotics studied were penicillin, streptomycin, tetracycline and erythromycin.

The half-life of penicillin G (crystalline sodium salt) after intravenous administration averaged 30 minutes in two normal subjects; it was 7 to 10 hours in two oliguric individuals with a urinary output less than 400 ml. per day. During the diuretic phase, the half-life in three patients ranged from 3.5 to 6.5 hours.

The half-life of tetracycline hydrochloride in four oliguric patients ranged from 60 to 120 hours. In one patient, the value was 60 hours after diuresis began and later, as diuresis progressed, it was 14 hours. In normal controls the values were 7 to 8 hours.

Streptomycin was given intramuscularly in two oliguric patients. In one of them the concentration in the serum remained unchanged over a period of 3 days and in the other the level fell to one-half its maximum in 19 days.

The half-life of erythromycin in one oliguric patient was found to be about 5 hours when the urine output was less than 400 ml. per day. Normal values were 1.5 and 1.1 hours.

These data are considered to be of importance in providing a rational approach to antibiotic therapy during acute tubular necrosis and uremia and in further defining the role of the kidney in their excretion. Further studies are underway on the persistence of these and other antibiotics in the blood of patients with chronic renal disease, during hemodialysis with the artificial kidney and on the *in vitro* decay of these drugs.

Dietary Fats and Blood Lipids. PETER T. KUO and JOHN C. CARSON, Philadelphia, Pa. (Introduced by Calvin F. Kay).

Similar dietary programs have yielded divergent effects upon plasma lipids as reported by different investigators. That some apparent discrepancies are based upon factors other than quantitative or qualitative changes in dietary fat is indicated by this investigation. In 18 patients receiving a 20 Gm. fat per day diet continuously for 10 to 54 months, the mean cholesterol, phospholipid, and triglyceride fell from 356, 320, and 302 mg. per cent, respectively, to 266, 256, and 146 mg. per cent, respectively. Ten of these patients then received a supplement of 27 to 54 Gm. per day of soya-bean oil for 6 to 18 weeks. This did not significantly alter the lipid levels. Four patients were placed upon a 3 to 12 week program of isocaloric regular, "fat-free," and 45 to 70 per cent corn oil formula diets in various sequences. Lipids were regularly and comparably lowered by either a "fat-free" or corn oil program when preceded by a regular diet. However, a "fat-free" program preceded by a corn oil program resulted in a rise in lipid levels in two of the patients. In one patient on a "fat-free" diet preceded by a regular diet, fasting triglyceride was stable and low for nine weeks, then abruptly doubled. Lipid determinations every two hours on this patient showed two peaks, one at 8 a.m., when fasting levels are usually determined, and a 2 a.m. trough. When a corn oil program was substituted, a single plateau was sustained throughout the afternoon and evening, followed by a decline that reached bottom at 8 a.m. This gives the impression, from observation of fasting levels alone, that on corn oil feedings triglyceride levels are depressed. These studies indicate that fasting lipid levels may be a misleading index of dietary effects and that responses may be profoundly influenced by the nutritional program of the preceding control period.

Studies on the Genetics of Leukocyte Antigens. PARVIZ LALEZARI and THEODORE H. SPAET,* New York, N. Y.

Leukoagglutinins were studied by the technique of Dausset. Sera positive for leukoagglutinins were obtained from 30 patients who had received from 2 to 700 transfusions. These sera were specific for leukocytes in that no mixed agglutination with erythrocytes occurred, and no erythrocyte antibodies were demonstrable. No false positive leukoagglutination was seen in a large number of normal sera; and spontaneous leukoagglutination was not encountered. Sera were tested only with leukocytes from blood with compatible red cells.

The abnormal sera were tested with leukocytes obtained from six pairs of identical twins. In each case twin leukocytes showed identical agglutination response patterns, indicating similar antigenic composition. The patterns differed among the different pairs of twins.

Three of the abnormal sera were tested against the leukocytes of the patients' own family members. Two of these sera gave negative results with a high percentage of the leukocytes from relatives, although most leukocytes from nonrelated subjects were agglutinated. The third serum agglutinated leukocytes from all family members tested.

The abnormal sera were tested against leukocytes from a large panel of normal and unrelated subjects. None of the sera was positive for the entire panel of leukocytes; and none of the leukocyte suspensions was agglutinated by all of the abnormal sera. All subjects had a different pattern of response to the various sera; and no two sera behaved identically to the panel of leukocytes.

The data indicate that there is a multiplicity of leukocyte antigens, and that these are probably inherited according to Mendelian laws.

Seven Year Follow-up of One Type of Prolonged Intermittent Steroid Therapy in Nephrosis. KURT LANGE, EDWARD WASSERMAN, LAWRENCE B. SLOBODY, and EUGENE J. WENK, New York, N. Y. (Introduced by A. F. Coburn).

Forty-six cases, 35 children and 11 adults, with the nephrotic syndrome ("pure" nephrosis and the nephrotic stage of glomerulonephritis) were treated with the following regimen. There was an initial course of adrenocorticotrophic hormone (ACTH), 100 to 200 units per day, given for 10 to 21 days and after complete diuresis had occurred, maintenance therapy consisting of 300 to 400 mg. of cortisone per day for three successive days out of each week. This maintenance therapy was carried out for 12 to 18 months. The average time of observation for this group after onset of maintenance therapy was 43.8 months. Every case coming under observation since 1951 was treated with this regimen. Twenty additional cases (12 children and 8 adults) are still under therapy or are not under observation for a minimum of 20 months as yet. They were not included in the statistics. None of the latter group, however, died, and only one is edematous at present.

According to the life table statistical method, the mortality expected in the group on maintenance therapy (on the basis of the mortality occurring in a similar group not treated with steroids or treated with steroids only when edematous) would have been 12.8 deaths during the period of observation. There has been one death in our group on maintenance therapy. Tested by the chi-square method, the possibility of this distribution occurring by chance was found to be less than one in a thousand ($\chi^2 = 10.87$, $p < 0.001$).

Of the group of 25 patients in whom this type of therapy was started within the first six months of their disease, 20 are at present free of proteinuria and 5 have a 1 plus proteinuria. Of the group of 20 patients in whom this therapy was started more than six months after onset of the disease, only 5 have no proteinuria, while 14 have a 1 plus proteinuria and one has a 3 plus proteinuria and he is moderately uremic.

Thus *early* treatment with ACTH to induce complete diuresis followed by maintenance therapy with steroids seems indicated.

In four cases resistant to repeated courses of intramuscular ACTH, complete diuresis was achieved by the intravenous infusion of 30 to 40 units of ACTH, over an eight hour period each day for 10 to 12 days.

Uric Acid Riboside Phosphorylase in Human Tissues—Inhibition by Colchicine, and Other Properties. LEONARD LASTER and ALBERTA BLAIR, Bethesda, Md. (Introduced by J. E. Rall).

Uric acid (UA), although generally regarded as only an end-product of purine metabolism in man, does, however, appear to be subject to active transport and catabolism in human tissues. Since uric acid riboside (UAR) has been detected in human erythrocytes, and many purine interconversions involve their riboside or riboside forms, a study of the metabolism of UAR in mammalian tissues was undertaken.

Accordingly, an enzyme was purified one hundredfold from dog intestine that catalyzes the phosphorolysis of UAR as follows:

$$\text{UAR} + \text{Inorganic phosphate} \rightleftharpoons \text{UA} + \text{Ribose-1-phosphate} \quad (1)$$

It (URA-P'ase) occurs in human spleen, liver, kidney, stomach and ileum, as well as in organs of other mammals. Human spleen UAR-P'ase was purified five- to tenfold and its properties were studied. Dog, and quite probably human, UAR-P'ase appear to be distinct from nucleoside phosphorylase (assayed with inosine), an enzyme that catalyzes the phosphorolysis of other purine ribosides. Colchicine is an inhibitor of UAR-P'ase but does not inhibit nucleoside phosphorylase activity under similar experimental conditions.

In equal concentrations, colchicine, phenylbutazone and a phenylsulfoxy derivative of phenylbutazone, G-28315 (1,2-diphenyl-3,5-dioxo-4-(β-phenylsulfoxy ethyl)-pyrazolidine), inhibited dog UAR-P'ase 45, 30, and 6 per cent, respectively, a relationship that correlates interestingly with their therapeutic effectiveness in acute gouty arthritis. Similar concentrations of probenecid and pyrazine monocarboxylamide, compounds affecting primarily renal disposition of urate, did not inhibit. Thiouracil and propylthiouracil were more potent inhibitors of human UAR-P'ase than was colchicine.

The findings in this study raise the question of whether previously unrecognized pathways of metabolism related to UA, such as reaction (*1*), are involved in the hereditary defect of purine metabolism in gout and in the pathogenesis and therapy of acute gouty arthritis. Clinical extension of these investigations would seem profitable for insight into human disorders of purine metabolism.

Reappraisal of Renal Hemoglobin Excretion: The Differential Transport of Free and Protein-Bound Hemoglobin. WILLOUGHBY LATHEM,* Pittsburgh, Pa.

The assumption that hemoglobin circulating in plasma exists altogether in a free, unbound state and freely traverses the glomerular membrane has provided the conceptual basis for studies of renal hemoglobin transport. The demonstration of hemoglobin binding by plasma protein(s) and the circulation of both free (unbound) and protein-bound hemoglobin have rendered this assumption invalid. In the present study the characteristics of hemoglobin excretion were reexamined, employing methods which permitted differentiation between the renal transport of free and protein-bound hemoglobin.

Hemoglobin was administered intravenously to normal subjects in multiple injections, raising progressively (three hour period) the plasma hemoglobin concentration to 200 to 300 mg. per cent. Urine and plasma were obtained for hemoglobin analysis following each injection, allowing 30 minutes for equilibration. The concentrations of free and protein-bound hemoglobin (PBH) were determined by paper electrophoresis, measuring the light absorption of the paper following staining with benzidine-hydrogen peroxide. Hemoglobinuria did not occur until the hemoglobin-binding capacity of plasma protein(s) was exceeded (124 ± 22 mg. per cent) and free hemoglobin

appeared in plasma. From the relationship between the changing plasma concentration and urinary excretory rate of free hemoglobin ($\Delta UV/\Delta P$), the glomerular clearance (C^G_{Hb}) of free hemoglobin was calculated and averaged 5.0 ± 1.1 (range, 3.9 to 6.8) ml. per minute. The tubular reabsorptive rate (T_{mHb}) of free hemoglobin was calculated as G^G_{Hb} times P_{Hb} U_{Hb} V and averaged 1.4 ± 1.0 (range, 0.0 to 2.8) mg. per minute. Methemalbumin, when present in plasma, was not excreted.

This study reveals: *a*) Hemoglobinuria does not occur until the binding capacity of plasma protein(s) is exceeded; *b*) The "renal threshold" is determined largely by this capacity; *c*) Free hemoglobin was excreted but PBH was not, indicating either absence of filtration or complete reabsorption of PBH; *d*) The excretory rate of free hemoglobin is determined largely by glomerular permeability, tubular reabsorption being small; *e*) Both permeability and reabsorption are less than previously estimated.

Observations on Hepatic Metabolism of Oxytetracycline in Man. CARROLL M. LEEVY, MYRA R. ZINKE, WOO YOON CHEY, JUNIUS SNELL, and ARTHUR ENGLISH, Jersey City and Maywood, N. J. (Introduced by Harold Jeghers).

Hepatic metabolism of nonradioactive and randomly labeled C^{14} oxytetracycline has been investigated in five normal subjects and four patients with moderately advanced Laennec's cirrhosis. Hepatic uptake and turnover of this antibiotic, and their effects on ammonium and oxygen metabolism over a four to six hour period, were studied utilizing the technique of hepatic vein catheterization. Two hundred and fifty milligrams of tagged oxytetracycline (specific activity about 0.1 microcuries per milligram) was given orally, or 100 milligrams of the nonradioactive antibiotic given parenterally. Antibiotic levels were determined from collected aliquots of intestinal juice, bile, urine and serum by microbiological and liquid scintillation radiochemical analyses. These levels were determined from percutaneous liver biopsies obtained one and six hours after oral administration of 250 milligrams of labeled oxytetracycline.

It has been previously demonstrated that radioactive oxytetracycline and tetracycline are concentrated in the liver of mice (Snell, Garkuscha and Allen). The present studies show that oxytetracycline also accumulates in the human liver. Initial serum levels of the antibiotic apparently result from lymphatic absorption as detection of oxytetracycline in hepatic venous blood is delayed; subsequent concentrations in peripheral blood appear to depend upon that released from hepatic stores. The uptake and egress of oxytetracycline were decreased in subjects with cirrhosis and correlated with estimated hepatic blood flow and biochemical function tests. There was a reduction in serum ammonium after either oral or parenteral administration of the antibiotic. This effect could not be wholly attributed to intestinal activity of oxytetracycline; following its parenteral use there was a decrease in hepatic venous ammonium before significant quantities of the antibiotic reached the intestine.

These data indicate that the liver serves as a storage center for oxytetracycline in man; intrahepatic metabolism of this antibiotic contributes to both its bacteriostatic action and effects on ammonium metabolism.

The Dependence of Plasma Potassium Concentration on Plasma Osmolarity, Blood pH, and Body Composition. J. LEIBMAN, L. W. BIRKENFELD, and I. S. EDELMAN,* San Francisco, Calif.

Plasma potassium concentration (K_p) may be influenced by body potassium content and blood pH. Theoretically, K_p might also vary with intracellular osmolarity, and instances of inverse correlations between K_p and plasma sodium concentration (Na_p) have been noted. An integrated account of the dependence of K_p on these factors, however, has not been achieved, nor have others been excluded. The present study is based on the proposal that the steady state concentration gradient of potassium across cell membranes is a function of body potassium, intracellular osmolarity and the partition of hydrogen ions in accordance with the Gibbs-Donnan effect. This concept was evaluated by simultaneous measurement of K_p, Na_p, arterial pH, total exchangeable potassium (K_e) and total body water (T.B.W.) in 30 patients with acid-base disturbances or abnormalities of K_p. Lean subjects were selected, and the ratio of K_e to "dry" body weight (body weight minus T.B.W.) was used as a measure of the potassium content of the lean body mass. Analysis of the data derived from these measurements revealed: a modest correlation between K_p and arterial hydrogen ion concentration ($[H^+]$) ($r = 0.47$); a similar limited correlation between K_p and K_e/"dry" body weight ($r = 0.41$); a somewhat higher correlation between K_p and the product $[H^+]$ times K_e/"dry" body weight ($r = 0.60$); a negligible correlation between K_p and Na_p ($r = -0.15$); and a significant but incomplete correlation between K_p and the ratio $[H^+]$ times K_e/"dry" body weight divided by Na_p ($r = 0.65$). Logarithmic coordinates did not modify these correlations. These results indicate a direct influence of K_e and extracellular pH on K_p and only an occasional relation to extracellular osmolarity. More strikingly, however, they demonstrate a hiatus in knowledge of some of the important parameters which control K_p in man.

Effects of Reduced Glomerular Filtration on Urine Concentration in the Presence of Antidiuretic Hormone. NORMAN G. LEVINSKY, DOUGLAS G. DAVIDSON, and ROBERT W. BERLINER,* Bethesda, Md.

Dogs in which the glomerular filtration rate (GFR) can be decreased reversibly in one kidney and urine collected separately from each kidney have been used to study the effects of reduced GFR on the elaboration of hypertonic urine. When the GFR of one kidney is lowered 10 to 25 per cent in dehydrated dogs infused with vasopressin, the concentration of the urine from that kid-

ney regularly becomes up to 40 per cent greater than that from the control kidney. Increases in urine osmolality are regularly noted even when the control concentration is as high as 1,800 to 2,000 mOsm. per Kg. H_2O. This indicates that the maximum urine concentrations found at very low osmolar excretions under ordinary conditions do not represent the maximum U/P ratio ("osmotic ceiling") which the kidney can achieve. When the reduction of GFR is 30 per cent or more, urine osmolality consistently falls markedly. In this circumstance analysis of renal tissue shows that urea concentration always and sodium (chloride) concentration usually is reduced in the medulla of the kidney whose GFR has been lowered. When urea does not contribute significantly to the urine osmolality and increased amounts of sodium salts reach the loop of Henle (mannitol diuresis), reductions of GFR of up to 70 per cent do not cause significant falls in urine concentration.

These data are consistent with the hypothesis, to be presented, that urine hypertonicity is developed by equilibration of fluid in the collecting ducts with hypertonic medullary interstitial fluid and that the medullary interstitial fluid is made hypertonic to plasma by deposition of sodium salts from water-impermeable Henle loops. Accumulation of urea in the same region, considered the result of passive diffusion from urea-permeable collecting ducts, makes possible the achievement of considerably higher osmolalities than could be developed in response to the interstitial sodium salts alone.

Effect of a Reduction in Filtered Load on Solute and Water Excretion in Hydropenic Man. MARVIN F. LEVITT, MARSHALL S. LEVY, and DEMETRA POLIMEROS, New York, N. Y. (Introduced by Alexander B. Gutman).

In eight normal subjects and four with advanced renal failure, the glomerular filtration rate (GFR) was reduced by venous obstruction of both lower extremities and/or the administration of a ganglionic blocking agent. For 18 hours prior to the study the patients were deprived of all fluid. During a two hour control and a comparable hypotensive period, throughout which intravenous vasopressin (Pitressin®) was administered at a rate of 200 milliunits per hour, renal clearances, urine osmolality, and the rates of solute and water excretion were measured.

In the normal subjects GFR was reduced to 60 per cent of control values with a concomitant fall in urine flow. The average urinary osmolality decreased 90 mOsm. per Kg. H_2O or 12 per cent from control levels, and the urea concentration fell 38 mOsm per Kg. H_2O or 20 per cent. The concentration of salt dropped markedly, the rate of chloride excretion falling to 10 per cent of control values. The average urinary potassium concentration rose 33 per cent producing a much smaller decrease in the rate of potassium excretion.

In the patients with renal failure the changes in GFR, chloride, water, and potassium excretion were similar to those described above. However, in these subjects the fall in GFR was associated with a rise in urea concentration of 40 mOsm. per Kg. H_2O and an increase in urine osmolality of 10 per cent.

An increase in urine urea and total solute concentration, as observed in the uremics, is expected when the quantity of isosmotic fluid reaching the concentrating segment is reduced. In the normals, the expected increment was obscured by a coincident diluting effect. The net dilution seems best explained by the continued back diffusion of urea at a site distal to that segment of the tubule where solute free water is extracted and maximum urinary concentration effected.

The Probable Identity of Glass Factor with Hageman Factor. JESSICA H. LEWIS * and WILLIAM R. MERCHANT, Pittsburgh, Pa.

Blood in contact with glass clots more rapidly than when in contact with a nonwettable surface such as silicone. This increased coaguability has been ascribed to activation of one or more of the various plasma coagulation factors or to removal of an inhibitor.

This glass-activated accelerator effect, herein called glass factor, may be demonstrated by mixing a small amount of glass treated material with normal silicone plasma and observing its effect on the recalcification time in a silicone tube. Plasma from patients suffering from deficiencies of AHF, PTC, proaccelerin, Stuart Factor and Factor VII shows normal glass factor. Normal plasma adsorbed with barium sulfate to remove prothrombin, proconvertin and PTC also shows normal glass factor content. Plasma from a patient with Hageman factor deficiency was devoid of this glass factor and a stored-frozen sample from a PTA deficiency had only minimal accelerating action.

When normal plasma was fractionated by continuous filter paper curtain electrophoresis some of the 32 fractions obtained (primarily in the γ area) showed the ability to accelerate the clotting of normal silicone plasma. The distribution of this "glass factor" corresponded almost exactly to that of the Hageman factor. On the other hand, when Hageman factor deficient plasma was so fractionated, neither Hageman factor nor glass factor activities were found in any of the fractions. The fractions from the Hageman deficient plasma were also assayed for anti-Hageman and antiglass factors, but no such activities could be detected. It is possible that an inhibitor could have been removed during the fractionation procedure.

A Circulating Antibody Directed Against Penicillin. ALLYN B. LEY, AMOS CAHAN, and KLAUS MAYER, New York, N. Y. (Introduced by Rulon W. Rawson).

It has been found that human erythrocytes incubated with high concentrations of penicillin (over 1 mg. per ml.) and subsequently washed and suspended in saline become agglutinable on exposure to the sera of certain individuals. All penicillin derivatives thus far tested, including synthetic penicillin, sensitize erythrocytes to react

with these sera. The inactivation of penicillin by penicillinase completely inhibits the sensitization.

The agglutinating factor in these sera resides in the gamma globulin fraction and retains its potency after heating at 56° C. for two hours. The prior addition of penicillin to the sera inhibits the agglutination of previously "penicillinized" erythrocytes.

Among 413 hospital patients tested, 41 (9.9 per cent) showed the presence of the agglutinin in their sera. Among 1,893 blood donors, tested by a relatively insensitive technique, 4 (0.2 per cent) gave positive reactions. All positive reactors about whom information was available had received or were receiving penicillin at the time the test was done. Skin and conjunctival tests with penicillin were negative in those individuals tested. In a group of 21 patients who gave a history of clinical penicillin sensitivity, 7 (33 per cent) showed the agglutinin in their sera.

Penicillin added to these sera *in vitro* exerted as great an antimicrobial effect as when added to control sera.

Erythrocytes exposed to high concentrations of penicillin (about 50 mg. per ml.) rapidly disappeared after transfusion into a patient whose serum contained the agglutinin, whereas they disappeared at an approximately normal rate in a normal control. No evidence has been found, however, that comparable hemolysis occurs when penicillin is administered in the usual manner.

It is concluded that this agglutinin is an antibody specifically arising as a consequence of penicillin exposure. The clinical significance of the antibody remains to be determined.

Modification of Adrenal Steroid Patterns in Man by Means of an Inhibitor of 11 Beta-Hydroxylase. GRANT W. LIDDLE,* DONALD ISLAND, HERSCHEL ESTEP, and GORDON M. TOMKINS, Nashville, Tenn., and Bethesda, Md.

Chart and co-workers demonstrated that 2-methyl-1,2-bis(3-pyridyl)-1-propanone (SU-4885) decreases adrenal vein 17-hydroxycorticoids of dogs. The present investigation indicates that SU-4885 induces in man biochemical changes resembling those of hypertensive congenital adrenal hyperplasia, that this may be due to specific inhibition of 11 β-hydroxylation, and that this phenomenon can be turned to advantage in evaluating pituitary function.

Although in four surgical patients SU-4885 caused abrupt *decreases* in adrenal vein hydrocortisone, in nonstressed subjects SU-4885, after several hours, caused marked *increases* in blood and urinary 17-hydroxycorticoids and urinary 17-ketosteroids. Despite high 17-hydroxycorticoid levels, no depression of circulating eosinophils or other evidence of hydrocortisone excess occurred.

It was postulated that SU-4885 might be a specific inhibitor of 11 β-hydroxylation. As such it would inhibit secretion of hydrocortisone, induce a compensatory increase in adrenocorticotrophic hormone (ACTH) secretion, and thereby cause a late rise in 11-desoxycorticosteroids such as "compound S." Chemically this would resemble the situation described by Eberlein and Bongiovanni in hypertensive congenital adrenal hyperplasia. The following evidence supports this postulate: *1*) SU-4885 has no effect on steroid levels in Addisonian patients maintained on exogenous hydrocortisone. *2*) SU-4885 does not cause an increase in steroid levels in normal subjects treated with hydrocortisone to preclude a "compensatory" rise in ACTH secretion. *3*) The 17-hydroxycorticoid which becomes elevated following SU-4885 has the chromatographic and chromogenic properties of the 11-desoxysteroid "compound S" rather than hydrocortisone. *4*) SU-4885 inhibits *in vitro* activity of a partially purified 11 β-hydroxylase derived from bovine adrenals.

Since the late rise in steroid levels is mediated by increased secretion of ACTH, SU-4885 has been used to test pituitary "reserve." Patients with pituitary tumors or with previously irradiated pituitary glands have failed to show increased steroid levels following SU-4885, suggesting that pituitary "reserve" was limited even though steroid levels were adequate under nonstressful conditions and even though responsiveness to exogenous ACTH was not lost.

Diverse Distribution of Red Cells and Plasma Albumin in Anatomical Regions of the Kidney. LAWRENCE S. LILIENFIELD, NIELS A. LASSEN, and JOHN C. ROSE, Washington, D. C. (Introduced by Edward D. Freis).

Red cells labeled with Cr^{51} and albumin labeled with I^{131} were injected simultaneously intravenously in eight dogs. Four renal pedicles were ligated after three minutes and 12 at the end of one hour. Blood pressure remained at normal levels throughout the procedure. Both kidneys were removed, frozen quickly, and sliced with a band saw. Various anatomic sections were removed, weighed, and counted with a well-type scintillation counter. Recounting after three weeks permitted measurements of each isotope separately. Tissue radioactivities were compared to radioactivity of arterial blood.

Red cells occupied 5.5 ml. per 100 Gm. outer cortex and 6.6 ml. per 100 Gm. of inner cortex. The anatomically highly vascular outer medulla contained 11.4 ml. red cells per 100 Gm. while the inner medulla contained 6.6 ml. The greatest variability was found in the papillae. In many animals the papillae contained less than 0.1 ml. red cells per 100 Gm. The average papillary red cell content was 3.7 ml. per 100 Gm.

One hour volumes of distribution of albumin were 23.5, 32.5 and 37.6 ml. per 100 Gm. of cortex, medulla and papilla, respectively. Papillary albumin was 85 per cent equilibrated in three minutes. All quantitative data were verified by radioautography.

It is concluded that: *1*) These data contradict the red cell concentration-plasma skimming theory of autoregulation, which predicts a higher red cell concentration in outer than inner cortex. *2*) The papillary red cell content is highly variable but on a comparative basis the renal papilla is extraordinarily deficient in red cells. *3*) Amounts and rate of albumin accumulation indicate that renal plasma albumin circulates extravascularly (a source of error in total plasma volume measurements). *4*) The

large amount of albumin in the papilla suggests that some is concentrated above that of plasma and might therefore be involved in a mechanism for the concentration of urine.

The Hemopoietic Effect of Batyl Alcohol. JAMES W. LINMAN, Chicago, Ill. (Introduced by Frank H. Bethell).

Observations previously reported by us indicate that there are at least two plasma stimulatory factors concerned with control of erythropoiesis. One accelerates erythroblastic cellular division and is thermostable, ether-soluble, and most likely a lipid. The other factor, which is relatively thermolabile, insoluble in ether, and probably protein in nature, augments hemoglobin synthesis. Batyl alcohol, the monoglycerol ether of *n*-octadecyl alcohol, was isolated from yellow bone marrow in 1941 by Holmes and co-workers, and a few reports have indicated that this compound possesses erythropoietic and leukopoietic activity. Similarities in chemical and physical characteristics of batyl alcohol and the thermostable plasma factor suggested a possible relationship between them.

The groups of normal rats were given 20 daily injections of either 12.5 mg. or 25.0 mg. of batyl alcohol in peanut oil, and a control group received peanut oil alone. The animals injected with the batyl alcohol developed reticulocytosis, erythrocytosis and myeloid erythrocytic hyperplasia. The newly formed erythrocytes were microcytic and showed decreased osmotic resistance. There was no increase in hemoglobin or hematocrit levels. This unique erythropoietic response was identical to that previously observed following the administration of the thermostable plasma erythropoietic factor. Batyl alcohol also induced thrombocytosis in the recipients. Although significant leukocytosis was not evident with the doses employed, other studies indicate that batyl alcohol has granulopoietic activity, but that a greater amount is required to evoke this response than is needed to induce erythrocytosis or thrombocytosis.

These observations are in accord with the hypothesis that all aspects of myelopoiesis are under the influence of humoral regulatory mechanisms. Hemoglobin synthesis appears to be governed by a relatively thermolabile factor. A thermostable, ether-soluble factor, which may be batyl alcohol or some related compound, accelerates erythroblastic cellular division and may also exert some degree of control over thrombopoiesis and granulopoiesis.

A Study of the Dynamics of Strontium and Calcium Metabolism and Radioelement Removal. W. B. LOONEY, C. J. MALETSKOS, MARIE HELMICK, JOHN REARDON, JONATHAN COHEN, and WARREN R. GUILD, Boston, Mass. (Introduced by Paul Zamecnik).

Ca^{40}, Ca^{45}, Sr^{85}, and Sr^{89} have been removed from the plasma of 18 dogs by circulating the blood for 1.5 to 7 hours through either an ion-exchange column or the artificial kidney. These procedures starting one hour after radioisotope administration removed 30 to 40 per cent of the injected dose; starting 0.5 to 3 days after administration removed 2.5 to 11.8 per cent of the injected dose; and starting 7 to 10 days after administration removed 0.24 to 2.4 per cent of the retailed dose. The mathematical analysis of the removal of the isotopes in nine dogs, starting one hour after administration demonstrated that 80 to 90 per cent of the dose was in a hypothetical compartment of bone which had a half-time of removal of 8 to 16 hours; the remainder was in a compartment approximating in size the extracellular space. The half-time removal from this compartment was 15 to 30 minutes.

The maximum sustained rate of mobilization of Ca^{40} from the skeletons of 10 dogs was in the order of 1 to 2 mg. per minute. During this time the serum calcium concentration was maintained constant at 1.5 mEq. per L.

The reduction in specific activity of tail bone segments taken at varying intervals during the removal period indicated that radiostrontium and radiocalcium were being removed from bone. Maintaining serum calcium concentrations by infusing Ca^{40} in the venous return did not alter the rate of radiostrontium removal, suggesting that strontium equilibrium is not primarily influenced by coexisting calcium levels. There was in general a reduction in renal excretion of both stable and radioactive cation during the period of radioelement removal. The reduction in the clearance of strontium was by as much as a factor of 20 to 30 while the reduction in the clearance of Ca^{40} and Ca^{45} was usually by a factor of 5. Reduction of the fecal excretion of radiostrontium and calcium was more marked than urinary excretion.

The application of this technique to the clinical removal of bone seeking radio elements will be discussed.

Isolation of the Rheumatoid Factor. JOSEPH LOSPALLUTO, ARTHUR LEWIS, and MORRIS ZIFF,* New York, N. Y.

A gamma globulin factor in the serum of most patients with rheumatoid arthritis has been under considerable study. This protein (rheumatoid factor) may be detected by a variety of methods, all of which involve reaction between the factor and other gamma globulins. Activity is manifested by agglutination of sensitized erythrocytes or latex particles or by formation of precipitates with Fraction II.

To isolate the factor, ion exchange chromatography (Sober and Peterson) was employed. Rheumatoid serum or euglobulin was applied to columns of diethylaminoethyl-cellulose (DEAE-cellulose), an anion exchanger, and fractionated by elution with buffers of decreasing pH and increasing concentration. Proteins fractionated in this manner appear in effluents in order of decreasing isoelectric point, gamma globulin being the first component to be eluted in a fraction recovered at pH 7. In the region of pH 5, however, we have observed the appearance of a second gamma globulin component distinct from the main bulk of gamma globulin. The rheumatoid factor was consistently recovered in this fraction. Since the rheumatoid factor is a macroglobulin, this observation indicated that the anion exchanger separated gamma globu-

lins on the basis of molecular size. Additional evidence for this has been obtained from ultracentrifugal experiments. This unusual chromatographic behavior has made possible the isolation of the rheumatoid factor in a highly purified state.

The factor is first precipitated from serum by the addition of normal Fraction II and the precipitate redissolved in 4 M urea. This solution is chromatographed on DEAE cellulose. Two components appear, one at pH 7 and a second at pH 5. The first is normal gamma globulin which can replace Fraction II as precipitant for the factor. The second is gamma globulin which is precipitated by Fraction II in a concentration of approximately 25 μg. per ml., agglutinates sensitized sheep cells in a concentration of approximately 1 μg. per ml., and demonstrates other reactions of the rheumatoid factor in high titer.

Penicillin Treatment of Experimental Infections Produced by Penicillin-Resistant Staphylococci. DONALD B. LOURIA, New York, N. Y. (Introduced by David E. Rogers).

Whether penicillin can affect the course of penicillin-resistant staphylococcal infections has been a subject of considerable controversy. In an attempt to gain information on this problem, experiments have been performed in which penicillin has been used to treat an experimental mouse infection produced by a strain of staphylococci insusceptible to high concentrations of penicillin *in vitro*.

Although eradication of the infection was not achieved, it could be demonstrated that penicillin treatment produced a significant increase in survival time even when given in dosages which resulted in blood levels well below the *in vitro* sensitivity of the microorganism. When larger concentrations of penicillin were administered, producing blood levels exceeding the *in vitro* sensitivity of the microorganism, the majority of animals were protected from death. Quantitative cultures performed on surviving mice revealed a marked decline or apparent eradication of kidney populations of staphylococci.

The protective effect was most marked when penicillin was given within six hours. When therapy was delayed for 24 hours, allowing early renal abscess formation to occur, protection was significantly reduced.

In vitro studies suggest a possible explanation for these results. When relatively small numbers of staphylococci were exposed to penicillin, significant killing was demonstrated at low penicillin concentrations. However, when the inoculum was increased to levels approximating those found in staphylococcal abscesses, no killing occurred despite massive penicillin concentrations.

These studies suggest that penicillin treatment may modify experimental infections produced by penicillin-resistant staphylococci when organ titers are relatively low. The insusceptibility of large staphylococcal populations to massive concentrations of penicillin suggests that staphylococci in abscess cavities may be relatively unaffected by penicillin therapy. The small protective effect of delayed penicillin treatment may thus be due to prevention of blood stream seeding rather than destruction of microorganisms within established abscess cavities.

Pressure-Volume Relationships of the Lung in Patients with Cardiorespiratory Diseases. FRANK W. LOVEJOY, Rochester, N. Y. (Introduced by Lawrence E. Young).

A method has been devised of recording both transpulmonary pressure and tidal volume on the X–Y axes of an oscilloscope. The resulting loop is photographed with a Polaroid camera. Compliance of the lung can be calculated from the point of zero velocity of the loop. The elastic work during inspiration can be estimated from the area of the right-angled triangle of which the compliance line is the hypotenuse. Nonelastic work is represented by the area of the loop. The total work of breathing is considered to be the area of the loop plus any part of the triangle outside the loop. Elastic and nonelastic work are related as a fraction of total work. The resulting work ratios have been compared with a battery of pulmonary function tests in 30 patients.

Values for compliance and the work ratios so obtained were similar to the values for these parameters obtained from loops constructed from pneumotachygraph records and esophageal pressures. The ratios of elastic work to total work and of nonelastic work to total work correlated very significantly with the one second timed vital capacity, maximum breathing capacity (per cent of predicted), ratio of residual volume to total capacity and with the air velocity index. There was no correlation of the work ratios to vital capacity or compliance. Compliance was usually reduced in patients with pulmonary emphysema or heart disease. In emphysema, the ratio of nonelastic work to total work was always markedly increased with a concomitant reduction of elastic work. In heart disease, the pressure-volume loops were relatively normal, but compliance was reduced.

This simple method of recording pressure-volume loops and the resulting work ratios is at least equal to a battery of pulmonary function tests in explaining the dyspnea of patients with cardiorespiratory disease.

Urinary Excretion of Indoles Causing False-Positive Watson-Schwartz Tests for Porphobilinogen. GEORGE D. LUDWIG, Philadelphia, Pa. (Introduced by Francis C. Wood).

Porphobilinogenuria, a pathognomonic sign of acute porphyria, is commonly detected by means of the Watson-Schwartz test, with formation of a chloroform-insoluble red complex with Ehrlich's benzaldehyde reagent (EBR). Chromogens responsible for occasional false-positive reactions have never been satisfactorily identified. These investigations were prompted by finding positive tests in conditions in which porphyria was simulated but excluded by *1*) quantitative porphyrin assays, *2*) evidence confirming other diseases, and *3*) proof by chemical, chromatographic, and spectrophotometric studies that the chromogen was not porphobilinogen (PBG) but an indolic compound.

In one such patient with hyperpigmentation, hepatic and neurological dysfunction, and dark urine, hemochromatosis was confirmed by iron studies, hepatic biopsy, and autopsy findings. The chromogen proved to be melanogen and melagin was isolated from the urine. Proof that melanogen (5,6-dihydroxyindole) can produce a positive Watson-Schwartz test was provided by synthesizing this compound from DOPA. In contrast to unsubstituted indole, which forms a chloroform-soluble pigment, 5,6-dihydroxyindole yielded an aqueous-soluble red aldehyde complex with a broad absorption maximum at about 556 mμ. The additional absorption peak at 520 mμ, characteristic of PBG-aldehyde complex, was absent. Subsequently, a positive Watson-Schwartz test was obtained with urine containing melanogen from a patient with metastatic melanoma.

Porphyria was excluded in another patient who manifested acute mental changes, hepatic dysfunction, cutaneous lesions, and a positive Watson-Schwartz test. A positive urorosein reaction and chemical evidence of increased indoleacetic acid and indican excretion was found. Two-dimensional paper chromatography confirmed these findings and demonstrated the presence of an additional unidentified indole. The patient's history and clinical features suggested pellagra, and dramatic response to nicotinamide was obtained with disappearance of excessive indoluria.

Urine from two patients with carcinoid syndrome gave negative tests, although higher concentrations of indoleacetic and 5-hydroxyindoleacetic acids yielded pink aqueous-soluble EBR complexes.

Excretion of Formiminoglutamic Acid in Folic Acid Deficiency States. A. LEONARD LUHBY, JACK M. COOPERMAN, DAVID N. TELLER, and ALVIN M. DONNENFELD, New York, N. Y. (Introduced by Milton Mendlowitz).

It has been demonstrated that in folic acid (PGA) deficiency, a major biochemical defect is the inability of the organism to degrade formiminoglutamic acid (FIGLU) to glutamic acid through the enzymatic transfer of the formimino (CH = NH) group. Broquist and Luhby have shown that in PGA deficiency induced by folic acid antagonist therapy in humans, FIGLU accumulates in the urine.

The following studies were undertaken to determine whether other PGA deficiency states could be recognized by the urinary excretion of formiminoglutamic acid.

Clinical subjects were patients with a variety of anemias suspected of being at least partially due to PGA deficiency. All initial urines showed no FIGLU excretion and each subject was given a metabolic load of 8 to 12 grams per day of the amino acid from which formiminoglutamic normally arises. Normal control subjects did not excrete FIGLU under these conditions. FIGLU was determined by microbiological assay using *Lactobacillus arabinosus*.

Results showed that in moderate macrocytic anemia of pregnancy, which did not respond to parenteral vitamin B_{12} and intravenous iron therapy, large amounts of FIGLU were excreted. PGA instituted while the metabolic loading was continued abolished the FIGLU excretion and corrected the anemia. In cases of megaloblastic anemia associated with sprue or malabsorption syndrome, FIGLU was also excreted. However, no FIGLU was found under these conditions in patients with Addisonian pernicious anemia in relapse, anemia associated with malignancy and iron deficiency anemia.

In several patients with anemia and toxemia of pregnancy, exhibiting no blood or marrow evidence of megaloblastosis, FIGLU was excreted. In one patient to whom two 10 milligram daily doses of folic acid were administered while the metabolic loading was continued, the FIGLU excretion was abolished, but reappeared when the PGA was discontinued.

The excretion of FIGLU in these patients demonstrates biochemical PGA deficiency. It can be used as a new clinical criterion of such states.

Observations on the Physiologic Changes Accompanying Hyperlipemia in Rabbits. WILLIAM S. LYNN, CARL WEBER, ROSE H. BROWN, MARGARET WELCH, and ASHTON B. MORRISON, Durham, N. C. (Introduced by Grace P. Kerby).

Experiments designed to study the physiologic effects of hyperlipemia and the mechanisms by which the hyperlipemia may be corrected were performed on rabbits fed diets containing 1 per cent amorphous cholesterol.

Rabbits with blood cholesterol values of 1,000 mg. per cent or higher were shown to drink and excrete two to three times as much water as animals fed stock diets. The rate of urinary (but not fecal) excretion of tracer doses of K^{42}, $S^{35}O_4$, P^{32} and Ca^{45} was two to four times greater than the controls. Na^{22}, however, was excreted at a slower rate. Urinary acid mucopolysaccharides precipitated by quaternary ammonium bromide and characterized by paper chromatography were similarly increased, as was the rate of incorporation thereinto of intraperitoneally injected $S^{35}O_4$. If the hyperlipemia persisted for three months or longer, excretion of all of the urinary components mentioned lessened. Pathologically the animals showed arcus senilis, aortic atherosclerosis and a very characteristic renal lesion consisting of a 2 to 3 mm. halo of extracellular fat at the corticomedullary junction, extending into the medulla.

Factors which facilitated clearing of the high blood fat after cessation of sterol ingestion were increased water intake (600 to 1,000 ml. daily) or addition of KCl (0.5 per cent) and K_2HPO_4 (0.5 per cent) to the drinking water. Factors which retarded the clearing process included dietary addition to saturated or unsaturated fats (from 1 to 20 per cent of the diet) or addition of glucose (7 per cent) to the drinking water.

Observations on the ability of these hyperlipemic animals to oxidize trace amounts of tube-fed saturated and unsaturated radioactive fatty acids to CO_2 indicated two facts: *1*) Unsaturated fatty acids (linoleic and linolenic) were oxidized more rapidly than stearic, and *2*) the hyperlipemic animals oxidized all three acids at a re-

duced rate. However, mitochondria (kidney or liver) from these animals oxidized fatty acids to CO_2 normally.

Thus, dietary hyperlipemia in the rabbit has been shown to produce profound effects on water, salt, polysaccharide and fat metabolism.

The Intracellular Osmolarity of Mammalian Tissues. LEROY H. MAFFLY and ALEXANDER LEAF,* Boston, Mass.

Because of its clinical importance in determining fluid distribution in patients as well as its basic importance to the regulation of cell volume, there has been considerable interest in learning the effective osmolarity within cells. Previous investigators have reported that both the freezing point and melting point depression of various mammalian tissues is one and one-half to two times greater than that of serum. From this it has been concluded that the osmolarity of the intracellular fluid is considerably greater than that of extracellular fluids. The methods used, however, have been criticized because of autolysis which will occur during the interval between removal of the tissue and the endpoint of the measurement.

In the present work, melting point measurements were made on tissue frozen in liquid nitrogen immediately upon removal from anesthetized animals. The frozen tissue was then pulverized and suspended in chilled, inert, liquid silicone. The suspension was then rapidly stirred and its temperature increase measured by a thermistor.

The theoretical melting curve of a solution was shown to be a rectangular hyperbola. Actual tissue melting curves were found to be closely superimposable on the theoretical curve. When the melting curves of various tissues (muscle, liver, heart and brain) were compared with the animals' serum, no significant differences were found. Whole kidney was approximately 10 per cent more concentrated than serum, a difference attributed to its content of hypertonic urine. When frozen tissue was exposed to room temperature for 10 or 20 minutes the subsequent measurement showed a marked increase in osmolarity. This confirms that rapid autolysis will lead to spurious results.

These direct measurements demonstrate that the osmolarity, or more correctly the water activity, within mammalian cells is the same as that of the extracellular fluids.

Adenosine Triphosphate Generation in Relation to the Pathway of Glucose Utilization in Human Erythrocytes. PAUL A. MARKS, ANNE B. JOHNSON, and JULIA BANKS, New York, N. Y. (Introduced by Alfred Gellhorn).

Adenosine triphosphate (ATP) formation was studied in relation to the pathways of glucose utilization in mature human erythrocytes (RBC). The Embden Meyerhof (EMP) and pentose phosphate pathways (PPP), but not the Krebs cycle, are active in RBC. ATP generation may be coupled, in the EMP, to oxidation of 3-phosphoglyceraldehyde or electron transfer from reduced diphosphopyridine nucleotide (DPNH) and, in the PPP, to electron transfer from reduced triphosphopyridine nucleotide (TPNH). ATP concentrations were determined under conditions of increased and decreased glucose breakdown via EMP or PPP.

Stimulation of RBC glycolysis by addition of glucose resulted in markedly higher levels of ATP. Blocking glycolysis via the EMP with iodoacetic acid (IAA) caused rapid decreases in ATP concentrations to undetectable levels (less than 10^{-6} M per Gm. hemoglobin). IAA neither inhibited glucose-6-p dehydrogenase activity nor glucose oxidation via the PPP.

A relationship between ATP levels and glycolysis via the EMP, but not via the PPP, was further indicated by the following findings: *1*) RBC with an hereditary deficiency in glucose-6-p dehydrogenase had markedly decreased rates of oxygen (O_2) consumption and somewhat reduced rates of 1-C^{14}-glucose oxidation to $C^{14}O_2$ but normal ATP levels. *2*) RBC stored in heparinized plasma for two days had little or no detectable ATP although their ability to oxidize 1-C^{14}-glucose to $C^{14}O_2$, a reaction associated with TPNH generation, decreased only 30 to 40 per cent.

In addition, methylene blue concentrations resulting in a ten- to twentyfold rise in RBC O_2 consumption increased 1-C-^{14}-glucose oxidation to $C^{14}O_2$ but had no effect on ATP concentrations.

These data indicate that ATP generation, essential to the maintenance of RBC integrity, is dependent on glucose utilization via the EMP. The fact that ATP generation in RBC is independent of O_2 consumption suggests that its formation is linked primarily to the oxidation of 3-phosphoglyceraldehyde and not of DPNH or TPNH.

Relationship Between Red Cell and Plasma Volume During Recovery from Pernicious Anemia and Megaloblastic Anemia of Folic Acid Deficiency. RALPH W. MASSIE, Nashville, Tenn. (Introduced by Hugh J. Morgan).

There is poor correlation between the prompt reticulocytosis which appears after initial treatment of Addisonian pernicious anemia and the frequently delayed elevation of hematocrit. This could be explained by concomitant increases in red cell volume and in plasma volume during the early phase of recovery. Thus, equiproportional increases in red cell volume and plasma volume would not alter the hematocrit. Since 1900, many techniques have been employed to measure the blood volume in pernicious anemia and very conflicting values have been reported Low, normal and rarely even elevated values have been observed in untreated patients. Frequent determinations of blood volume during convalescence have apparently never been performed. The reported data, however, are conflicting.

Three patients with pernicious anemia and one patient with megaloblastic anemia secondary to folic acid deficiency were studied. Frequent blood volume determinations were performed by dilution of chromium^{51} labeled

erythrocytes. Reticulocyte counts and hematocrits were performed daily.

The blood volume in severe untreated pernicious anemia and megaloblastic anemia of folic acid deficiency is at times markedly diminished. Part of the deficit of blood volume is a deficit in plasma volume. The routine hematocrit determinations in this situation fail to reveal the profound deficits in circulating red blood cells that exist.

The increase in red blood cell volume during the first three weeks of treatment is rapid and almost linear.

During the first two weeks of treatment, the blood volumes returned to normal or slightly above normal. Both plasma and red cell volumes increased during this time.

The failure of the hematocrit to rise in proportion to the increase in total red cells is due to an early equiproportional rise in plasma volume.

The final rise in hematocrit is accompanied by a decrease in plasma volume, with the total blood volume remaining constant during this stage of recovery.

The Nature of the Sodium Diuresis Produced by an "Aldosterone Antagonist," Spirolactone. WALLACE W. MCCRORY and WALTER R. EBERLEIN, Philadelphia, Pa. (Introduced by Henry L. Barnett).

A new synthetic steroid (SC-8109, Searle; spirolactone), described as an aldosterone antagonist, was administered orally in equivalent dosage (25 mg. per Kg. body weight) to four children free of renal or adrenal disease, and to a 12 year old male with chronic hypokalemic alkalosis who had been shown not to have hyperaldosteronism by means of urinary aldosterone assays and exploratory laparotomy. Renal function of the latter subject was normal as judged by renal clearance measurements (C_{in}, C_{pah} and Tm_{pah}), urinary solute concentrating ability and response to a five day load of ammonium chloride (175 mEq. per 1.73 M.^{2} surface area per day). Urine was collected from each subject for 48 hours before and 48 to 72 hours after administration of the synthetic steroid. The rate of excretion of sodium, potassium, chloride, titratable acid and ammonia and the urinary pH were measured. All subjects were maintained on a normal salt intake. The administration of the synthetic steroid resulted in an increased urinary sodium excretion, an increase in urinary pH and a decrease in excretion of titratable acid in all subjects. The urinary excretion of potassium remained unchanged in the control subjects. The patient with alkalosis, however, excreted significantly less potassium and chloride. These results are indicative of altered renal tubular hydrogen ion excretion and suggest that the steroid induces sodium excretion by mechanisms other than aldosterone antagonism *per se*. The effects of prolonged administration of spirolactone to normal subjects and the patient with unexplained alkalosis will be described.

A Mechanism of Renal Hypertension in the Human. JOHN P. MERRILL,* ALBERTO GUINAND-BALDO, and CARMELO GIORDANO, Boston, Mass.

Recent experimental work in animals has suggested that renal hypertension may be associated with the absence of some metabolic or excretory function of the kidney rather than its production of a pressor substance. A unique opportunity to test his hypothesis in the human has been provided by observations on identical twins with hypertension and renal disease in whom a third normal kidney has been successfully transplanted. Four patients with severe renal failure and hypertension received a successful renal homograft from an identical twin. In all patients the blood pressure decreased rapidly toward normal at a time when the patient possessed two severely damaged but one normal kidney. The average blood pressure preoperatively was 210/140; two weeks postoperatively, 150/95. Surgical stress of a similar magnitude (*i.e.*, the transplantation of a cadaver kidney as a true homograft in another patient with hypertension) did not result in decrease in blood pressure. The rate of blood pressure decrease in the twins was not dependent upon changes in weight or sodium balance but roughly correlated with the rapidity with which the transplanted kidney function improved. In two patients the infusion of *l*-norepinephrine at constant rates before and after the removal of the two damaged kidneys was studied. The healthy identical twin donor served as control. Before transplantation the response to norepinephrine was markedly greater than in the normal twin. The addition of a third but normal kidney, markedly decreased the blood pressure "ceiling" to norepinephrine, although it remained higher than in the control twin. Both before and after transplantation this effect was enhanced by a low sodium diet. These findings suggest that the kidneys may play a role in hypertension by modifying the response to normally occurring pressor amines.

Increased Binding of Vitamin B_{12} by Serum Mucoproteins in Chronic Myelogenous Leukemia. AARON MILLER and JOHN F. SULLIVAN, Boston, Mass. (Introduced by C. P. Emerson).

The serum in chronic myelogenous leukemia (CML) has an increased binding capacity for vitamin B_{12} both *in vivo* and *in vitro*. We have studied the *in vitro* binding of added cobalt^{60}-labeled vitamin B_{12}^* by normal and CML sera with dialysis techniques.

B_{12}^* binding occurred within one minute and thereafter did not exchange with added carrier B_{12}. Binding was unaffected by pH changes from 6 to 12 but was decreased at lower pH levels (pH 3.2, normal sera binding decreased 77 per cent, CML sera 10 per cent). A large fraction (average, 69 per cent) of added radiovitamin "bound" by normal sera after dialysis at pH 7.3 was measured as "unbound" by *Euglena gracilis* assay whereas a smaller fraction (average, 11 per cent) was so measured in CML sera. At the pH of this assay (3.5) 48 per cent of the radiovitamin which had been bound in normal sera at pH 7.3 became dialyzable compared to 10 per cent in CML sera. Prior addition of pseudovitamin B_{12} or hydroxo-cobalamin decreased B_{12}^* binding in both

sera; dimethylbenzimidazole, cyanide or agents combining with sulfhydryl groups had no effect.

Normal serum mucoproteins, remaining after sulfosalicylic acid precipitation of proteins, bound an average of 0.24 millimicrograms B_{12}^* per milliliter (13 per cent of total serum binding). Binding by CML mucoproteins was increased (5.14 millimicrograms B_{12}^* per milliliter or 59 per cent of total serum binding), which entirely accounted for increased B_{12}^* binding in some CML sera. CML mucoprotein B_{12}^* binding was unchanged at pH 3.2. Precipitation of CML mucoprotein-B_{12}^* complex with phosphotungstic acid resulted in 95 per cent recovery of radiovitamin in the precipitate.

The observed differences in vitamin B_{12} binding between normal and leukemic sera in respect to pH change and *Euglena gracilis* assay may be explained by mucoprotein binding of vitamin B_{12}. The increased serum vitamin B_{12} binding in chronic myelogenous leukemia may be entirely due to this mucoprotein binding.

Hemolysis in Uremia: Prevention of Intracorpuscular Defect by Renal Tissue. E. E. MUIRHEAD * and J. A. STIRMAN, Dallas, Texas.

Under certain conditions a hemolytic component may contribute greatly to the anemia associated with renal disease and uremia. Hemolysis has been studied in uremia.

Erythrocytes obtained four days following bilateral nephrectomy of the dog were transfused into recipients compatible by the antiglobulin technique and the life span was measured simultaneously by the Ashby and radiochromium methods. Comparable results indicating a shortened life span were obtained by the two methods. This approach supported the use of the radiochromium method under these conditions.

Erythrocytes obtained four days following bilateral nephrectomy of dogs were transfused into normal recipients on 30 occasions. A prominently shortened life span was determined. The results differed significantly from those obtained when the erythrocytes of normal dogs were reintroduced into their normal environment or when these cells were transfused into normal recipients. In seven experiments the same shortened life span was observed in the nephrectomized dog as in the normal recipient. These findings indicated an intracorpuscular defect. By this method this defect reached a maximum 24 hours following the nephrectomy.

In five experiments the ureter was connected to the vena cava and the opposite kidney was removed. Four days later the erythrocytes from these animals were transfused into normal recipients. The life span approached normal values. There was a significant difference between the life span curves following nephrectomy and those following ureterocaval anastomosis.

The results indicated the rapid development of an intracorpuscular defect following nephrectomy. Under the same degree of excretory renal insufficiency (uremia) protection against such a defect by intact renal tissue was demonstrated. Thus the stability of the erythrocytes in the circulation of the dog appears to be due in part to nonexcretory renal function(s).

The Effect of Potassium Depletion and Repletion on Aldosterone Excretion. ALEX F. MULLER, ELIZABETH L. MANNING, and ANNE M. RIONDEL, Geneva, Switzerland. (Introduced by Marian W. Ropes).

The mechanism by which potassium loading increases and potassium depletion decreases urinary aldosterone has not been established. Some claim that changes in serum potassium regulate hormone activity since serum sodium and the state of hydration could not be correlated with the observed variations in aldosterone. The observation that urinary aldosterone increases with potassium loading without changes in external sodium balance and serum potassium has led some workers to infer that the potassium ions act directly on hormone production. More recent experiments established a close relationship between the effects of potassium and body fluid shifts; yet the authors concluded that their studies were not consistent with the hypothesis that all changes occurring in aldosterone secretion during potassium depletion and repletion were regulated by extracellular fluid volume.

The present metabolic studies were carried out in normal subjects in an attempt to elucidate the various factors involved in the regulation of aldosterone during potassium depletion and repletion. Hypokalemia was produced either by administering exchange resins or by withdrawing potassium from the diet.

The diminished aldosterone output was always accompanied by relative sodium retention and hemodilution as measured by hematocrit and total protein changes. Further expansion of the plasma and extracellular fluid will occur with increasing sodium intake despite a falling urinary aldosterone. When potassium loading produces hemoconcentration by a shift or a loss of sodium and water, only then does aldosterone rise. Sodium excretion may increase despite rising urinary levels of aldosterone.

The changes in urinary aldosterone occurring during these experiments can be explained on the basis of variations in the intravascular volume without invoking a direct effect by the potassium ion.

The Relation of Thyroidal Hormone Level to Epinephrine Response. JOHN F. MURRAY and JOHN J. KELLY, JR., Brooklyn, N. Y. (Introduced by William Dock).

The hemodynamic and metabolic responses to epinephrine are strikingly altered by variations in the level of the thyroid hormones. Little clinical use has been made of the phenomenon of increased sensitivity of the hyperthyroid patient to epinephrine because no previous study attempted a quantitative separation of the hyperthyroid from the euthyroid response to epinephrine.

Intravenous epinephrine was administered to 28 euthyroid subjects, 22 normal and 6 hypermetabolic, and to 11 patients with thyrotoxicosis. Differences in the magnitude of increase of the oxygen consumption, pulse rate, and blood pressure clearly separate these groups. Nor-

mals tolerated infusion rates of epinephrine up to 0.2 micrograms per Kg. per minute before experiencing symptoms of epinephrine toxicity. Hyperthyroid patients could only withstand a fraction of this dose. The sensitivity of the thyrotoxic subject to epinephrine is not due to hypermetabolism because the response of the hypermetabolic but euthyroid group was no greater than that of the normals.

Analysis of the normals' and thyrotoxic subjects' response to an infusion rate of epinephrine of 0.05 μg. per Kg. per minute was made. This dosage was chosen because it was capable of producing striking changes in the hyperthyroid subjects and yet not likely to produce disturbing symptoms. The mean change of O_2 consumption in the normal group was +5 per cent with an S.D. of $\pm$ 5 per cent. Three of 14 values of the hyperthyroid group fell within one standard deviation of this.

The increase in heart rate times pulse pressure provided a sharper separation of the two groups. The mean for the euthyroid was +25 per cent with an S.D. of $\pm$ 12 per cent with no values from the hyperthyroid group falling within two standard deviations.

The epinephrine test is a simple test which appears to provide a high degree of specificity in distinguishing hyperthyroidism from other conditions.

Studies on the Influence of Cortisone on Serum Calcium Homeostasis. W. P. LAIRD MYERS and WALTER LAWRENCE, JR., New York, N. Y. (Introduced by David A. Karnofsky).

Balance studies have demonstrated that cortisone may induce a negative calcium balance but these studies have not aided in understanding the role of the adrenals in homeostatic mechanisms governing the serum calcium level. Control of this level has been defined in terms of an equilibrium existing between bone and extracellular calcium. That the adrenals may exert an influence on this equilibrium is suggested by the occasional occurrence of hypercalcemia in adrenal insufficiency in both man and experimental animals.

The present investigations, therefore, were undertaken to define more precisely the role of cortisone in serum calcium homeostasis: *1*) Cortisone administration to a patient with hypoparathyroidism (controlled by vitamin D and calcium lactate) caused hypocalcemia with no change in the calcium balance. *2*) The influence of cortisone withdrawal was studied in an adrenalectomized, thyro-parathyroidectomized dog. Coincident with the development of adrenal insufficiency, hypercalcemia was noted. These changes were reversed when cortisone was again administered. This study was repeated under balance conditions with the same results but with no appreciable alteration in the consistently negative calcium and phosphorus balances. *3*) Cortisone has been reported as having produced hypercalcemia in nephrectomized dogs. The effect of cortisone was therefore studied in a nephrectomized dog who had undergone prior thyro-parathyroidectomy and under these circumstances hypercalcemia did not occur.

These observations suggest that cortisone, in addition to its action on calcium balance, may also exert an influence on the equilibrium between bone and extracellular calcium in a direction opposite to that of parathormone and vitamin D. The fact that hypocalcemia does not develop ordinarily with cortisone administration may be accounted for by compensatory activity of the parathyroids. That cortisone lack can lead to hypercalcemia in the dog in the absence of the parathyroids indicates that its action is not that of direct antagonism to parathormone.

The Demonstration of a Succinoxidase Inhibitor in Gastrointestinal Mucosa. M. NAKAMURA, P. PICHETTE, S. BROITMAN, A. BEZMAN, N. ZAMCHECK, and J. VITALE, Boston, Mass. (Introduced by Charles S. Davidson).

The study of the gut mucosa was stimulated by the hitherto unexplained variability of the succinoxidase activity of the rat intestinal mucosa despite its relative constancy in other tissues such as heart, liver, and kidney. Thus, the oxidation of succinate [$QO_2(N)$] by intestinal mucosa of 41 rats varied from zero (30 rats) to 50 to 100 (7 rats) to 100 to 210 (4 rats). On the other hand, xanthine oxidase activity of rat intestinal mucosa was quite constant, and succinoxidase of guinea pig intestinal mucosa was similarly constant.

The addition of rat intestinal mucosal homogenate caused a decrease in oxygen uptake of guinea pig intestinal mucosa. These observations suggested that an inhibitor of the succinoxidase system resided in the intestinal mucosa of rats and was absent from that of guinea pig. Similarly, the oxygen uptake of rat heart homogenate in the presence of succinate was decreased by the addition of rat intestinal homogenate. On the other hand, guinea pig homogenate did not affect the activity of rat heart homogenate. In general, the lower the enzyme activity of a given rat intestinal mucosa, the greater its inhibition of heart enzyme activity. The addition of rat serum to rat intestinal homogenate increased oxygen uptake. Similarly, serum reversed the inhibitory effect of the intestine on heart enzyme.

The inhibitor, soluble in alkali and fat solvents, proved to be a fatty acid. Fatty acids are normally found in the intestinal mucosa of the rat. Rat heart succinoxidase activity was also inhibited by rat gastric mucosa. Human gastric mucosa has failed to date to demonstrate any inhibitory effect on rat heart succinoxidase activity.

Failure of Aldosterone to Maintain Sodium Retention in Normal Subjects and Addisonian Patients. DON H. NELSON * and J. THOMAS AUGUST, Boston, Mass.

Initial sodium retention followed by sodium diuresis has been reported in men and dogs receiving desoxycorticosterone or fluorohydrocortisone. This effect has not been reported in patients with Addison's disease or in normal subjects receiving aldosterone. Sodium diuresis occurring with extracellular fluid volume expansion has been attributed to a decrease in endogenous aldosterone produc-

tion or possibly to increased secretion of other adrenal steroids. Two normal subjects were given 3 to 6 mg. of *dl*-aldosterone diacetate in sesame oil intramuscularly daily in divided doses for 14 to 26 days while being maintained on a constant diet. Initial marked sodium retention with weight gain of 2 to 3 Kg. was followed by a spontaneous increase in sodium excretion and no further gain in weight during the remaining period of aldosterone administration. All subjects showed sodium retention in excess of water retention without an increase in plasma sodium concentration. There was no significant increase in urinary 17-hydroxycorticoid excretion during the period of increased sodium excretion but secretion of adrenal steroids not measured by the techniques employed could not be ruled out. Aldosterone was then given to two well-documented Addisonian patients on constant cortisone therapy, one of whom in addition received vasopressin (Pitressin®). Both patients showed a response similar to the normal subjects', and a natriuresis of over 300 mEq. occurred in the Pitressin® treated subject without diminution of urinary output or further weight gain. These data indicate that in normal subjects and cortisone treated Addisonian patients, sodium diuresis may occur during volume loading despite continued administration of aldosterone.

The Effect of Parathyroidectomy on the Availability of Bone Sodium. GEORGE NICHOLS, JR. and NANCY NICHOLS, Boston, Mass. (Introduced by K. Emerson, Jr.).

Previous work by the authors and others indicates that sodium moves out of bone mineral into the extracellular fluid in response to acute sodium depletion and/or metabolic acidosis. We have postulated that this might result in a substitution of calcium for sodium at the surface of bone crystal as suggested by Neuman's *in vitro* studies of hydroxyl apatite. Citrate appears to be concerned in mobilizing calcium from bone into extracellular fluid, and the production of this acid by bone is enhanced by parathyroid hormone. These findings suggested that parathyroidectomized animals would have normal or low bone sodium and that acute sodium depletion might increase their susceptibility to tetany due to migration of calcium from extracellular fluid into sites in bone crystal previously occupied by sodium.

To test this hypothesis, plasma and bone from normal and parathyroidectomized rats were analyzed for water, sodium, chloride, calcium, and plasma pH. Animals from each group were sodium depleted by intraperitoneal dialysis against ammonium chloride solution 24 hours prior to sacrifice. Parathyroidectomy produced a 47 per cent fall in plasma Ca but no change in sodium or pH. In contrast, bone sodium increased 8 per cent ($p < 0.001$) without any significant change in calcium. Sodium depletion of normal animals, confirming previous findings, caused plasma pH to decrease 0.05 without change in sodium or calcium, while bone sodium decreased 4 per cent. In parathyroidectomized animals pH decreased 0.10, plasma sodium fell significantly, and Ca rose 15 per cent. Furthermore, bone sodium remained unchanged and no tetany was seen.

These findings suggest that parathormone is necessary for the mobilization of sodium from bone mineral perhaps through unknown modifications in bone matrix or by the production of local acidosis due to increased citrate formation. Moreover, in bone, its action on sodium metabolism appears more important than its effect on calcium.

Experimental Inapparent Poliomyelitis: Factors Involved in Reinfection of Humans with Attenuated Poliovirus. JAMES C. NIEDERMAN, New Haven, Conn. (Introduced by John R. Paul).

Investigation of the use of attenuated strains of polioviruses has not only pointed the way to new methods of immunization against this disease but has also added considerable knowledge to the picture of subclinical inapparent poliomyelitis infection. The studies to be reported have been concerned with the capacity for reinfection in individuals repeatedly fed attenuated virus, and with the relation of such repeated infection to the maintenance of immunity. Major questions to be answered were: *a*) What is the role of the dosage of virus in reinfection, and *b*) what are the effects of pre-existing antibody levels in the host when rechallenged with poliovirus.

Eleven individuals, aged 6 to 16 years, who had previously been fed Sabin's attenuated LSc strain of Type I poliovirus, were rechallenged with the same large dose of the same virus 16 weeks after their first exposure. On this rechallenge, virus excretion in the stool occurred intermittently in all 11 individuals, but such excretion was shorter and less continuous than on initial feeding. A delayed antibody rise was induced in 5 individuals, all of whom had a prefeeding neutralizing antibody titer of 256 or less. Six individuals (most of whom had antibody titers of 256 or more) failed to develop antibody rises in spite of virus excretion.

In order to see how readily this reinfection could be repeated, these same 11 individuals and 5 others were again challenged with four different doses of the same attenuated virus, ranging from large to small. The results indicate that there is a direct relationship between the amount of virus fed and the ease of reinfection as measured by virus excretion. With large virus doses reinfection readily occurred, while with smaller doses reinfection infrequently occurred even though the antibody levels in the hosts were all approximately of the same order.

Blood Coagulation and Blood Lipids. SAUL NITZBERG, ROBERT GOLDSTEIN, and MICHAEL A. PEYMAN, Boston, Mass. (Introduced by Samuel Proger).

In fasting and postprandial specimens, we have studied whole blood clotting time in glass and silicone, clotting time or recalcified plasma, heparin tolerance test, thromboplastin dilution test, thrombo-elastography and Stypven time. The serum lipids measured were total fats, fatty acids, cholesterol, phospholipids, beta-lipoproteins, and occasionally ethanolamine phosphatide.

Generally, we have not confirmed reports of blood hypercoagulability following ingestion of fats. The exception was the Stypven time, which was usually decreased postprandially. The artificiality of this test, however, makes its value questionable. Also, there appeared to be a trend toward increased coagulability in some normal subjects postprandially, as shown by the heparin tolerance test and thrombo-elastography. However, this tendency was not constant on repeated testing.

There was no close correlation between changes in blood coagulability and the degree and character of the postprandial hyperlipemia as chemically determined.

Blood coagulability was not significantly different in 38 patients whose serum cholesterols were below 200 mg. per cent, as compared with 32 patients whose values were above 300 mg. per cent.

In 10 patients with hypercholesteremia, coagulation studies are being performed both when the serum lipids are elevated and when they are lowered after the daily ingestion of unsaturated fatty acids. The results will be reported.

Measurement of Coronary Blood Flow and Myocardial Rubidium Uptake With Rb^{86}. DAVID NOLTING, ROBERT MACK, ERNST LUTHY, MORTON KIRSCH, and CHARLES HOGANCAMP, St. Louis, Mo. (Introduced by R. J. Bing).

The dynamics of myocardial Rb^{86} uptake and its use in the estimation of coronary blood flow in dogs were studied. Myocardial extraction and uptake of Rb^{86} were determined with coronary sinus catheterization. Coronary blood flow, determined with nitrous oxide, served as reference. Myocardial extraction of Rb^{86} remained constant at changing arterial concentrations; as coronary blood flow decreased, the per cent myocardial extraction of rubidium rose. This suggests diffusion of Rb^{86} into a large miscible pool. Coronary blood flow using Rb^{86} was determined by the Fick principle or by the method of Love and Burch. Coronary A–V rubidium differences can be calculated without coronary sinus catheterization since the percentage extraction of Rb^{86} by the heart remains constant (approximately 43 per cent). Myocardial uptake of Rb^{86} can be calculated: a) from the slope of precordial radioactivity, since slope is proportional to the myocardial rubidium uptake [uptake equals flow (N_2O) times myocardial rubidium extraction]; and b) by a proportionality constant obtained from the ratio of precordial activity to radioactivity of heart homogenates obtained after termination of the experiment, or from precordial activity obtained after insertion of balloons filled with known concentrations of Rb^{86} into the ventricles. A straight line relationship exists between precordial count and specific activity of Rb^{86}. Values for coronary flow determined with nitrous oxide show good agreement with those derived from the use of Rb^{86} using the Fick principle; this is particularly the case at low coronary blood flows. The method based on the Fick principle appears to be of promise for the determination of coronary blood flow in man.

Studies of the Activities of Steroid Hormones on Electrolyte Balance and the Constituents of Protoplasm: The Effects of 16 Alpha-Hydroxylation of 21-Carbon Steroids. MAURICE M. PECHET,* EVELYN L. CARROLL, MARY MITCHELL, and MARY JO WEGNER, Boston, Mass.

Previous studies have demonstrated that 1,2-dehydrogenation of parent steroids, cortisone and hydrocortisone, results in an increase in anti-anabolic and anti-inflammatory activities and causes a decrease in sodium-retaining properties. 9α-Fluoronation of parent steroids causes a marked increase in sodium-retaining properties. The effects of 16α-hydroxylation on the metabolic activities and on the renal electrolyte activities of 1,2-dehydro-9α-fluorohydrocortisone were investigated with complete balance studies in Addisonian subjects.

1,2-Dehydro-9α-fluoro-16α-hydroxyhydrocortisone (9α-fluoro-16α-hydroxyprednisolone; triamcinolone) in doses of 30 mg. daily caused marked losses of nitrogen and phosphorus. The loss of phosphorus was commensurate with the loss of nitrogen. In doses of 20 and 30 mg. daily, there was 1) a prompt increase in excretion of calcium, 2) a loss of sodium, and 3) a marked loss of potassium. The loss of potassium was much greater than that predicted from the loss of nitrogen and was not accompanied by parallel changes in serum potassium.

In doses of 30 mg. daily 1,2-dehydro-9α-fluoro-16α-hydroxyhydrocortisone did not offset the sodium retention induced by 150 micrograms daily of 1,2-dehydro-9α-fluorohydrocortisone (dose ratio, 200:1); in doses of 20 mg. daily 1,2-dehydro-9α-fluoro-16α-hydroxyhydrocortisone did offset, after 48 hours, the sodium retention induced by 500 micrograms daily of aldosterone (dose ratio, 40:1).

Thus, 16α-hydroxylation of 1,2-dehydro-9α-fluorohydrocortisone results in a loss of the sodium-retaining property and a retention of the potassium-losing property of the parent steroid. Pronounced weakness and asthenia were associated with the administration of 1,2-dehydro-9α-fluoro-16α-hydroxyhydrocortisone (triamcinolone) and are probably due to potassium loss. Potassium loss persisting concomitantly with sodium loss has not been observed heretofore with steroids possessing anti-inflammatory properties.

Clinical Trials of Five Strains of Orally Administered Attenuated Polio Virus. STANLEY A. PLOTKIN, HILARY KOPROWSKI, KLAUS HUMMELER, AGNES FLACK, and JOSEPH STOKES, JR., Philadelphia, Pa. (Introduced by Frederick C. Robbins).

Five strains of attenuated polio virus have been used in the vaccination of a group of infants. The five strains used were: CHAT, (Type I), Calf (Type I), Jackson (Type II), P-712 (Type II), and Fox (Type III). All strains had been attenuated by passage through mouse brain, tissue culture, and chick embryo, and were the progeny of a single plaque. The vaccines were prepared in monkey kidney tissue culture and dispensed in tissue culture fluid diluted in milk. In none of the infants were

there any illnesses attributable to the viruses administered. Vaccinations with CHAT, Calf, P-712, and Fox strains were effective at widely varying dosages. Jackson strain, however, appeared to be a poor antigen.

The presence of high titers of passive maternal antibodies and/or young age seemed to exert an inhibiting effect on the process of vaccination. Age was not always important, as illustrated by six infants less than two weeks of age who were successfully vaccinated. Follow-up antibody determination on infants immunized with attenuated strains of living polio virus in 1955 and 1956 revealed persistence of antibodies for periods up to 28 months. Six infants were vaccinated against all three types of polio. When the strains were fed at three week intervals, no interference between strains was observed.

In a small town near Philadelphia, 18 families are participating in an open field trial of CHAT, P-712, and Fox strains. One child without antibodies was vaccinated in each family, while stools were collected from all members of the family in an effort to observe spread of fecal virus.

On the Electronmicroscopic Recognition and Clinical Cure of Lipoid Nephrosis in Adults. VICTOR E. POLLAK, GIUSEPPE FOLLI, CONRAD L. PIRANI, ROSS T. W. REID, and ROBERT C. MUEHRCKE, Chicago, Ill. (Introduced by Robert M. Kark).

Lipoid nephrosis is a nonfatal illness characterized by the nephrotic syndrome in which tubular but not glomerular changes would be found post mortem were the patient to die of a complicating disease. Of 96 adults previously studied by renal biopsy and light microscopy, 34 had glomerulonephritis (membranous or proliferative) and 11 had tubular disease with surprisingly little or no glomerular damage.

To elucidate this further, electronmicroscopic, clinical and metabolic observations were made on 10 nephrotics: three children with lipoid nephrosis, three adults with membranous glomerulonephritis (Ellis Type II, Longcope Type B), and four adults with little or no glomerular damage by light microscopy. Four healthy control subjects were studied. To insure adequate sampling five to seven glomeruli were studied for each biopsy. Serial studies were made after dextran or prednisone diuresis.

In membranous glomerulonephritis the lamina densa (basement membrane) was greatly thickened (eight times) and split longitudinally. Endothelial and epithelial cells were normal. Studies on children partly confirmed Farquhar's observations, *viz.*, the basement membrane and endothelial cells were normal, but the foot processes of the epithelial cells were fused into a continuous layer of cytoplasm, and many trabeculae were collapsed. An identical picture was observed in adults. After dextran diuresis in one patient epithelial cell abnormalities were unchanged. Four patients, treated with prednisone, diuresed. Epithelial cell healing was seen in second biopsies, which were completely normal when proteinuria had disappeared. Prednisone has been used for three years in one patient, who is well clinically, biochemically and electronmicroscopically, as are others treated for shorter periods.

These observations indicate that: *1*) Membranous glomerulonephritis and lipoid nephrosis are distinct disease entities. *2*) Lipoid nephrosis occurs in adults as well as children. *3*) Lipoid nephrosis is a reversible disease, at least in early stages. *4*) In lipoid nephrosis diuresis *per se* is not responsible for restoration of glomerular lesions to normal.

Determination of the Rate of Insulin Destruction In Vivo. THADDEUS E. PROUT and IUEAN E. EVANS, Baltimore, Md. (Introduced by Samuel P. Asper, Jr.).

Insulin disappears rapidly from the intravascular space following the intravenous administration of insulin labeled with iodine^{131} (I^{131}). The half-life of insulin I^{131} *in vivo* has been shown to be approximately 25 minutes. This rapid decline in the plasma concentration of insulin I^{131} has been attributed to the removal of insulin from the circulation by the binding of insulin to tissue and by proteolytic destruction of insulin, principally in the liver. Whether or not degradation accompanies tissue binding *in vivo* has not been clearly shown.

Twenty-two normal male rabbits have been given insulin I^{131} by intravenous injection. The rate of disappearance of insulin I^{131} has been followed by measuring the decline of radioactivity of the insulin separated from plasma by electrophoresis. The metabolism of the injected radioinsulin has been determined quantitatively by calculating the amount of free I^{131} which has been either separated from the injected insulin or excreted in the urine. The influence of crystalline-zinc insulin (CZI) in doses of 10, 100, and 1,000 units per Kg. on the rate of disappearance and metabolism of insulin I^{131} has been studied.

Approximately 90 per cent of the radioinsulin is metabolized during the 80 minutes following injection. Neither the quantity metabolized nor the rate of disappearance of radioinsulin from the serum is altered by the intravenous administration of CZI in doses of 10 or 100 units per Kg. The half-life of the radioinsulin is increased to 53 minutes and metabolism is decreased to 48 per cent in 80 minutes by the injection of CZI in dosage of 1,000 units per Kg.

It is concluded from these studies that sequestration of insulin in peripheral tissue does not appear to protect the insulin from degradation. The ability of proteolytic enzymes to destroy insulin is not effected by the injection of 100 units of insulin per Kg. body weight.

Effect of Growth Hormone on Plasma Fatty Acids. M. S. RABEN * and C. H. HOLLENBERG, Boston, Mass.

Values of plasma unesterified fatty acids varied with fasting, feeding, glucose and insulin, as previously observed by others, in accordance with the concept that the value is an index of fat mobilization. Fasting values of normal and diabetic dogs regularly increased 100 per cent or more following partially purified porcine and purified

bovine growth hormone preparations. Growth hormone did not prevent the precipitous fall in concentration induced by glucose or glucose-insulin infusions, and differed from epinephrine which raised the value even under these circumstances. Growth hormone was effective for longer than 18 hours, in contrast to the brief action of epinephrine. Human growth hormone, prepared by the glacial acetic acid method and free of other pituitary hormones, raised fasting values in normal and hypopituitary human subjects. One and 2 mg. doses were effective in some normal adult subjects, and 4 mg. was regularly effective, doubling the value in four hours. No change occurred in this period without the hormone. A 4 mg. dose influenced the fatty acid concentration for at least 24 hours in hypopituitary subjects, but for shorter periods in normal individuals. Blood sugar concentration was not affected. Simian growth hormone was also effective but 30 mg. doses of equally purified porcine and bovine preparations were ineffective in this, as in other effects, in man. The findings suggest that growth hormone participates in the regulation of fat mobilization in man, and a physiological importance is inferred from the similarity of the dose promoting growth and increasing fatty acids. Two mg. of human growth hormone three times a week for 11 months to a pituitary dwarf produced a growth rate of 2.6 inches per year and raised the values of serum inorganic phorphorus and alkaline phosphatase.

Ventilatory Response to CO_2 in Patients with Congestive Heart Failure. LLOYD H. RAMSEY, JOHN GRISCOM, and JAMES SNELL, Nashville, Tenn. (Introduced by C. R. Park).

An increase in minute ventilation (V_E), often associated with the sensation of dyspnea, is a well documented occurrence in congestive heart failure. Since the hyperpnea is associated with low or low normal arterial CO_2 tension (Pa_{CO_2}) and normal oxygen saturation it is generally accepted that the hyperpnea is a result of reflex rather than chemical mechanisms. However, earlier studies indicate that such patients are even more sensitive than normal subjects to the inhalation of CO_2. This finding would indicate persistence of chemical control mechanisms and raise doubt concerning the singular importance of reflex mechanisms in the production of the hyperpnea. Unfortunately, these earlier studies which implied an increased sensitivity to CO_2 in heart failure are difficult to interpret because oxygen tensions were allowed to decrease simultaneously with the increase in CO_2.

Use of an open circuit method has allowed collection of V_E in normal and congestive failure subjects exposed to 2, 3 and 4 per cent CO_2 while keeping oxygen concentration at that of room air. Pa_{CO_2} was calculated from pH and CO_2 content of arterial blood.

The increase in V_E following 20 minutes' inhalation of each of the three CO_2 mixtures was considerably greater in the congestive failure group than in the normal subjects. This difference in response was greater in cardiac subjects with the higher resting V_E and although quite variable tended also to be greater in the patients with the lower Pa_{CO_2}.

Of considerable interest was the finding of a greatly increased ratio of physiologic dead space to alveolar ventilation (V_D/V_A) in the cardiac at rest which persisted at the higher levels of ventilation induced by CO_2. The V_A was only minimally increased at rest and the stepwise increase with CO_2 was not significantly different from the normal controls. Thus the difference in ventilation consisted almost entirely of dead space ventilation at rest and in response to CO_2. This increase in V_D can best be explained by a relatively low pulmonary blood flow in relation to ventilation. The work required for this dead space ventilation may well be a major factor in the production of the symptom of dyspnea.

The Effect of Isotonic Expansion of the Extracellular Fluid Volume on the Natriuretic Response to Infusions of Saline in Postoperative Patients. RUSSELL E. RANDALL, JR. and SOLOMON PAPPER, Boston, Mass. (Introduced by Maurice B. Strauss).

Since the "effective" extracellular fluid volume is a major determinant of renal sodium excretion in normal individuals, a study was devised to ascertain whether a decrease in this volume might be involved in the phenomenon of sodium retention commonly observed following major surgical procedures.

The renal excretion of sodium before and after the intravenous infusion of 2,000 ml. of 0.9 per cent sodium chloride solution over a 90 minute period was measured in three patients on the second to fourth day prior to a major orthopedic surgical procedure and on the second postoperative day. In each case the rate of sodium excretion *before* the challenging infusion was lower postoperatively than preoperatively. In two of the three cases the natriuretic response to the infusion postoperatively was only one-third of the preoperative response. That endogenous adrenal cortical activity is not involved in this phenomenon is suggested by the fact that a patient with Addison's disease maintained on a constant dose of hydrocortisone exhibited a similar response following cholecystectomy.

In three other patients, studied both before and after similar major orthopedic surgery, isotonic saline was infused in the postoperative study until the basal rate of sodium excretion increased to the preoperative level. At this time the 2,000 ml. challenging infusion was administered. The natriuretic response in each case was equal to or greater than that of the preoperative study.

These observations are consistent with the concept that a postoperative decrease in the "effective" extracellular fluid volume may be responsible for both sodium retention and the decreased natriuretic response to challenge following surgery.

An Enzyme in Plasma Inactivating Hageman Factor. OSCAR D. RATNOFF,* Cleveland, Ohio.

Blood circulating within the vascular tree is fluid; yet, when transferred to glass, clots readily. Earlier studies,

based on the work of Shafrir, deVries and Margolis, demonstrated that the clot-promoting effect of glass requires the presence of Hageman factor. Two types of inhibitory activity directed against Hageman factor may help maintain the fluidity of blood within its vessels. Previously, evidence was provided that glass freed Hageman factor from inhibition, initiating coagulation. Now experiments are described which suggest that plasma also contains an enzyme inactivating this freed Hageman factor.

Margolis reported that the clot-promoting factor in glass-treated plasma deteriorated rapidly; loss of activity seemed due to an antagonist in plasma. The deterioration of the clot-promoting factor in glass-treated plasma has been confirmed. The kinetics of inactivation of the clot-promoting property are enzymatic. Plasma also inactivates concentrated Hageman factor enzymatically, supporting evidence that Hageman factor is the clot-promoting factor in glass-treated plasma. The enzyme is present in normal plasma, 56° C.-heated plasma, delipidized plasma and plasma from patients deficient in Hageman factor. Its action is uninfluenced by glass or decalcification. The enzyme is concentrated in a fraction of plasma soluble successively at half-saturation with ammonium sulfate and upon dialysis at pH 5.2, ionic strength 0.02; it is found in Cohn fraction IV-4.

The following tentative hypothesis is suggested. The fluidity of blood is partly maintained by inhibitors directed against Hageman factor. Exposure of blood to glass converts Hageman factor to an active form, presumably releasing it from inhibition. The freed Hageman factor then initiates clotting. Once active, Hageman factor is enzymatically destroyed, preventing its continued action. The possible relationship between the formation of intravascular thrombi and the balance between the vascular surface and the inhibitors directed against Hageman factor remains to be defined.

Aminonucleoside Glomerulonephritis: Morphologic and Metabolic Studies. LILLIAN RECANT,* BENJAMIN A. BOROWSKY, and DAVID M. KESSNER, St. Louis, Mo.

Chronic glomerulonephritis has been induced in rats by administration of the aminonucleoside of puromycin (6-dimethyl-aminopurine-3-amino-D-ribose). Daily injections of 500 μg. per 100 Gm. rat for 50 to 70 days were required for development of the lesion. Morphologic changes in the kidney included hyalinization of the basement membranes of the glomeruli, proliferative changes in Bowman's capsule leading to crescent formation, and dilated tubules with protein casts. Previous observations indicated that larger doses of aminonucleoside given for two weeks induce a classical nephrotic syndrome in rats. Morphologic changes in such animals are minimal.

To obtain evidence of the mechanism of production of this experimental kidney disease, two types of studies were designed: *1*) determination of chemical configuration requisite for induction of the lesion, and *2*) effect of aminonucleoside on adenosine metabolism. In the first project, the following analogues of aminonucleoside were studied: 3-amino-D-ribose, 6-dimethyl-aminopurine, 6-dimethyl-adenosine, and 3-aminoadenosine. Despite the fact that these compounds contained one or the other basic moiety of aminonucleoside, none were found to produce nephrosis. The second study was prompted by the chemical similarity of aminonucleoside and the naturally occurring metabolite, adenosine. A yeast system known to produce acid labile phosphate (Po) in the presence of adenosine was utilized. With adenosine as substrate, 31.0 μM of Po was formed. The simultaneous addition of aminonucleoside and adenosine in molar ratios of 1:1 and 2:1 resulted in the formation of 34.5 μM and 6.8 μM of Po, respectively. When the concentration of aminonucleoside was raised to five times that of adenosine, complete inhibition of Po formation resulted. Paper chromatographic analysis and enzymatic assay identified the Po as adenosine triphosphate (ATP).

It is suggested that there exists a causal relationship between inhibition of ATP formation and the ability of aminonucleoside to induce experimental renal disease.

The Circulatory Dynamics in Pulmonary Emphysema During Treadmill Exercise. JOHN T. REEVES, ROBERT F. GROVER, GILES F. FILLEY, and S. GILBERT BLOUNT, JR.,* Denver, Colo.

To determine the effects of exertion on the cardiac output in emphysema, seven patients were studied by cardiac catheterization at rest and during exercise on the treadmill. All patients were evaluated clinically and by ventilatory, carbon monoxide and previous treadmill studies. Mild or severe diffuse obstructive emphysema was the primary or only disease present, and no patient had clinical heart failure at the time of study.

The results are compared to those in normal subjects and in other patients with emphysema, during rest and exercise, from this and other laboratories. In our patients the resting cardiac index was in the normal or low normal range. For the work performed and the oxygen taken up during exercise, the cardiac index was at least two S.D. below the mean normal in all but one instance, and in no patient was the index greater than normal. The increased demand for oxygen by the exercising tissues was met by a widening of the A-V difference. The mean exercise pulmonary artery pressures ranged from 36 to 105 mm. Hg, and the total pulmonary resistance ranged from 610 to 2,500 dynes-second-cm.$^{-5}$-M.^{2}. By determining the oxygen uptake, the oxygen content of arterial and mixed venous blood, and the pulmonary artery pressure at frequent intervals, it was found that a "steady state" existed in these patients after about five minutes of exercise.

It appears that in addition to disturbances in the transport of oxygen to the pulmonary capillary blood, these patients with emphysema show impairment in oxygen transport from the lungs to the tissues by virtue of an inadequate cardiac output during exercise.

A Study of the Adjustments to Sodium- and Water-Retaining Hormones in Normal Subjects. ARNOLD S.

RELMAN,* WILLIAM K. STEWART, and WILLIAM B. SCHWARTZ,* Boston, Mass.

In an attempt to clarify the mechanisms through which normal subjects "escape" from the effects of sodium- and water-retaining hormones, balance studies have been carried out in normals given desoxycorticosterone acetate (DCA) (20 mg. daily) or fluorohydrocortisone (5 to 10 mg. daily), with or without added vasopressin (ADH). Daily weights, blood pressure, sodium balance and endogenous creatinine and osmolar clearances were measured.

1. With intakes of 180 to 200 mEq. per day, urine sodium did not fall below 50 to 80 mEq. per day, even when the steroids were combined.

2. The phase of sodium retention lasted a few days and, after accumulation of 350 to 500 mEq., all subjects came into equilibrium or began "unloading," despite continued steroid. In only 2 of 17 experiments did mild hypertension appear, and in none was there overt edema.

3. Simultaneous DCA and ADH (10 units per day I.M.) did not enhance sodium retention; rather, "escape" occurred more promptly after only 70 to 200 mEq. sodium retention.

4. Continuous administration of DCA for 10 to 14 days while on a low sodium diet did not prevent the steroid from producing transient sodium retention when salt was then added to the diet. This was demonstrated by comparing the response to increased sodium intake with and without DCA.

5. In most experiments endogenous creatinine clearance tended to rise before or during "escape," but large changes in sodium excretion were sometimes unrelated to creatinine clearance.

It is concluded that: *a*) By themselves, hormones, even at very large doses, are not adequate to cause overt clinical edema and sodium-free urine. *b*) There is no evidence for the development of "resistance" to the tubular sodium-retaining effect of chronically administered DCA. *c*) While these observations suggest that an increase in GFR may be important in the "escape" from steroidal sodium retention, the present data do not permit any definitive evaluation of this factor.

Changes in Wedged Hepatic Venous Pressure and Hepatic Blood Flow Accompanying Clinical Improvement in Cirrhosis. TELFER B. REYNOLDS,* ALAN G. REDEKER, and HERMAN M. GELLER, Los Angeles, Calif.

To evaluate the natural history of portal hypertension, portal venous pressure has been assessed by recording wedged hepatic venous pressure (WHVP) during periods of apparent clinical improvement in patients with alcoholic cirrhosis. Nine patients were studied during their first episode of ascites and again from two to six months later when ascites had spontaneously disappeared. A rise in serum albumin accompanied the loss of ascites in each patient and icterus, present in four of them at the time of the initial study, subsided.

In three patients WHVP increased minimally (from a mean of 19.3 to a mean of 20.5 mm. Hg) despite the clinical improvement. Hemorrhage from esophageal varices occurred soon after the disappearance of ascites in each of these patients and in two instances was fatal.

In the remaining six patients significant falls in WHVP were noted at the time of apparent clinical improvement (from a mean of 15.5 to a mean of 9.8 mm. Hg). Radiologic evidence of esophageal varices has not appeared in any of these patients.

The fall in WHVP in six out of nine patients suggests that portal hypertension is not fixed and may decrease concomitantly with clinical improvement in cirrhosis. This observation may have some bearing on the problem of the "prophylactic" portacaval shunt.

The fall in WHVP was probably due to decreased hepatic vascular resistance since evidence of increased collateral flow around the liver did not develop and estimated hepatic blood flow, measured in four patients, increased from a mean of 1,200 ml. per minute to a mean of 1,435 ml. per minute.

Blood Sugar Regulation During Sleep in Normal and Diabetic Subjects. EUGENE D. ROBIN, DAVID M. TRAVIS, DESMOND G. JULIAN, CHARLES H. CRUMP, and BURIS BOSHELL, Boston, Mass. (Introduced by C. Sidney Burwell).

Ideal control of diabetes mellitus means that blood sugar levels of diabetics conform to diurnal curves of normals. Diurnal curves should include blood sugar changes occurring during sleep. Previous data on these changes are scanty. Therefore, blood sugar regulation during sleep was studied in normals and in diabetics.

Determinations of hourly blood sugar concentrations in normals during sleep reveal little variation from hour to hour. The greatest difference between hourly blood sugar concentrations in any subject was 26 mg. per cent. The mean standard deviation for the group was 5.3 mg. per cent. Results of glucose tolerance tests performed during the waking and sleeping state were essentially the same.

Thus, sleep is associated with an effective homeostatic mechanism for blood sugar regulation. However, blood sugar constancy does not depend on sleep *per se*. Studies of hourly blood sugar levels in nonsleeping normals reclining and fasted at night reveal the same constancy. This constancy is based on an effective regulating mechanism operating at a time when demands on this mechanism are minimal.

Diabetics are insulin deficient and lack an effective mechanism for blood sugar regulation. Wide variations in hourly blood sugars are found in both untreated and insulin-treated diabetics.

No untreated diabetic showed even approximately constant hourly blood sugar levels at night. Variations of as much as 91 mg. per cent were found. The mean standard deviation for the group was 19.4 mg. per cent.

Nor did insulin provide an effective regulating mechanism, since insulin-treated diabetics showed marked variations in hourly blood sugar levels.

Patterns of variation included hypoglycemia during

sleep, progressive hyperglycemia, and hyperglycemia for several hours followed by normoglycemia.

Insulin is a biochemical, but not a physiological, substitute for a sugar regulating mechanism. Ideal control necessitates consideration of the diabetic during sleep as well as during wakefulness.

Hormonal Influences on Intracellular Proteolytic Enzyme Activity. HERBERT G. ROSE, MARY C. ROBERTSON, and THEODORE B. SCHWARTZ,* Chicago, Ill.

A method has been devised for the assay of proteolytic enzyme activity in which cellular integrity is preserved. Since hormonal activity might, under some circumstances, require intact cells, such a system may help to resolve discrepancies that have arisen in substantiating the hypothesis that hormones act upon enzymes. The surviving rat diaphragm has been used as an enzyme preparation to hydrolyze the synthetic substrates glycylglycine and L-leucylglycine, thereby measuring dipeptidase and leucine aminopeptidase activities, respectively. Enzymatic behavior of this preparation is very similar to that of purified enzymes. A seasonal variation in enzyme activity was noted with a peak in summer and a fall in winter. In a number of situations enzyme activity changes occur in directions consistent with a functional role in the regulation of protein catabolism. Thus fasting increases aminopeptidase activity (controls, 43.8 ± 1.4 μM leucylglycine hydrolyzed per gram diaphragm per hour; fasted, 57.4 ± 2.1). Cortisone administration also increases activity (controls, 47.1 ± 2.1; injected, 60.6 ± 3.1), while adrenalectomy decreases activity (controls, 45.0 ± 2.5; adrenalectomized, 28.0 ± 1.2). Similarly, thyroidectomy reduces activity (controls, 57.2 ± 4.2; thyroidectomy, 39.8 ± 4.0), but only inconsistent increases could be obtained after triiodothyronine administration. In most instances, the changes observed also remained significant when referred to diaphragm dry weight, diaphragm nitrogen, and total diaphragm weight. It is concluded that hormonal and metabolic stimuli provoke changes in intracellular peptidase activity consonant with what would be predicted from studies conducted on a physiologic level.

Thiocyanate-Dischargeable Iodide in the Dog Thyroid Gland. I. N. ROSENBERG,* J. C. ATHANS, A. BEHAR, and C. B. TISCH, Boston, Mass.

Arterial and thyroid venous plasma concentrations of radioactive iodine were measured in acute experiments in the dog before and after the intravenous administration of thiocyanate or perchlorate. Radioactive iodine (50 to 300 microcuries I^{131}) was injected intravenously and two hours to seven days later, the animal being anesthetized with pentobarbital, one lobe of the thyroid was removed; the other was dissected so that its only visible remaining connections to the circulation were the vascular pedicle at the upper pole and the inferior thyroid vein, and a polyethylene catheter was inserted in the latter. Consecutive blood samples were then drawn from the inferior thyroid vein in heparin-coated syringes, while femoral arterial blood samples were obtained intermittently throughout the duration of the experiment (40 to 60 minutes). The plasma concentrations of total and of trichloroacetic acid-precipitable I^{131} were measured, and the acid-soluble I^{131} (presumably iodide) obtained by difference.

In experiments performed two to four hours after I^{131} administration, the accumulated glandular radioactivity was small and the arterio-thyroid venous iodide^{131} concentration difference was large; the arteriovenous difference was promptly abolished by thiocyanate administration, but only occasionally and transiently did the venous iodide^{131} concentration exceed the arterial. If thiourea was injected a few minutes before thiocyanate, the latter induced values of venous iodide^{131} concentration considerably larger than the arterial values. In experiments done three to seven days after I^{131} administration, glandular uptake was large, arteriovenous iodide^{131} differences were usually small, and perchlorate or thiocyanate administration led to a prompt and often sustained increase in the venous concentration of iodide^{131} so that the values were appreciably greater than the corresponding arterial values.

These results suggest the presence within the gland of inorganic iodide not newly entered from the plasma.

A Study of the Means by which Exercise Increases the Pulmonary Diffusing Capacity for Carbon Monoxide. JOSEPH C. ROSS, REGINA FRAYSER, and JOHN B. HICKAM,* Durham, N. C.

The diffusing capacity of the lung for O_2 and CO normally increases during exercise, as measured by various techniques. This is usually thought to be related to changes in the pulmonary circulation. Conceivably, exercise may increase diffusing capacity by *1*) effects secondary to increased cardiac output, such as enlargement of the pulmonary capillary bed, or *2*) effects secondary to increased ventilation, such as improving the distribution of blood and gas within the lungs. To separate these factors, measurements were made of the pulmonary diffusing capacity for CO by the "steady state" method of Filley (D_{co}) and the cardiac output by the dye method during procedures designed to alter pulmonary ventilation with minimal effects on output and to alter output and pulmonary vascular pressures with minimal effects on ventilation. Observations were made on normal, recumbent males.

In 7 subjects, leg exercise increased D_{co} from a resting mean of 30 to 49 ml. per mm. Hg per minute, cardiac output from 7.5 to 13.0 L. per minute, and ventilation from 8.5 to 22.2 L. per minute. In 7 subjects, the cardiac output was increased by drugs (epinephrine or norepinephrine and atropine) from a resting mean of 7.7 L. per minute to 13.3 L. per minute, but mean D_{co} and ventilation did not change. In 7 subjects, ventilation was voluntarily increased from a resting mean of 7.2 L. per minute to 21.8 L. per minute. There was negligible change in output, but D_{co} rose from the resting mean of 27 to 47 ml. per mm. Hg per minute. In 9 subjects, central venous pressure was increased by 5 to 10 mm. Hg with albumin infusion and G-suit inflation, but D_{co} did not in-

crease unless ventilation also increased. In a total of 67 simultaneous observations of D_{co}, output, and ventilation in 24 subjects, D_{co} and ventilation showed a highly significant positive correlation ($r = 0.0830$), but D_{co} and output were not significantly correlated.

"Steady state" D_{co} increases during exercise because ventilation increases; it appears insensitive to changes in output and pressure.

Systemic and Coronary Hemodynamics in Normal Men and Women. GEORGE G. ROWE, CESAR A. CASTILLO, GEORGE M. MAXWELL, D. J. FREEMAN, DOUGLAS H. WHITE, and CHARLES W. CRUMPTON,* Madison, Wis.

Systemic and coronary hemodynamics were determined by the Fick principle and N_2O methods, respectively, in 15 normal men and 15 normal women. Ages averaged 30 years for the men and 26 for the women but there was sufficient overlap in the groups that there was no significant age difference ($p < 0.1$).

Systemic hemodynamics were similar in that cardiac index and rate, right and left ventricular work index (LVWI), pulmonary and peripheral arterial pressures were not significantly different.

Coronary hemodynamics differed considerably. Coronary blood flow per 100 Gm. of left ventricular myocardium per minute (CBF) was 26.5 per cent greater in the women ($p < 0.001$) and their coronary vascular resistance was 35 per cent less ($p < 0.01$). Myocardial oxygen consumption ($CMRO_2$) and carbon dioxide liberation were not significantly different. Although the hemoglobin and hence the arterial (AO_2) and mixed venous oxygen content were less in the women ($p < 0.001$), the product of CBF times AO_2 was greater (+16.6 per cent, $p < 0.05$) indicating that more O_2 was available to the female heart. Females also had more CBF per kilogram meter of LVWI (+26 per cent, $p < 0.01$) and a reduced LVWI to $CMRO_2$ ratio (−28 per cent, $p < 0.05$).

This study may be interpreted to mean that the heart of women is better perfused by blood than the heart of man and perhaps this is associated with the known lower incidences of coronary artery disease in females.

Chronic Salivary Gland Virus Infection in Children. WALLACE P. ROWE, Bethesda, Md. (Introduced by Harry Eagle).

Salivary gland virus is known to cause cytomegalic inclusion disease of the newborn, but infection in older individuals is poorly understood. Serological studies indicate that infection is highly prevalent. Using tissue culture techniques for serological and virus isolation studies, a survey was made of infection in lower economic group children, aged four months to four years. It was found that 18 per cent of children in the Washington, D. C., area had antibodies, while in other countries the proportion of positives reached 60 to 80 per cent. Four per cent of clinic children had virus in their saliva as determined by a single test. Since virus was found only in children with complement fixing antibody, a longitudinal study was made of antibody positive children in a receiving home nursery. When repeated tests were made, virus was recovered from the saliva on one or more occasions from two-thirds of the children with antibody. Twelve children excreted virus for more than a month, and several were demonstrated to excrete virus over periods of at least five to eight months. On the basis of antibody studies, some of the virus positive children had been infected for at least 12 to 24 months. Ten of 11 children who were excreting virus in their saliva also had demonstrable virus in their urine, although inclusion cells were not found in repeated smears of the urinary sediment of 7. Careful clinical studies of 16 oral carriers showed a variety of abnormalities, mostly of a nondescript nature. The only finding which appeared to be in excess of the abnormalities in other children in the same population was white cells or casts in the urinary sediment of 6, including two cases with marked elevation of Addis counts.

Intestinal Similarities Between Celiac Disease and Idiopathic Sprue. CYRUS E. RUBIN, LLOYD L. BRANDBORG, PATRICIA C. PHELPS, and HAWLEY C. TAYLOR, JR., Seattle, Wash. (Introduced by Robert H. Williams).

To investigate the possible relationship between celiac disease and idiopathic sprue, 110 suction biopsies of the small bowel were performed in 65 patients comprising idiopathic sprue, suitably aged controls, and active, latent, and adult celiac disease with well established diagnoses.

A flexible tube, 4 mm. in diameter, was designed for safe biopsy in infants and adults. Under fluoroscopic control a mucosal knuckle was excised with measured negative pressure. The gross specimen's absorptive surface and vasculature were photographed and examined microscopically during fixation. In serial sections the percentage of epithelial cells observed in a total count of epithelial cells plus a band of muscularis was estimated by a modified Chalkley technique employing a mold-counting micrometer grid.

The surface in normal controls (adults, 25; children, 14) was covered with well vascularized slender villi. In 10 sprues, 2 of 3 adult, 1 of 9 juvenile, and 1 of 4 "cured" celiacs, villi were absent, vessels reduced in number and the surface flattened and knobby. Steatorrhea uniformly accompanied the latter findings. Histologically there was loss of villi, altered surface epithelium, chronic inflammatory infiltration, and crypt abnormalities suggesting retardation of mucosal replacement. A varied spectrum from normal to moderately severe mucosal alterations was evident in the remaining celiacs: adults, 1 moderate; "cured," 2 normal, 1 moderate; active, 2 mild, 6 moderate.

In sections there was a marked reduction from the normal length of absorptive epithelium in severe lesions. To test this visual impression the modified Chalkley counts described above were blindly made by a nonclinical observer. The difference between normal and severely abnormal groups was highly significant statistically by a chi-square test, with $p < 0.0001$.

The similarity of the intestinal lesions in idiopathic sprue and celiac disease suggests that they are the same

process. More prolonged observations will test this theory.

Transcortin: A Corticosteroid-Binding Plasma Protein. AVERY A. SANDBERG and W. ROY SLAUNWHITE, JR., Buffalo, N. Y. (Introduced by Frank H. Tyler).

Previous work in our laboratory indicated that human serum albumin (HSA) accounts for the preponderant part of the binding of estrone, estradiol, progesterone and testosterone in the blood. Cortisol and corticosterone were bound to plasma to a much greater extent, however, than they were to HSA. This indicates the presence of a protein(s), with great avidity for cortisol and corticosterone. By analogy to transferrin, we designate it as transcortin. The present studies are based on experiments utilizing equilibrium dialysis of 1 per cent HSA containing C^{14}-labeled and carrier steroid against 1:5 dilutions of plasma in saline. The results indicate that 75 to 90 per cent of tracer doses of cortisol and 60 to 75 per cent of corticosterone are bound by transcortin under these conditions. Undiluted plasma bound 100 per cent of tracer amounts of cortisol. The binding capacity of plasma transcortin was measured by adding increasing amounts of carrier cortisol to the HSA and was shown to be approximately 20 μg. per 100 ml. of undiluted plasma. Thus, transcortin would seem to be nearly saturated at normal plasma levels of cortisol. Testosterone, progesterone and 11β-hydroxyandrostenedione showed little (12 to 32 per cent) and estrogens no binding to plasma proteins other than HSA.

An hypothesis can therefore be formulated regarding the significance of changes of transcortin concentration. If one assumes that transcortin-bound cortisol is inactive, the pharmacological and physicological effects of cortisol may be a function of the steroid not bound by transcortin. Thus, reduction of transcortin concentration may account in some measure for manifestations in patients with Cushing's disease having apparently normal adrenals; and increased levels of transcortin for the high levels of cortisol in pregnancy without manifestations of hyperadrenocorticism. The development of methods for measuring transcortin concentration will shed light on this hypothesis.

An Explanation for and Experimental Correction of the Abnormal Water Retention in Cirrhosis. HAROLD P. SCHEDL and FREDERIC C. BARTTER,* Bethesda, Md.

Diuresis was induced in patients with hepatic cirrhosis and in normal subjects with isotonic dextrose given intravenously. That the impaired water diuresis of cirrhotics was *not* due to excessive or persistent antidiuretic hormone (ADH) was confirmed by the observations that *1*) urine tonicity fell readily below that of serum, *2*) physiologic amounts of administered vasopressin (Pitressin®) could prevent this fall, and *3*) administered Pitressin® had only normal duration of action.

If "free" water is generated by tubular reabsorption of sodium, the minimal free water clearance (C_{FH_2O}) and resultant tendency to hyponatremia of cirrhotics is paradoxical. Their almost quantitative reabsorption of filtered sodium would imply *greater* than normal C_{FH_2O}. Excessive proximal reabsorption of glomerular filtrate in these patients could prevent diuresis by withholding sodium and water from distal sites where free water could be generated.

These hypotheses were tested by repeating the infusions with volume kept constant, but with progressively greater amounts of mannitol substituted for dextrose. With increasing mannitol proportions, normals showed the expected progressive fall in C_{FH_2O}, which reached negative values with isotonic mannitol. In contrast, cirrhotics showed increases in C_{FH_2O} up to seven times that with isotonic dextrose; even when only isotonic mannitol (and thus no free water) was given, C_{FH_2O} was as much as twice that with isotonic dextrose.

As further evidence for increased cation transfer at distal tubular sites, potassium excretion increased with mannitol in cirrhotics, but not in normals.

These findings support the concept that retention of water loads and hyponatremia in cirrhotics result not from ADH but from excessive proximal reabsorption of sodium and water. Delivery of a sufficient volume of glomerular filtrate from proximal to distal sites with "osmotic" diuretics produces more free water in cirrhotics than in normals under identical conditions.

ECHO-9 Virus Exanthema. WILLIAM F. SCHERER, JAMES PRINCE, and JOSEPH ST. GEME, Minneapolis, Minn. (Introduced by Jerome T. Syverton).

During the summer of 1957, an epidemic of exanthema became manifest in Minnesota. Patients presented fever and a generalized morbilliform rash which lasted from one to three days. Family infections and spread within neighborhoods were common. Although rash was the only symptom in many cases, a spectrum of disease was displayed which ranged from inapparent asymptomatic infection to skin rash with little disability, to skin rash and fever, to skin rash and aseptic meningitis, and finally, to aseptic meningitis without rash. In an attempt to determine the etiology of this syndrome, specimens from a selected group of patients were studied by tissue culture techniques.

In 21 patients ECHO-9 virus neutralizing antibodies appeared during convalescence and in 15 of the 21 patients ECHO-9 virus was recovered from throat swabs, feces and/or cerebrospinal fluids. The Minnesota strain of ECHO-9 virus was cytopathogenic for human foreskin fibroblasts; it differed from the prototypic strain of ECHO-9 virus and resembled the European, English and Canadian strains in its pathogenicity for newborn mice.

Compensatory Mechanisms in Primaquine-Sensitive Erythrocytes. STANLEY L. SCHRIER and ROBERT W. KELLERMEYER, Chicago, Ill. (Introduced by Alf S. Alving).

"Primaquine-type" drug-induced hemolytic anemia occurs in otherwise healthy Negroes, and rarely in Cauca-

sians. While drug-sensitive erythrocytes are normal morphologically and immunologically, they undergo acute intravascular hemolysis when exposed *in vivo* to primaquine, sulfanilamide, nitrofurantoin (Furadantin®), furazolidone (Furoxone®), other drugs and fava beans. Characteristically these erythrocytes have a normal oxidized glutathione content but a low, unstable reduced glutathione content. We believe that the primary enzymic abnormality is a genetically determined deficiency of glucose-6-phosphate dehydrogenase, with compensatory increases in glutathione reductase and aldolase. Erythrocytes deficient in glucose-6-phosphate dehydrogenase might be deprived of energy resulting from triphosphopyridine nucleotide (TPN) reduction in the oxidative hexosemonophosphate shunt, and, therefore, obliged to derive energy from diphosphopyridine nucleotide (DPN) reduction.

Aldolase and glyceraldehyde-phosphate dehydrogenase provide substrate for, and catalyze, the initial oxidative phosphorylation in anaerobic glycolysis (Embden-Meyerhof pathway), providing energy in the form of reduced DPN and high energy phosphate bonds. These enzymes were assayed in hemolysates by modifications of the Warburg and Christian method.

Aldolase was increased in hemolysates from 10 primaquine-sensitive individuals when compared with 13 normals, independent of variations in the following reaction conditions: time, amount of hemolysate, temperature, pH, substrate concentration, aerobiosis and anaerobiosis. In contrast, glyceraldehyde-phosphate dehydrogenase in hemolysates of primaquine-sensitive erythrocytes was normal.

Glyceraldehyde-phosphate dehydrogenase has been called a "pacemaker of anaerobic glycolysis." Its substrate, triosephosphate, does not accumulate in erythrocytes, while the substrate for the preceding reaction, fructose-diphosphate, is present in considerable amounts. We propose that increased aldolase could convert fructose-diphosphate to additional triosephosphate, which glyceraldehyde-phosphate dehydrogenase could use to reduce DPN. Thus erythrocytes deficient in glucose-6-phosphate dehydrogenase theoretically could utilize energy sources of anaerobic glycolysis more effectively and maintain normal life spans unless challenged by hemolytic drug.

The Renal Response to Acetazolamide (Diamox®) During the Metabolic Acidosis of Salicylate Intoxication. ROBERT SCHWARTZ, FRANCIS X. FELLERS, JOHN KNAPP, and SUMNER YAFFE, Boston, Mass. (Introduced by Charles A. Janeway).

These studies were initiated to determine the extent to which alkalinization of urine could be achieved rapidly (since salicylate excretion is a function of urinary pH) without further compromising acid-base homeostasis during metabolic acidosis.

Three infants with salicylate levels greater than 25 mg. per cent and with advanced metabolic acidosis were investigated for the effects of salicylate intoxication on the excretion of individual cations (Na, K, Ca, Mg, and NH_4), anions (organic acids, HCO_3, Cl, phosphate) urinary pH, and salicylates. During control observations while sodium bicarbonate was infused, glomerular filtration rate (GFR) (creatinine or inulin) was depressed to half of normal in two infants and renal plasma flow (PAH) was depressed in two. Control urine pH's ranged from 5.07 to 5.54 and less than 2 per cent of the filtered nonbound salicylate was excreted. Although ammonium and titrable acid resulted in buffer conservation of 101 to 146 μEq. per minute per M.^{2}, organic acids (ketones) (174 to 512 μEq. per minute per M.^{2}) contributed significantly to net buffer cation loss.

Diamox® (5 to 10 mg. per Kg.) was given intravenously after several control periods and while bicarbonate infusion continued. The latter prevented progression of the acidosis and in most cases the serum CO_2 content actually increased. With an increase in urine flow and urinary pH (maximally 7.21 to 7.70), salicylate excretion increased markedly from excretion/filtration (E/F) less than 0.02 to maximum E/F 0.28, 0.64, 0.83. Salicylurate and other salicylate conjugates were not affected. Ammonium and titrable acidity were diminished, while Na and particularly K increased. Although filtered bicarbonate was low (less than 10 mEq. per L.) and GFR depressed, bicarbonate excretion increased to as much as 30 per cent of filtered load. Organic acid excretion remained elevated throughout and buffer cation loss (organic acid plus bicarbonate) after Diamox® was two to three times the control values.

The variability in buffer cation loss after blocking the major renal compensatory mechanism precludes reasonable prediction of buffer needs to maintain homeostasis.

The Kinetics of Renal Bicarbonate Reabsorption During Acute Respiratory Acidosis. WILLIAM B. SCHWARTZ,* ADRIEN FALBRIARD, and GUY LEMIEUX, Boston, Mass.

The kinetics of renal bicarbonate reabsorption have been studied during acute sustained respiratory acidosis (pCO_2 80 to 110 mm. Hg) in anesthetized dogs whose plasma bicarbonate levels were elevated by infusion of sodium bicarbonate. With progressive elevation of plasma bicarbonate concentration, there was a curvilinear rise in the rate of reabsorption which at the highest plasma levels studied (55 to 60 mEq. per L.) reached an average level of 3.8 mEq. per 100 ml. glomerular filtration (GFR). Frank excretion of bicarbonate began at plasma levels of 26 to 30 mEq. per L. The data indicate that elevation of pCO_2 does not simply increase the reabsorptive capacity to a single new constant rate, but rather suggests that during acute sustained respiratory acidosis the reabsorption of bicarbonate is a function of plasma bicarbonate concentration.

The pattern of bicarbonate reabsorption is similar to that which has been observed previously with partial inhibition of carbonic anhydrase in animals with metabolic acidosis. Analysis of these latter data in terms of Michaelis-Menten theory yielded findings consistent with the hypothesis that enzyme activity had become rate-limiting in the reabsorptive process.

The present data were plotted in a similar fashion according to the Lineweaver-Burk modification of the Michaelis-Menten equation on the assumption that filtrate bicarbonate concentration was equal to (or approximated) some constant and direct function of S for the overall reabsorptive process and that reabsorption per 100 ml. GFR was equal to v. The fit of the data with Michaelis-Menten kinetics was consistent with the hypothesis that in acute respiratory acidosis carbonic anhydrase becomes rate-limiting in the reabsorptive process, and this interpretation provides a tentative explanation for the increasing rate of bicarbonate reabsorption seen with elevation of the plasma bicarbonate level. As a corollary, the slower and constant reabsorptive rate, independent of plasma bicarbonate concentration, which is seen with normal pCO_2 would appear to be directly determined by carbon dioxide tension which seems to prevent full utilization of enzyme capacity.

A Model for the Appraisal of Glucose Metabolism. J. C. SEED, F. S. ACTON, and A. J. STUNKARD, New York, N. Y., Princeton, N. J., and Philadelphia, Pa. (Introduced by F. D. W. Lukens).

The interpretation and correlation of data on carbohydrate metabolism can be simplified by plotting separately the behavior of the major factors concerned with glucose homeostasis. The combination of such plots gives a reasonably complete description of glucose homeostasis.

Currently available data permit one to plot as a series of straight lines, the major components of the system mediating glucose homeostasis. Such straight lines, representing a series of linear differential equations, were plotted as follows: *1*) glucose utilization (dx/dt) against glucose concentration (x) for liver, muscle (periphery) and brain, *2*) glucose production by the liver, and *3*) the rate of glucose diffusion in a two compartment system. Inspection of such plots, which are based on experimental data, allow the prediction of changes in any element of the system when another element is altered, as for example, by a glucose load, the administration of insulin, or muscular exercise. Results of this inspection may be given more precise form by combining and integrating the differential equations by the techniques of numerical calculus.

The equation obtained by this integration has already proven useful in clinical work. It has greatly facilitated interpretation of the intravenous glucose tolerance test. Comparison of the integrated equation to the raw data of the test permits the calculation of the values for the individual physiological functions which are required to produce the final shape of the curve. The equation also accounts for the hypoglycemia occurring during the intravenous glucose tolerance test and for the fact that this hypoglycemia is proportional to the rate of fall of blood glucose concentration.

The Biosynthesis and Metabolism of L-Fucose in Man. STANTON SEGAL and YALE J. TOPPER, Bethesda, Md. (Introduced by Truman Schnabel, Jr.).

A significant amount of L-fucose (6-deoxy-L-galactose) is known to occur in man, not only as a constituent of blood group polysaccharides in red blood cells, saliva, gastric mucin and ovarian cysts, but also as the free sugar in urine and milk. This sugar is of interest physiologically because of its role in blood group specificity and biochemically because of its unusual structure. To study fucose biosynthesis, glucose-6-C^{14} was administered intravenously to two normal postpartum lactating females. Milk was collected and pooled five hours later. Fucose and lactose were isolated and chemically degraded. The pattern of C^{14} labeling in fucose was found to be similar to that in the other hexoses, suggesting the direct conversion of glucose to fucose with the hexose carbon skeleton remaining intact. Such a transformation involves the reduction of carbon atom 6 and the inversion of the configuration of C-2, C-3 and C-5. Metabolism of fucose was studied by the intravenous administration of fucose-1-C^{14} to a normal subject. Within six hours 57 per cent of retained C^{14} was excreted as expired $C^{14}O_2$, peak labeling of $C^{14}O_2$ being observed within 30 minutes. Thirty-two per cent of the C^{14} dose was excreted in the urine, mostly as unaltered fucose. *In vitro* studies to delineate the pathway of fucose catabolism in mammalian tissues are being undertaken with rabbit and beef tissue. Fucose appears to be metabolized to CO_2 chiefly by liver and kidney, with very little catabolism occurring in brain, skeletal muscle, spleen, or red blood cells. These studies have demonstrated that despite its unusual configuration, L-fucose is both synthesized and rapidly metabolized by man.

Relationships of Hypertension and Renal Impairment to Experimental Chronic Pyelonephritis in Rats. ALVIN P. SHAPIRO, Pittsburgh, Pa. (Introduced by I. Arthur Mirsky).

Chronic pyelonephritis was produced in over 100 rats by kidney massage and intracardiac inoculation of bacteria, as previously described. Animals infected repeatedly with *Escherichia coli, Proteus morgagni,* and *Streptococcus zymogenes,* were followed as long as one year, and developed predominantly tubular lesions while glomeruli were relatively intact. Excretory function was significantly impaired. Average osmolal concentration after 24 hours of dehydration was 1,300 milliosmols per Kg. (normal, 2,100 milliosmols per Kg.). Azotemia was absent until osmolality fell below 60 per cent of normal. Despite these changes, vascular disease and significant hypertension did not develop.

Chronic pyelonephritis is considered an important cause of hypertension in man, although many patients with the disease remain normotensive. Absence of hypertension in experimental chronic pyelonephritis, despite otherwise typical pathologic and functional changes, suggested increased susceptibility to renal infection as an alternative explanation for the clinical association between pyelonephritis and hypertension. This possibility has been studied as follows:

Inoculation of *E. coli, without renal massage,* produced

no gross pathologic lesions in kidneys of 15 normotensive rats and positive renal cultures in 20 per cent with only one culture demonstrating heavier than 1 plus growth. This result duplicated previous studies in which the necessity of kidney massage for production of *E. coli* pyelonephritis in normal rats was established. On the other hand, in 17 rats made hypertensive with desoxycorticosterone acetate (DCA) and salt, inoculation of *E. coli, without massage,* resulted in gross lesions in 30 per cent of the kidneys, and positive cultures in 38 per cent, three-fourths of these showing heavier than 1 plus growth. With massage, gross lesions after *E. coli* inoculation occurred in 54 per cent of normotensive and 75 per cent of hypertensive kidneys.

Chronic pyelonephritis in rats produces renal lesions which are functionally and pathologically tubular in character, but hypertensive disease does not result. The data suggest that the relationship between chronic pyelonephritis and hypertension may at least partially represent increased susceptibility of the hypertensive kidney to pyelonephritis.

Hemadsorption in the Diagnosis and Study of Viral Infections. ALEXIS SHELOKOV, Bethesda, Md. (Introduced by Joseph E. Smadel).

Erythrocytes of certain animal species adsorb onto the surface of monolayer tissue cultures infected with the hemagglutinating viruses. The reaction is inhibited by specific antibody and can be used in neutralization tests for serologic diagnosis and virus identification.

Hemadsorption technique, employing monkey kidney cell cultures and guinea pig erythrocytes, applied to 93 throat washings from patients suspected of influenza, yielded 21 strains of Asian virus. Moreover, in some instances new isolates were recognized and definitively typed within two days after initiating testing. Only 10 strains were recovered from these specimens when the usual chick embryo procedure was used.

In addition to influenza viruses, hemadsorption is associated with the following: mumps, CA (croup-associated), vaccinia and two newly discovered agents recovered from children with respiratory disease which are designated "hemadsorption viruses 1 and 2." Polioviruses, ECHO viruses types 1 through 11, adenoviruses types 1, 2, 4, 6, 7 and 8, Coxsackie viruses A9, B-1 through B-5, and herpes simplex virus do not display hemadsorption.

Hemadsorption provides a simple, rapid means for diagnosing and studying a number of viral diseases.

A Study of the Transplacental Transfer of Thyroid Hormones in Sheep. ROBERT H. SHEPPARD, V. MARILYN TURNER, and WILLIAM M. PAUL, Toronto, Canada. (Introduced by W. Hurst Brown).

Evidence for or against the passage of thyroid hormones across the placenta is conflicting. Most studies conducted to date have been indirect, *e.g.,* attempting with thyroid hormone to prevent the formation of thiouracil or percholate fetal goiters. When I^{131} labeled hormones have been used, samples were usually too small or activity too low for adequate chromatography or autoradiography.

It is possible, in the sheep, to place a cannula directly into a sufficiently large fetal artery that adequate blood samples can be withdrawn without interfering with the normal placental circulation, and without leakage. This was done in a series of 15 experiments and radioactive iodine (I^{131}) alone or I^{131} labeled L-thyroxine or L-triiodothyronine was administered on different occasions to mother or to fetus. Serial blood samples were taken from each circulation up to six hours from the time of injection. Utilizing an ethanol extraction technique and paper chromatography in a butanol-dioxane-ammonia solvent system, L-thyroxine, L-triiodothyronine and iodide were separated. The radioactivity was measured by cutting each chromatogram into a series of transverse strips and by counting each strip in a well-type scintillation counter. The peaks of radioactivity were identified by the use of suitable added standards.

Almost immediately, I^{131} administered as sodium iodide passed across the placenta. After administering labeled hormone, some radioactivity could be detected in the opposite circulation. However, on chromatography, all of this activity was found to be in the iodide area and was presumably the result of hormone deiodination. None was present in the L-thyroxine or L-triiodothyronine areas. Thus, in these acute experiments, no transmission of labeled thyroid hormones from mother to fetus or from fetus to mother was demonstrated.

The Appearance of a Fibrinolysin Activator in the Blood of Patients with Enhanced Fibrinolytic Activity. SOL SHERRY,* ROBERT I. LINDEMEYER, ANTHONY P. FLETCHER, and NORMA ALKJAERSIG, St. Louis, Mo.

Increased fibrinolytic activity in the human circulation has been reported following electroshock therapy, pyrogen injections, intense exercise, epinephrine, and ischemia. The mechanism underlying this increased activity has not been investigated. Two possible explanations have been advanced: a reduction in the antifibrinolytic activity of blood, or the appearance in the blood of an activator of the naturally occurring profibrinolysin.

Utilizing a variety of methods, a study has been made of the state of fibrinolytic activity in the blood of patients subjected to the various stresses mentioned above. The lysis time of a clot formed from the plasma euglobulin fraction and the ability of the plasma euglobulin to lyse preformed I^{131} tagged human plasma clots proved to be most sensitive for demonstrating the appearance of increased fibrinolytic activity.

Increased fibrinolytic activity could be demonstrated in almost all patients subjected to electroshock, pyrogen administration, or intense exercise, but was observed with less consistency following epinephrine and local ischemia. With the latter, fibrinolytic activity, when observed, was most striking in the venous blood draining the ischemic area, confirming the recent observations of Kwaan that fibrinolytic activity can be locally produced.

The production of intense fibrinolytic activity in the blood of these patients was associated with the appearance of a fibrinolysin activator and without significant reduction in antifibrinolytic activity, profibrinolysin or fibrinogen concentration. The presence of an activator was demonstrated by activation experiments employing purified profibrinolysin as the substrate and the plasma euglobulin as the source of activator. These observations support the view that under stress situations of the type described, circulating fibrinolytic activity increases due to the release of a fibrinolysin activator.

Inhibition of Water Reabsorption in the Intact Perfused Renal Tubule by Ouabain and Dinitrophenol: Evidence for Active Sodium Transport. JOSEPH C. SHIPP, Boston, Mass. (Introduced by Eugene Eppinger).

The following experiments were designed to study water and sodium reabsorption by the renal tubule. The effects of ouabain and dinitrophenol on water reabsorption were studied. Ouabain, a cardiac glycoside, is known to inhibit cation transport in isolated tissues. Dinitrophenol reduces the available metabolic energy required for active transport.

Collection and stopped flow perfusion experiments were performed on the intact proximal renal tubule of anesthetized Necturus. Classical micropuncture techniques of Richards were used. C^{14} labeled inulin served as an indicator of net water movement. In stopped flow perfusions a tubule was blocked with oil near the glomerulus and distally; the tubule was filled and the perfusate removed after 20 minutes.

In six experiments, fluid collected from the distal 20 per cent of the proximal tubule showed a tubular fluid ÷ serum inulin ratio of 1.52, indicating a mean water reabsorption of 33 per cent. Similar collections in four animals which had received ouabain (0.01 mg. per animal) intravenously showed a decrease in net water reabsorption to 15 per cent. In control studies stopped flow perfusions showed a mean water reabsorption of 28 per cent, in good agreement with the collection data. In seven perfusion experiments, ouabain in the perfusate (1.4×10^{-4} M per L.) reduced net water reabsorption to 10.2 per cent, indicating direct inhibition of the mechanism responsible for water reabsorption. This direct action on tubular transport may contribute to the diuretic effect of cardiac glycosides in man. In seven experiments where dinitrophenol (2×10^{-4} M per L.) was included in the perfusate, water reabsorption was reduced to 9.8 per cent. This suggests that water transport is effected by a mechanism whose energy supply is susceptible to dinitrophenol inhibition.

The present observations of water movement in a renal tubule known to have a transtubular electrical potential, negative on the luminal side, are consistent with the hypothesis that active Na transport is responsible for water reabsorption.

Thyroid Secretion of Nonthyroxine Iodine. D. W. SLINGERLAND, E. S. DELL, D. E. GRAHAM, A. P. TRAKAS, and B. A. BURROWS,* Boston, Mass.

Measurements were made in hyperthyroid patients and euthyroid subjects of thyroidal radioiodine, serum protein-bound radioidine (PBI^{131}) and chemical iodine (PBI^{127}) concentrations and urinary radioiodine excretion (URI^{131}) following a tracer I^{131} (500 μc). After five to seven days of equilibration the administration of methimazole (Tapazole®) resulted in a rapid increase in URI^{131}, predictable from a complete block of thyroid uptake of "recycled" iodide in hyperthyroidism, but greater than the predicted value in normal subjects.

Usually, during the course of Tapazole®, the ratio of daily URI^{131} to PBI^{131} concentration gradually diminished. In some patients this ratio reached a constant value, although PBI^{127} continued to fall. In several patients this ratio decreased abruptly by as much as 60 per cent with the administration of iodide, with a slowing of the apparent discharge of thyroidal radioiodine, but without appreciable changes in PBI^{131} or PBI^{127}. If the later urinary radioiodine is derived solely from the peripheral degradation of PBI^{131}, then the earlier ratio (significantly higher than the final ratio) of URI^{131} must represent iodine from some source other than circulating hormonal radioiodine.

These findings were confirmed by repeated short-term studies with I^{132}: Serum inorganic I^{127} and I^{131} (calculated from serum I^{132} levels and urinary iodine specific activities) increased sharply with Tapazole® and the serum inorganic I^{131} fell with iodide administration. Studies of I^{131}-thyroxine degradation in myxedema did not reveal any peripheral effects of Tapazole®, iodides, or triiodothyronine.

This study suggests that iodide or organic iodine, other than thyroxine, is being secreted from the thyroid gland of hyperthyroid patients in significant amounts; that antithyroid compounds (including iodide) may suppress the release of this material; that antithyroid compounds may block the intrathyroidal cycling of iodide released by deiodinating mechanisms in normal subjects; and that a defect in reutilization of such intrathyroidal iodine may exist in untreated hyperthyroidism.

Pyrimidine Metabolism in Man: The Biosynthesis of Orotic Acid. LLOYD H. SMITH, JR. and FAITH BAKER, Boston, Mass. (Introduced by Edward Bland).

Considerable information is available concerning purine metabolism in man. This has been obtained primarily by the study of uric acid excretion and the rate of incorporation of isotopic precursors into it. In contrast almost no information is available concerning pyrimidine metabolism in man, due in large measure to the absence of a pyrimidine excretory product comparable to uric acid. The potential importance of methods for assaying pyrimidine metabolism in man is apparent from a consideration of the genetic and controlling functions of nucleic acids, the role of pyrimidine nucleotides in intermediary metabolism, and the increasing interest in pyrimidine analogues as anti-neoplastic agents.

In this overall study of pyrimidine metabolism, initial attention has been directed to the synthetic pathway. Using C^{14}-labeled precursors the following enzymatic steps

in pyrimidine synthesis have been identified in circulating human blood cells:

I Carbamylphosphate + aspartate $\rightleftharpoons$ carbamylaspartate.

II Carbamylaspartate $\overset{-H_2O}{\rightleftharpoons}$ dihydroorotate.

III Dihydroorotate $\overset{-2H}{\rightleftharpoons}$ orotate.

The enzymes involved in I and II are found in solution in red cell hemolysates and white cell sonicates, in which their biochemical characteristics have been determined. The enzyme of III has not been obtained in solution, has been found to be localized in white cell nuclei, and is virtually absent from circulating erythrocytes. Step III can be demonstrated, however, in nucleated avian erythrocytes. For each step the normal range of enzyme activity per unit number of cells has been determined. Free carbamylaspartate, dihydroorotate, and orotate could not be demonstrated in plasma or erythrocytes. The usefulness of these assay systems for the *in vitro* evaluation of a newly synthesized inhibitor of pyrimidine formation, *l*-carbamyl cysteic acid, has been demonstrated. These studies are currently being extended to leukemic cells.

Metabolism of Cortisone by Malignant Prostatic Tissue. JOSEPH E. SOKAL and W. ROY SLAUNWHITE, JR., Buffalo, N. Y. (Introduced by Philip K. Bondy).

When patients with advanced carcinoma of the prostate were given 300 mg. of cortisone acetate per day, a striking rise in their total urinary 17-ketosteroid output was observed; this phenomenon did not occur in normal men or in patients with debilitating non-neoplastic diseases (Sokal *et al.*, Yale J. Biol. Med. 1953, **25**, 320). This finding has been confirmed in an additional group of patients. This effect was not observed in most patients with other cancers, but was seen in some. Next, extraction and fractionation of the 17-ketosteroids excreted during baseline and cortisone periods was accomplished in three consecutive patients with carcinoma of the prostate. It was found that the difference between these patients and controls is due principally to a rise in 11-ketoetiocholanolone excretion, which averaged 14.3 mg. per 24 hours more than the baseline excretion, as compared with 2.0 mg. per 24 hours for controls with cancer of the lung.

In order to determine whether this phenomenon represents a host change in patients with prostatic carcinoma or is a specific manifestation of the metabolic activity of the tumor, slices of prostatic adenocarcinoma obtained at surgery were incubated with radioactive cortisone *in vitro*. The conversion of cortisone to adrenosterone was demonstrated in both of two experiments. Presumably this compound is further metabolized, probably in the liver, to the 17-ketosteroids identified in the urine. Thus the abnormal urinary ketosteroid pattern observed in patients with prostatic carcinoma during cortisone administration reflects a hitherto unsuspected metabolic activity of this tumor. The conversion observed in these experiments has not previously been reported for human tissue *in vitro*.

Studies of Properdin in Cancer Patients. CHESTER M. SOUTHAM, New York, N. Y. (Introduced by J. H. Burchenal).

A survey of serum properdin levels in over 150 patients with neoplastic diseases of many types confirms the previously reported increased frequency of low properdin levels (less than 2 units per ml. by Pillemer's zymosan technique). The total data, as well as serial determinations in individual cancer patients, indicate that properdin depression tends to correlate with severity of disease, but several unexplained discrepancies have been found.

Sera from several cancer patients gave a zone effect in successive serum dilutions in the zymosan assay. This may be caused by properdin inhibitors, which have been demonstrated in two sera, or by a high titer of endogenous complement (C'3) which obscures the properdin-zymosan reaction.

Administration of bacterial polysaccharides (Pyrexal®) failed to elicit the expected rise in serum properdin in cancer patients.

Human properdin administered intravenously in doses of 500 units per Kg. or more to patients with properdin deficiency was detected only briefly (up to two hours) in serum. When similar doses were given daily for five days this disappearance from the blood stream became less rapid, and low levels of properdin remained detectable in serum for about a week thereafter. The data suggest that administered properdin was rapidly bound to as-yet-unidentified tissue sites rather than excreted or metabolized.

The present studies do not permit any evaluation of possible therapeutic effects.

The Role of pH Gradients in the Distribution of Ammonia Between Blood and Cerebrospinal Fluid (CSF), Brain, and Muscle. JAMES STABENAU, KENNETH S WARREN, and DAVID P. RALL, Bethesda, Md. (Introduced by Jacob Robbins).

A pH gradient normally exists between blood and cerebrospinal fluid, with blood about 0.1 units alkaline to CSF. Similar gradients presumably exist between blood and muscle or brain, and can influence the distribution of compounds which are partially ionized at body pH, since only the un-ionized form is freely diffusible. We have examined the distribution of one such compound, ammonia, between blood and CSF, muscle, or brain in anesthetized dogs infused with ammonium bicarbonate. The control CSF/blood ammonia ratio was 0.6; brain/blood ammonia was 0.8. After NaOH infusion blood pH rose to 7.7 while CSF pH remained essentially constant. With this larger gradient, CSF/blood ammonia rose to 1.3, and brain/blood ammonia became 2.0. HCl infusion lowered blood pH to 7.1; CSF pH again remained unaltered. With a reversed pH gradient CSF/blood ammonia fell to 0.4, and brain/blood ammonia became 0.9. Respiratory alkalosis and acidosis gave similar data, and muscle ratios were even more striking than brain ratios. If only un-ionized ammonia diffuses freely between blood and CSF, the ratio of total ammonia between these compartments is the in-

verse ratio of the percent un-ionized ammonia (which is pH dependent) in each compartment. Assuming that pH determines the CSF/blood ratios, "apparent free plasma ammonia" was calculated and used to show that the blood/tissue ratios obtained experimentally were to be expected from known estimates of tissue pH. These experiments indicate the variations observable between blood and tissue ammonia. Since ammonia toxicity presumably occurs within the tissues, recognition of the influence of pH gradients on ammonia distribution may allow estimation of brain ammonia concentration, and the role of ammonia in hepatic coma may be more clearly evaluated.

The Effect of Intravenously Administered Ceruloplasmin on Copper Absorption in a Patient with Wilson's Disease. IRMIN STERNLIEB, ANATOL G. MORELL, and I. HERBERT SCHEINBERG,* New York, N. Y.

Two features of the disordered copper metabolism which characterizes Wilson's disease are marked deficiency, or absence, of the plasma copper-protein ceruloplasmin, and abnormal absorption of orally administered copper. Several investigators have postulated that ceruloplasmin deficiency is the primary inherited defect which results in abnormal and excessive copper absorption. This paper reports results of experiments designed to test this hypothesis.

When radioactive copper^{64} is orally administered to normal subjects the serum concentration of copper^{64} rises promptly, falls and rises a second time several hours later. The initial copper^{64} appearing in the serum is essentially all loosely bound to albumin, while the second elevation in copper^{64} concentration is due to ceruloplasmin copper^{64}. The latter may result from *1*) copper^{64} incorporation in newly synthesized ceruloplasmin, and/or *2*) exchange of copper^{64} with copper in pre-existing ceruloplasmin.

When copper^{64} is administered to patients with Wilson's disease the initial rise and fall in serum copper^{64} concentration appear, but there is no second elevation.

A patient with Wilson's disease was given 2.3 mg. of copper as cupric^{64} acetate in each of two experiments two weeks apart. Twenty-one hours prior to the second experiment 1.35 Gm. of purified ceruloplasmin was administered intravenously and this produced a normal serum ceruloplasmin concentration of 25 mg. per 100 ml. Copper^{64} concentrations in serum and in ceruloplasmin were measured at intervals. In both experiments serum copper^{64} rose initially, and fell, but there was essentially no secondary rise and no copper^{64} in ceruloplasmin. In both experiments 42 per cent of the administered copper^{64} was recovered in stools and urine. These results suggest that: *1*) Exogenous ceruloplasmin does not alter the manner or amount of copper absorption in patients with Wilson's disease, and *2*) these patients seem unable to effect exchange of exogenous ceruloplasmin copper with orally administered copper^{64}.

The Existence of a Large, Slowly-Exchangeable Pool of Body Sodium. DAVID H. P. STREETEN, ABRAHAM RAPOPORT, and WILLIAM S. WILSON, Ann Arbor, Mich. (Introduced by Jerome W. Conn).

Injected radiosodium exchanges with a pool comprising 70 per cent of total body sodium in 24 hours. Observed expansion of this pool (Na_e) over the ensuing six days has been assumed to result from unmeasured extrarenal sodium losses. Sodium unexchanged at 24 hours has been considered relatively inert and unexchangeable.

In seven normal, young adult males, Na_e has been estimated from daily serum and urinary counts for one to four weeks after administration of 3 μc. $Na^{22}Cl$. Sodium intake was 6.9 mEq. per day in two subjects, to minimize extrarenal losses. Two others received constant, normal sodium intake, and were not allowed to wash for two periods totaling two weeks. Determinations of stable sodium and of radioactivity were performed on ashed stools and on concentrates of bath fluid used for the final washing of the body, clothing, plastic stockings and bed clothes. In the three remaining subjects similar studies of losses of sodium from feces and skin were restricted to four day periods.

Na_e increased exponentially throughout the study in all subjects, mean increments varying from 17.0 to 66.7 mEq. per day. These changes did not result from extrarenal sodium losses which were below 5 mEq. per day in six subjects and 6.8 mEq. per day in the seventh. Mean Na_e increased from 42.6 mEq. per Kg. at 24 hours to 50.8 mEq. per Kg. after two weeks. In two subjects, final values of Na_e approached reported estimates of the total amount of body sodium. Since the expansion of Na_e had not ceased at the end of the observation periods, it is concluded that most, if not all, body sodium is eventually exchangeable in young adult males. The slowly exchangeable component of the sodium pool is probably in bone. In studies of sodium metabolism the significance of a slowly versus a rapidly expanding Na_e is obvious.

Relation of Size of Ventricular Septal Defects to Circulatory Dynamics. H. J. C. SWAN, ROGER M. SAVARD, and JOHN W. KIRKLIN, Rochester, Minn. (Introduced by Earl H. Wood).

Ventricular septal defects were measured at operation in 43 and at necropsy in 2 patients in whom circulatory dynamics had been determined previously at cardiac catheterization. A critical area for such defects should exist above which pressures in the right and left ventricles would equalize, while less area would offer sufficient impedance to blood flow between the ventricles to prevent equalization of pressures.

Systolic pressures were significantly less in the right ventricle than in the left in 11 of 13 patients with defects of less than 0.9 sq. cm. per M.^{2} of body surface and an inverse correlation was demonstrated between size of the defect and the left-to-right systolic pressure gradient. Systolic pressures were similar in the left and right ventricles of 21 of 24 patients with defects that exceeded 1.2 sq. cm. per M.^{2}, and this relationship did not vary as defect size increased. Calculated resistance to flow across

the defect (pressure/flow index) was related in the theoretically expected manner to defect area. The ratio of pulmonary to systemic vascular resistance (*i.e.*, the relative resistance to blood flow through the pulmonary vessels independent of body size) showed a poor positive correlation in patients with defects of 1.0 sq. cm. per M.^{2} or less, and no correlation was evident in patients with larger defects. In this latter group there was no correlation between defect area and magnitude of left-to-right shunt.

It is concluded that in the absence of high pulmonary vascular resistance, ventricular septal defects of less than 1.0 sq. cm. per M.^{2} offer sufficient impedance to flow across the defect to prevent equalization of right and left ventricular systolic pressures. Defects of larger size virtually equalize ventricular pressures, and other factors, chiefly pulmonary vascular resistance, determine pulmonary blood flow and intracardiac shunts.

Homografts of Bone Marrow in Dogs After Lethal Total-Body Radiation. E. DONNALL THOMAS, HARRY L. LOCHTE, JR., ALFRED JARETZKI, III, CHARLES A. ASHLEY, and OTTO D. SAHLER, Cooperstown, N. Y. (Introduced by Joseph W. Ferrebee).

Life-saving homografts of bone marrow after lethal total-body radiation usually succeed in rodents but fail in dogs. Presumably a major problem in the dog is an active immune mechanism relatively resistant to radiation. To overcome this resistance the following procedures have been employed: *1*) Radiation dose has been increased beyond the LD_{100} range. *2*) The dose has been divided and given over a three day period. *3*) Recipients have been splenectomized and given adrenocorticotrophic hormone (ACTH) before radiation. *4*) Both donors and recipients have been of the same breed. *5*) The dose of bone marrow has been kept above 5 billion cells.

By a combination of these procedures and with the use of antibiotics and good nursing care, successful marrow homografts have been produced in six of seven dogs given 800 to 1,200 r. Successful grafts are attended by a return to the peripheral blood of all three formed elements and by an appearance of female leucocytes in male dogs that received marrow from female donors.

Inhibition of immune mechanisms to the degree required for a successful homograft implies loss of other immune responses. Greatly increased liability to infection is observed in irradiated dogs even after the formed elements of the peripheral blood have returned to normal. For this reason, transplantation of splenic tissue from adult or fetal donors is being studied as a means for re-establishing immunologic responsiveness and resistance to infection.

The bearing of these studies upon work with irradiated patients and upon homografts of other organs will be discussed.

The Relaxation Pressure-Volume Diagram in Pulmonary Disease. ER YI TING and HAROLD A. LYONS, Brooklyn, N. Y. (Introduced by Perrin H. Long).

The relaxation pressure diagram for normal human subjects has been constructed by Rahn and his associates as a method for the study of the mechanics of respiration. No previous reports of its use in the study of pulmonary disease have been made.

This study concerns itself with the determination of the relaxation pressure-volume diagram (P_R) in patients who have three distinct forms of respiratory impairment, *i.e.*, obstructive disease, restrictive disease, and combined disease of the lung. The results in each type of pulmonary disease are compared to the results found in the normal subjects.

After the total lung capacity and its subdivisions were measured, the intrapulmonary pressure (P_R, the total pressure) and the intraesophageal pressure (P_L) were measured. Relaxation pressure-volume curves were constructed and a similar curve for lung tension was plotted, and by subtraction of lung pressure P_L from P_R, the thoracic wall pressure (P_C) was obtained. The data so obtained gave reproducible curves.

In the restrictive disease group, the pressure-volume diagram was markedly different from that of the normal subjects. The curves are flattened and shift downward and to the right. In patients with obstructive disease, for example, emphysema, there is also a marked alteration in the P_R curve. It assumes a concave shape with a shift upward and to the left. With combined obstructive and restrictive disease of the lung, the P_R curve is raised on a higher level than normal, but in an area lying between the obstructive and restrictive type of curves. It can be shown that these findings may be due to one of two factors or a combination of: *1*) alteration of the lung pressure (P_L), when the disease is parenchymal, or *2*) alteration of the chest wall curve (P_C), when the disease is of the chest wall as in trauma or kyphoscoliosis.

The study also demonstrated that the reduced compliance of the lung is due to either parenchymal or thoracic change.

The significance of these results will be discussed. An analysis of the contributing factors involved in the alterations of the mechanics of respiration has not been given the attention it deserves. Some of the data suggest that some current thoughts of the stress-strain properties of the lung may have to be modified.

Urinary Excretion of Delta-Aminolevulinic Acid in Various Hematological Disorders. GARSON H. TISHKOFF, WILLIAM MCFARLAND, and LOUIS J. FAENZA, Boston, Mass. (Introduced by William Dameshek).

The role of δ-aminolevulinic acid (ALA) as a precursor in the biosynthesis of heme has been recognized. This metabolite has also been demonstrated as a constituent of normal urine. On the assumption that the excretion of ALA is primarily a reflection of erythroid biosynthetic function, an attempt has been made to correlate urinary levels of ALA with bone marrow erythroid activity in various blood disorders.

Various hematological disorders affecting erythroid tissue were studied, including erythroid hyperplasia, ery-

throid hypoplasia, maturation arrest and malignant proliferation. The results are expressed as micromols of ALA $\times 10^{-3}$ per ml. of urine. The mean normal urinary level in 16 subjects was 30.5 ± 2.9 as determined on random urine specimens. Elevated levels of 150 to 350 were found in various porphyrinurias. These included one patient with acute intermittent porphyrinuria and three patients with lead intoxication. Patients with marked erythroid hyperplasia as a result of hemolytic disease, such as hereditary spherocytosis, Cooley's anemia and acquired hemolytic anemia, evidenced values in normal or near normal range. A case of untreated pernicious anemia with maturation arrest also showed a normal urinary level of ALA.

Low values of ALA were seen in 14 patients with hypoplastic anemia. In these, urinary levels ranged from 1.7 to 12.0. Four patients with myelofibrosis and myeloid metaplasia showed decreased values ranging from 3.3 to 6.6. Low values of ALA were also observed in patients with malignant proliferation, either as a result of acute leukemia, erythroleukemia or metastatic carcinoma.

It is suggested that determinations of urinary δ-aminolevulinic acid may be of value in assessing the degree of bone marrow injury in such conditions as hypoplastic anemia, including those due to large doses of ionizing radiation, and myelofibrosis.

The Effect of pH on Norepinephrine-Induced Contractions of Arterial Smooth Muscle. LOUIS TOBIAN,* STEPHEN MARTIN, and WILLIAM EILERS, Minneapolis, Minn.

Spirally cut strips of rat aorta were mounted under 0.5 Gm. tension in Krebe-Henseleit medium with 25 mEq. of bicarbonate per liter. The bath was alternately aerated with gas containing either 4.5 or 11 per cent CO_2, giving pH's of 7.5 and 7.2, respectively. These alternating changes of pH did not by themselves change the tension of the strips. Then norepinephrine (10^{-9}) was added to the bath at pH 7.5 and the increased tension was measured isometrically. Then the norepinephrine was washed out, allowing the tension to return to the 0.5 Gm. baseline; the pH was changed to 7.2 and norepinephrine (10^{-9}) was again added. These alternations of pH were continued consecutively for five successive contractions in each of six strips. At pH 7.5 the strips developed an average additional tension of 0.30 Gm. with norepinephrine, while at pH 7.2 the average additional tension was only 0.17 Gm. The acid medium caused an inhibition of contraction in every strip, averaging 43 per cent inhibition. In a similarly arranged study with six other strips, the pH was alternately changed from 7.5 to 7.2 by alternating media with either 28 mEq. or 14 mEq. of bicarbonate per liter, respectively, while keeping the CO_2 constant. Again the average increase in tension with norepinephrine at pH 7.5 was 37 per cent greater than that at pH 7.2. In three other strips the pH was alternated between 7.35 and 7.15 by changing the bicarbonate concentration while keeping the CO_2 constant. The increase in tension with norepinephrine was 38 per cent greater at 7.35 than at 7.15. Summarizing, in every strip of every study the contraction was invariably diminished in the more acid medium ($p = 0.00001$). This would suggest that acidotic patients can be rendered more sensitive to norepinephrine by correcting their acidosis.

Effects of "Dry" Heat Exposure on Splanchnic Flow and Resistance in Man. ELMERICE TRAKS and SALVATORE M. SANCETTA, Cleveland, Ohio. (Introduced by George J. Gabuzda).

Previous observations have shown that an elevation in the ambient temperature produces musculo-cutaneous vasodilatation. Our data have demonstrated decreases in the brachial and pulmonary artery pressures, total systemic and pulmonary resistances, with no change in cardiac output in man during a two hour exposure to ambient temperature of 37° C. and relative humidity of 40 per cent. It has been generally assumed that this vasodilatation is accompanied by visceral vasoconstriction. The following experiments were designed to test this hypothesis.

Splanchnic blood flows (BSP extraction) were determined in a group of six subjects during a two hour exposure to 23° C. and 40 per cent humidity. These served as controls for the method. In 10 subjects, following basal determinations at the above environment, flows were determined at the end of one and two hours' exposure to ambient temperature of 37° C. and humidity of 40 per cent. Both groups included patients with normal and enlarged left ventricles.

The group of subjects observed at the comfortable environment had average flows of 746, 772, and 721 ml. per $M.^2$ per minute, and splanchnic resistances of 7.6, 7.7, and 7.9 resistance units for the initial, one hour and two hour determinations, respectively. Those exposed to heat had average flows of 731, 778, and 741 ml. per $M.^2$ per minute, and resistances of 9.0, 7.5, and 5.9 units for the control, one hour and two hour periods. Only the changes in resistance in the latter patients were statistically significant ($p = < 0.001$). Three of the ten patients exposed to heat were in left ventricular failure; their data did not differ from the other seven not in failure.

The data indicate that vasodilatation rather than vasoconstriction occurs in the splanchnic bed when ambient temperature is acutely elevated and humidity is maintained at a relatively low level.

Production of Free and Conjugated Corticosteroids by Perfused Human Placentas. PHILIP TROEN, Boston, Mass. (Introduced by A. Stone Freedberg).

The contribution of the human placenta to synthesis or metabolism of corticosteroids has not been clear. Indirect evidence has been adduced for placental production of corticosteroids as a cause of the elevated plasma corticosteroid levels observed during pregnancy. Other workers, however, have attributed this change to decreased corticosteroid metabolism.

Perfusion of the human placenta by a technique developed in this laboratory seemed a promising direct ap-

proach to this problem. Validity of the methodology has been supported by our recent demonstration of continuing placental metabolism in the form of citrate utilization during perfusion for 12 hours.

Ten placentas from normal pregnancies were obtained immediately after delivery and perfused with a modified Tyrode's solution containing citric acid. Seven placentas were perfused 12 hours, two 8 hours, and one 6 hours. The perfusate was analyzed for free and conjugated corticosteroids using dichloromethane extraction, β-glucuronidase hydrolysis, and the Porter-Silber (P-S) reaction.

Mean cumulative production (in cortisol equivalents) of total P-S material per placenta during perfusion for 12 hours was 8.2 mg. (range, 0.75 to 14.9 mg.). Mean hourly production per placenta increased linearly from 0.13 mg. during the first hour to 1.5 mg. during the twelfth hour. Mean cumulative ratio of free to conjugated material was 2 (range, 0.15 to 4.5). Extraction of placental homogenates yielded less than one-twentieth the amount obtained from perfusates, thus ruling out release of preformed P-S material.

The nature of the P-S material was studied. Ethyl acetate extracts of perfusates were analyzed by countercurrent distribution (3 solvent systems, 100 tube apparatus) and by paper chromatography (Bush B-5 system) combined with ultraviolet absorption, P-S reaction, and blue tetrazolium staining. Preliminary results are consistent with the presence in perfusates of cortisone, cortisol, tetrahydrocortisone, tetrahydrocortisol, compound S and as yet unidentified polar material.

These results indicate the human placenta synthesizes and conjugates corticosteroids.

Platelet Thromboplastic Factor: Its Chemical Nature, In Vitro Activity, and the Identification of Similar Thromboplastic Substances in Red Blood Cells. STANLEY B. TROUP and CLAUDE F. REED, Rochester, N. Y. (Introduced by S. N. Swisher).

A substance supplied by normal platelets is required for the formation of thromboplastin during the clotting process. This substance is known to be or to contain phospholipid, and it has been suggested that it is a single phospholipid, phosphatidylethanolamine. Quick has also demonstrated the presence of a factor in normal red blood cells that can contribute to thromboplastin formation.

Methods recently developed allow the extraction and separation of the unaltered lipids from platelets and red cells, and the identification and measurement of the components. The component lipids from platelets and red cells have been isolated, highly purified, and their thromboplastin generating activity measured.

The following observations have been made: *1*) The composition of the lipid fraction of platelets and red cells is similar. *2*) The red cell lipid fraction contributes to thromboplastin generation and probably is the red cell factor described by Quick. Red cell phospholipids are fully as active as those found in platelets. *3*) Of the various phospholipids, only phosphatidylserine gives maximal thromboplastic effect at a concentration of 2 micrograms per ml. of platelet-free plasma. Phosphatidylethanolamine can also give a maximal effect, but requires a much larger amount. *4*) One microgram of the total lipid extract per ml. of platelet-free plasma augments thromboplastin generation although this extract contains only 7 per cent phosphatidylserine.

The unique role of phosphatidylserine and phosphatidylethanolamine as cellular components of the thromboplastin generation system is suggested by their virtual absence from normal plasma. The hemorrhagic tendency or thromboses frequently seen in disorders involving intravascular hemolysis may be related to the release of the lipid substances from the lysed red cells.

Hypercalciuria Associated with Reduction in Corticoid Therapy After Prolonged Administration of Prednisone and After Bilateral Adrenalectomy for Cushing's Syndrome. STEWART G. TUTTLE and WM. G. FIGUEROA, Los Angeles, Calif. (Introduced by John S. Lawrence).

This study documents exchanges of calcium, phosphorus and nitrogen during the recovery phase of two men with Cushing's Syndrome. Disease in the first resulted from three years' therapy of pemphigus vulgaris with cortisone and prednisone; the second was a case of spontaneously occurring bilateral adrenal cortical hyperplasia. Balance data have been obtained: 670 days in the first patient, and 225 days pre- and postadrenalectomy in the second. While on a constant regimen slow reduction of a dose of prednisone in Case 1 from 25 to 5 mg. per day resulted in a fall in urinary calcium and storage of nitrogen and phosphorus. However, at a dosage of 3 mg. per day, although there was no recurrence of skin lesions, symptoms of corticoid withdrawal were noted and were accompanied by hypercalciuria, increased fecal calcium, negative calcium balance and a small rise in serum inorganic phosphorus. These were controlled by increase in the dose of prednisone to 5 to 7 mg. per day. Findings similar to the above were observed during cortisone replacement therapy in the postoperative studies made on the second patient. When receiving 75 to 100 mg. cortisone per day calcium, phosphorus and nitrogen were retained and urinary calcium was normal. Reduction of the dose of cortisone to 37.5 mg. per day produced mild symptoms of adrenal insufficiency, calciuria of 400 to 500 mg. per day and an increase in fecal calcium. The balance of nitrogen remained strongly positive. Hypercalciuria disappeared when 75 mg. per day dose of cortisone was restored. The complete dichotomy between tissue synthesis (positive nitrogen balance) and loss of calcium in this latter instance seems to cast doubt on the hypothesis that a protein anabolic effect necessarily protects the skeleton against decalcification. Apparently a deficiency as well as an excess of cortical hormones may affect the calcium balance adversely.

The Use of a Patient-Cycled Respirator to Evaluate Mechanical and Central Factors in the Hypercapnia of Emphysema. JOHN M. TYLER and BIRGER GRAPE, Boston, Mass. (Introduced by Thomas C. Chalmers).

The relative importance of mechanical versus central factors in the ventilatory drive of nine emphysematous hypercapnic patients has been studied, using a patient-cycled respirator. A signal proportional to air-flow (obtained by electrical differentiation of a signal from a pneumograph around the patient's chest) is led to a servo-amplifier which controls a valve between a blower and the body respirator, producing an applied pressure proportional to flow. Since this affords continual assistance in overcoming flow resistance and thereby reduces the work of breathing, it would be anticipitated that the ventilatory response to hypercapnia, both resting and increased by inspired CO_2, would be augmented to the extent that mechanical factors limit this response.

The ventilatory response to inspired CO_2 was tested during unassisted and assisted breathing. Arterial pCO_2 (Pa_{CO_2}) was estimated by the rebreathing method of Collier (mean difference in 22 simultaneous comparisons with arterial pCO_2, $+0.4 \pm 2.5$ mm. Hg [1 SD]). Three patients showed a marked augmentation in response to CO_2 during assisted breathing. In these patients, assistance during room air breathing caused hyperventilation. One patient had a severely depressed response to CO_2 which was not changed by assistance. While breathing room air, assistance caused his minute ventilation (V_E), oxygen consumption (V_{O_2}), and carbon dioxide production, (V_{CO_2}), to decline without altering Pa_{CO_2}. It is concluded that in this patient both resting ventilation and the response to inspired CO_2 were governed by a depressed respiratory center, while in others mechanical factors may be a determining influence.

In three patients assistance caused a significant, sustained fall in V_E, V_{O_2}, and V_{CO_2}. Despite the fact that estimated V_A was unchanged or decreased, Pa_{CO_2} declined 7 mm. Hg in two of these. This is consistent with the suggestion of Riley that the metabolic cost of breathing may play an important role in the hypercapnia of emphysema.

Specific Desensitization of the Delayed Hypersensitive State. JONATHAN W. UHR, New York, N. Y. (Introduced by H. S. Lawrence).

The delayed hypersensitive state can be induced to specific protein antigens in guinea pigs or in man by intradermal injection of minute amounts of antigen in the form of an immune precipitate. The degree of sensitization is enhanced if killed tubercle bacilli are incorporated into the suspension containing the specific precipitate. Active antibody production is suppressed under these conditions so that detectable antibody does not appear for two or more weeks after delayed skin reactivity has appeared.

In the present study guinea pigs were sensitized to two immunologically distinct proteins. One to two weeks later after maximal sensitization had been attained the animals were injected with a relatively large amount of one of the antigens. Afterwards, the animals were skin tested with 3 to 5 μg. of both antigens. Skin reactivity to the antigen which had been used for desensitization was specifically and completely abolished, but the same animals retained their skin reactivity to the second antigen to which they were sensitive. Although 2 mg. of specific antigen was required for complete desensitization, as little as 20 μg. sufficed to decrease the size of specific skin reactions in sensitized animals. The duration of the nonreactive state increased as larger amounts of desensitizing antigen were used. The return of delayed skin reactivity was usually associated with the appearance of circulating antibody. Desensitization could be accomplished by the specific antigen-antibody complex as well as by "free" antigen. The appearance of delayed skin reactions could be prevented in fully sensitized animals even when the desensitizing dose was given one or more hours *after* skin testing.

The ease of desensitization in these experimentally sensitized animals contrasts with prior studies reported in the literature in which specific desensitization of animals or man infected with tubercle bacilli was seldom successful.

Respiratory Adaptations in Induced Hyperthyroidism. HEINZ VALTIN and S. MARSH TENNEY,* Hanover, N. H.

In hyperthyroidism there is not only an increased tissue metabolic demand for oxygen but also a need for increased elimination of carbon dioxide. The respiratory apparatus could adapt by raising alveolar ventilation, or carbon dioxide tension, or both; the tissue need could be met by increasing blood flow, or lowering venous oxygen tension, or both.

In order to assess the individual factors which determine either full adaptation or compromise, hyperthyroidism was induced in normal human subjects and albino rats by the administration of triiodothyronine.

In man, a mean increase in basal oxygen consumption of 35 per cent was attended by an equivalent rise in alveolar ventilation with resultant maintenance of normal alveolar gas tensions and respiratory exchange ratio. Ventilatory stimulus-response curves derived by varying concentrations of carbon dioxide in inspired air indicated no significant increase in "sensitivity" of the respiratory center during hyperthyroidism.

"Tissue-venous" gas tensions in rats were approximated by the subcutaneous air pocket technique. For a thyroid-induced mean increase in oxygen consumption of 50 per cent the carbon dioxide tension fell 2.5 mm. Hg and the oxygen tension rose 8 mm. Hg. Tissue gas tensions, when related to rat whole blood respiratory exchange ratio isopleths, reveal deviations from the mean line suggesting increase in tissue blood flow: oxygen consumption ratio.

These data may be interpreted as showing that, for the degree of induced hyperthyroidism studied, respiratory adaptation to the increased oxygen consumption and carbon dioxide production is complete. The tissue-venous gas tensions indicate that regional blood flow has overcompensated tissue oxygen need.

Significance of Virulence and Viability as Related to Quantitative Immunogenic Properties of Several Antituberculosis Vaccines. H. M. VANDIVIERE, Chapel Hill, N. C. (Introduced by H. S. Willis).

Standardization for potency and viability in suspensions of Mycobacteria has long been an enigma of import, both requiring prolonged periods and measurable in retrospect only. Cord-formation titration, omental index (potency) and formazan curve interpolation (viability) are described, with corroboration, as standardization methods. Several vaccines have been so standardized and their immunogenic capacities quantitatively investigated.

"Degree of protection" has challenge of "protected subjects" as the final critique. Thus, 3,000 guinea pigs were randomly vaccinated with homogenous suspensions of one of the several vaccines which were standardized before use. Experimental groups were characterized so that statistical significance would evolve from either constant viability with potency, the protean factor or conversely. Tuberculin testing of experimental and control allocates with volume of allergic response $[v = 1/6\ h\pi(h^2 + 3r^2)]$ and duration of allergy calculations were periodic. Ultimately quantitative challenge was accomplished.

Effect of time on immunity was studied by postvaccination challenge at disparate periods from 6 to 30 months. Short-term vaccination reveals negligible differences in protection conferred by the various vaccines, whereas groups challenged at 12 to 30 months present significant differences between strains. The weakest BCG afforded little more protection than no vaccine. Degree of protection by R_1Rv is significantly higher than that conferred by the best BCG. Increasing potency and/or viability evokes increasing protection, but increased viability will not completely compensate for potency nor is protection as long duration. Evaluation of disease is based on Feldman's index and comprehensive pathological evaluations from coded gross and microscopic preparations.

Prevaccination cord-formation titer and omental index with postvaccination volume of allergic response, duration of allergy and degree of protection stand in comport for each strain. The weakest vaccine gives the least response, the most potent evokes greatest response and the intermediates between, each having its own specific level.

Beginning human studies (2,500) are presented on high-incidence groups.

The Rheumatoid Factor in Rheumatoid Arthritis. JOHN H. VAUGHAN and JEAN HARRIS, Richmond, Va. (Introduced by John L. Patterson, Jr.).

The rheumatoid agglutinating factor is a protein migrating electrophoretically as a fast γ-globulin. It is capable of agglutinating antibody-coated cells and of precipitating with Cohn Fraction II. Studies in this laboratory have emphasized its sharp specificity, as evidenced particularly by its ability to react with cells sensitized only with certain human anti-Rh antibodies. The reaction with rabbit antibody or gamma globulin has been shown to follow the pattern of reaction of an antibody for its cross-reacting antigen.

With the demonstration (Franklin *et al.*) that the rheumatoid factor exists in plasma as a complex of a heavy γ-globulin (19S) with a smaller γ-globulin (7S), attempts were made at its separation by filtration. Using a membrane with a maximum pore diameter of 0.1 μ, the factor was found to be retained, while uncomplexed γ-globulin passed through. The isolated product has the electrophoretic mobility of a fast moving γ- or a β-globulin. It contains carbohydrate to about 5 per cent of the total protein. Agglutination of sensitized cells can be induced by 0.1 to 0.5 μg. N of the material.

After transfusion of a normal individual with 250 ml. of rheumatoid plasma, the factor could be detected in the recipient circulation for two weeks. On repeated weekly transfusions of 250 to 500 ml. for six weeks there was no evidence of the development of an accelerated rate of disappearance of the factor, indicating that the factor was not treated by the recipient as a foreign antigenic substance. There was no evidence of the development of arthritis in the recipient, even when an attempt was made to provoke such reaction by injecting sterile saline into the knee.

The data are regarded as strongly indicating that the rheumatoid factor is antibody primarily directed to a human gamma globulin, cross-reacting with rabbit γ-globulin, and developing because of autoimmunization secondary to the underlying disease.

A Biochemical Study of Certain Skeletal Muscle Constituents in Human Muscular Dystrophy. PAUL J. VIGNOS, JR., Cleveland, Ohio. (Introduced by Walter H. Pritchard).

Progressive muscular dystrophy is the most common primary myopathy and may be regarded as the prototype of primary degenerative muscle disease. The skeletal muscle of 17 ambulatory dystrophics with relatively mild disease was compared with 10 normal controls and a second control group of 8 patients with neurogenic muscle atrophy to determine whether biochemical alterations found are specific. Myosin and enzyme determinations were referred to a parenchymal tissue base.

Degeneration of muscle in progressive myopathy is associated with the appearance of connective tissue and fat cells. Diagnostic significance is attached to the finding of high muscle fat content by histologic examination in dystrophy. However, quantitative muscle fat determinations show neither increase nor significant difference from neural atrophy. A significant increase in collagen nitrogen was found in all diseases studied. This appears to be a more dependable indication of early muscle dysfunction than fat deposition. Water content in experimental muscle atrophy has shown varying results. In the clinical groups studied, juvenile dystrophy showed the only significant reduction in muscle water content.

Muscle glycolysis shows the greatest change in muscular dystrophy. A marked reduction to one-third of the normal values occurred. Two nonglycolytic enzymes in-

volved in phosphate energy transfer, creatine kinase and adenosine triphosphatase showed moderate statistically significant decreases. Succinic dehydrogenase was normal.

A defect in contractile protein of muscle has been suggested as an alternate etiologic concept in dystrophy. The content of the principal myofibrillar protein, myosin, showed an absolute decrease proportional to the reduction in nonglycolytic enzymes.

Previous work has shown that glycolysis is impaired in dystrophy. These data give further support to a serious glycolytic defect but suggest, also, that the biochemical alterations are much more complex and involve both myofibrillar and sarcoplasmic components. The biochemical changes lack specificity but may represent a stereotyped response to dissimilar metabolic errors.

Effect of Sulfate on Physical State and Renal Excretion of Divalent Cations. MACKENZIE WALSER and ANN BROWDER, Baltimore, Md. (Introduced by Gilbert H. Mudge).

Recent physicochemical studies have established that bi-bivalent salts are incompletely dissociated, *e.g.*, the dissociation constant at 40° C. and ionic strength 0.15 is 0.004 for $CaSO_4$ and 0.005 for $MgSO_4$. The concentration of free divalent ions in plasma and urine may therefore be influenced by the concentration of divalent ions of opposite charge. If tubular reabsorption operates upon free ions, the excretion rate of calcium and magnesium should be influenced by the filtrate concentration of sulfate and phosphate. The converse should also apply.

During sodium sulfate infusion in unanesthetized dogs, the ultrafiltrable fraction of plasma calcium and magnesium (determined at 37° C. and pCO_2 40 mm. Hg) increased from 40 to 65 per cent in controls to 70 to 95 per cent, without hypoproteinemia. Tetany occurred despite normal plasma calcium and magnesium when plasma sulfate exceeded 20 mM per L., but could be prevented by inducing hypercalcemia or hypermagnesemia.

The fraction of filtered calcium and magnesium excreted increased from less than 3 per cent and less than 6 per cent, respectively, in controls to 70 per cent with sulfate infusion alone. When plasma chloride was reduced further by peritoneal dialysis with sodium sulfate, 75 to 105 per cent of filtered calcium and magnesium were excreted at normal filtration rate, and creatinine U/P of 2 to 3. These results were nearly independent of variation of plasma calcium from 5 mg. per cent to 24 mg. per cent and variation in plasma magnesium from 2 mg. per cent to 9 mg. per cent.

These findings are pertinent to the reduced protein-binding of calcium in uremia, the therapeutic use of parenteral $MgSO_4$, and the treatment of hypercalcemia and abnormal accumulations of other divalent ions.

A New Bactericidal System in Normal Serum and Plasma. RALPH J. WEDGWOOD, Cleveland, Ohio. (Introduced by W. M. Wallace).

Serum of man and other mammals contains many bactericidal factors presumably related to natural resistance to infection. One, the properdin system, lyses various bacteria, inhibits some viruses and interacts with endotoxins. Another bactericidal system, similar to but distinct from the properdin system, has been found. This system, bactericidal against certain Gram-negative bacteria, has been found in sera and plasma of all mammals tested. Greater activity is found in plasma than serum. The system is heat labile in serum, more stable to heat in plasma. In addition to bactericidal activity, under certain conditions the system induces a transient, nongenetic change in colonial morphology of the test organism resembling transformation from rough to smooth.

Both effects are induced by "endpiece" of complement (supernate following dialysis, pH 5.6, ionic strength 0.02) and hydrazine or ion-exchanged plasma. The system thus appears distinct from the first or fourth components of complement, and from euglobulins, and does not require magnesium, calcium or properdin. Factors resembling the second and third components of complement may be needed.

This system has similarity to a system inactivating endotoxin (Landy) and also to a system cytotoxic for certain cell lines in tissue culture (Bolande). Thus, serum and plasma appear to contain at least two distinct systems which in addition to their bactericidal activity may interact with endotoxin. Since endotoxins and tolerance to endotoxins appear related to resistance and susceptibility to various traumata including Gram-negative infections and shock, the occurrence of serum systems interacting both with bacteria and endotoxins is of biologic interest.

Morphologic Changes in Dogs Following the Production of Shock with Endotoxin and Their Comparison to the Morphologic Changes Occurring During Shock Associated with Bacteremia in Patients. MAX H. WEIL, Minneapolis, Minn. (Introduced by Raymond E. Weston).

Pathomorphologic studies were made in 10 dogs after production of a fatal form of sudden circulatory collapse which followed a single intravenous injection of endotoxin derived from Gram-negative bacteria. Principal alterations involved the liver, gall bladder and intestinal tract. These included acute hepatic congestion, marked edema and hemorrhage of the gall bladder wall, and intestinal mucosal hemorrhages and ulcerations. Vascular lesions consisting of deposits of intraluminal and subendothelial hyaline fibrinoid material were observed in small blood vessels in the mucosa and submucosa of the intestine, in the efferent venules of the liver, and in the small branches of the pulmonary arteries.

For comparison, a review was made of the autopsy findings in 19 consecutive patients who died of bacteremia and shock in the University of Minnesota Hospitals. The principal lesions included bronchopneumonia and abscess formation, supperative pyelonephritis and abscesses, congestive splenomegaly and hepatomegaly and intestinal mucosal hemorrhages and ulcerations. Hyaline thrombi were observed in the lumina of coronary arteries, pulmo-

nary arteries, mucosal and submucosal blood vessels of the intestine and in the adrenal sinusoids.

The most noteworthy feature was the regularity with which lesions occurred in the gastrointestinal tract, in the patients as well as in the animals. In the dogs, previous studies had shown that the immediate reaction to endotoxin is brought about by obstruction of outflow of blood from the liver. Consequently, large quantities of blood are pooled in the liver and portal venous bed and shock ensues. The relationship between stasis of blood and the formation of hyaline thrombi or emboli has not been elucidated, as yet. It seems likely, however, that the deposition of intraluminal and subendothelial fibrinoid material would account for the localized ischemic lesions which occurred in both groups. These comparative studies indicate that parallel changes occur in human patients and in experimental animals.

Some Antileukemic Effects of a Sulfhydryl Inactivator Derived from the Active Principle of Garlic (Allium Sativum). AUSTIN S. WEISBERGER, Cleveland, Ohio.

Sulfhydryl (-SH) compounds have frequently been implicated in cell growth and division and may have a particularly important role in malignancies and leukemia. Agents which inactivate -SH groups often exert an inhibitory effect on malignant growth.

Allicin (allylthiosulfinic allyl ester) is a powerful bactericidal agent derived from garlic by the interaction of an enzyme and substrate present in garlic bulbs. This compound inactivates many -SH enzymes without affecting most other enzymes and combines readily with cysteine. Such reactivity with -SH compounds suggests that it may also have inhibitory effects on malignant growth. Accordingly, various alkyl thiosulfinic alkyl esters (methyl, ethyl, propyl and butyl analogs) were studied in animals. These compounds inhibit the growth of Sarcoma 180 in mice. They also produce a lymphopenia and decreased cellularity in lymph nodes when administered to mice for 10 days.

In vitro effects on human leukocytes include a progressive decrease in the influx of S^{35} L-cystine with increasing concentrations and depression of both aerobic and anaerobic glycolysis.

Some effects of synthetically prepared diethyl and dipropyl thiosulfinic esters have been observed in four patients with leukemia. One child in the terminal resistant phase of acute leukemia manifested a fall in leukocyte count and marked decrease in spleen size. The spleen had previously not responded to other therapy. One patient with advanced chronic myeloid leukemia exhibited a rapid fall in immature leukocytes and decrease in spleen size. These patients received 100 to 400 mg. of these compounds for eight days. One patient with monocytic leukemia and one with chronic lymphocytic leukemia exhibited no significant response in five days.

Duration and amount of therapy as well as effects of other analogs require further evaluation. However, these preliminary results indicate that such compounds (R-SO-S-R) capable of inactivating -SH groups merit further study in leukemia and related disorders.

Tissue Electrolytes in Hypertension Due to Excess Sodium Chloride Administration. JOHN M. WELLER, Ann Arbor, Mich. (Introduced by Sibley W. Hoobler).

The prolonged administration of excessive amounts of sodium chloride (as 2 per cent in their drinking water) to rats resulted in the inconstant occurrence of hypertension. Analysis of the tissues of a group of rats which developed more severe hypertension showed significant differences from that of a group which had virtually no hypertension. The hypertensive rats had marked hypernatremia and hyperchloremia which were not present in the normotensive ones. These changes were not simply due to dehydration as the extracellular phase, as calculated for skeletal muscle, was actually expanded. The skeletal muscle of hypertensive rats contained more sodium and less potassium than did that of the more normotensive animals. Heart muscle of the hypertensive group showed predominantly a potassium deficit. In the aorta of the hypertensive rats, the major finding was an elevation of the sodium content. In contrast to this the aortas of rats not developing hypertension showed some increase in both sodium and potassium content.

From these data it would appear that excess tissue sodium and expansion of the extracellular space need not, in themselves, lead to elevation of the blood pressure. When severe hypertension occurred, potassium deficiency was the distinguishing additional finding. These observations again stress the importance of the maintenance of a normal sodium : potassium ratio and the physiological significance of the antagonistic action of these ions.

Studies on the Nature and Incidence of Buerger's Disease. STANFORD WESSLER,* SI-CHUN MING, VICTOR GUREWICH, and DAVID G. FREIMAN, Boston, Mass.

Fifty years after its classic description, Buerger's disease (TAO) has come under fresh scrutiny. Although TAO could not be established on clinical grounds, the diagnosis was considered in 79 of 1,464 Beth Israel Hospital patients with arterial insufficiency. Among 33 of these 79 patients there were 4 necropsies, 39 amputations, and 19 vein biopsies. Controls included 66 injected limbs amputated for atherosclerotic gangrene, 102 vein biopsies, and 23 dogs with experimental venous thrombosis. Lesions were classified pathologically as acute, intermediate, or healed according to Buerger's criteria. No acute arterial lesions were found although marked vasculitis with thrombosis resembling TAO was seen in gangrenous areas in both the TAO and atherosclerotic groups. The necropsies and amputated limbs from the TAO group revealed that the arterial occlusion was caused by thrombosis usually secondary to embolism or atherosclerosis. Frequently associated with these lesions were vascularization of the arterial wall, venous thrombosis, and perivascular fibrosis involving contiguous nerves. These findings, generally considered characteristic of the inter-

mediate and healed stages of TAO, were also found among limbs amputated for atherosclerotic gangrene. Moreover, some of the histologic venous features were reproduced in the animals. Finally, the phlebitis often considered typical of Buerger's disease was found among vein biopsies from both the TAO and control groups. These data indicate that the specificity of TAO cannot be established histologically by demonstrating acute arteritis in areas of gangrene, acute phlebitis, or intermediate or healed arterial lesions as defined by Buerger. These latter arterial lesions are nonspecific and can be produced by simple intravascular thrombosis. Since Buerger's disease cannot be precisely defined clinically, proof of the diagnosis becomes exceedingly difficult, for it depends on the unequivocal demonstration of the acute arterial lesion in an area free of gangrene. No patient at the Beth Israel Hospital has satisfied this criterion.

Hepatic Storage and Biliary Transport of Bromsulfalein in Dog and Man. HENRY O. WHEELER, JAY I. MELTZER, ROBERT M. EPSTEIN, and STANLEY E. BRADLEY,* New York, N. Y.

Hepatic mechanisms for removal of plasma constituents were explored using bromsulfalein (BSP) as a test substance. In 18 anesthetized dogs, hepatic BSP removal rate (R) was calculated from values for total hepatic venous outflow (rotameter) and splanchnic BSP arteriovenous differences under various conditions of loading produced by appropriate BSP infusions. The data appeared to support the view that hepatic removal of BSP (and presumably other substances) involves two processes: *1*) biliary secretion at a rate limited by a transfer maximum (Tm) equal to the fixed rate observed at any constant plasma level above 3 mg. per cent; and *2*) hepatocellular storage (S) in an amount proportional to plasma concentration. The second affects total removal rate only when changes in plasma level permit net storage uptake or release and may be computed as the change in removal rate observed under these circumstances relative to the rate of change in plasma level (mg. per mg. per cent).

Since extrahepatic removal proved to be small, Tm and S could be measured without determination of blood flow, by solution of simultaneous equations derived by substitution of values obtained during at least two different constant infusions of BSP into the following general equation:

$R = Tm + S \cdot (\Delta P/\Delta T)$, where $(\Delta P/\Delta T)$ equals the rate of change in plasma concentration and R equals the corresponding removal rate (infusion rate minus $(\Delta P/\Delta T)$ times plasma volume).

In 11 anesthetized dogs Tm averaged $1.73 \pm$ S. D. 0.45 mg. per minute per 10 Kg. body weight, and S averaged 19.7 ± 6.8 mg. per mg. per cent per 10 Kg. indicating a higher concentration of BSP stored in hepatic parenchyma than in plasma. In 13 normal human subjects Tm averaged 8.41 ± 1.32 mg. per minute per 1.73 $M.^2$, and S averaged 60.0 ± 26.1 mg. per mg. per cent per 1.73 $M.^2$. Abnormal patterns observed in hepatic disease included virtually complete absence of biliary transport accompanied by normal storage, suggesting that separate mechanisms are involved in storage and transport.

Inhibition of Mycobacteriophage Propagation by Fresh Serum. ARTHUR C. WHITE and VERNON KNIGHT,* Nashville, Tenn.

Fresh human serum has been found to prevent the propagation of mycobacteriophage D-29 on human tuberculosis strain $H_{37}Rv$. This effect was observed in sera from tuberculin positive and negative donors, and from patients with active tuberculosis in concentrations of 10 per cent and greater. The effect was not removed from fresh serum by pretreatment with living tuberculosis cultures, saprophytic mycobacteria, or mycobacteriophage. The inhibitory effect was completely removed by treatment of serum with trypsin. Alpha and beta globulin fractions prepared from serum by ammonium sulfate precipitation showed considerable effect; albumin and gamma globulin showed little effect. The active component could be distinguished from properdin and complement. This reaction superficially resembles the inhibition of mycobacteriophage propagation by Tween 80 previously reported from this laboratory, but may be distinguished by the failure of serum to prevent propagation of mycobacteriophage on the saprophyte, ATCC 607. The effect of serum appears to be on the bacterium rather than on the phage since serum does not prevent phage propagation on a saprophytic mycobacterium. Inhibition of phage propagation by serum may explain the observed failure of large doses of mycobacteriophage to modify experimental tuberculous infection in mice. It is probable that the lack of increase in lytic particles in phage-bacterial systems in the presence of serum indicates failure of phage to infect the bacteria. If this is so, this and similar phage-bacterial systems could not exhibit transduction and related phenomena *in vivo* (heritable changes produced in bacteria by infection with phage, such as increase in penicillin resistance and increase in virulence); nor could bacteriophage treatment be expected to exert a suppressive effect on animal infection.

The Influence of Adrenocorticotrophic Hormone (ACTH) on the Release of Nonesterified Fatty Acids from Rat Adipose Tissue In Vitro. J. EARLE WHITE and FRANK L. ENGEL,* Durham, N. C.

Oxycel and peptide ACTH possess adipokinetic and ketogenic activities when assayed in rats and mice, and it has been shown that these effects are neither mediated by the adrenals nor due to contamination with other hormones. The present study supports the hypothesis that ACTH influences fat metabolism directly by demonstrating an accelerated release of nonesterified fatty acids (NEFA) from adipose tissue on incubation with ACTH *in vitro*.

Twenty to 30 mg. portions of intra-abdominal adipose tissue from fasted male rats were incubated in 1 ml. of heparinized rat plasma at 36° C. in air in a Dubnoff shaking incubator. Plasma NEFA were determined be-

fore and three hours after the addition of hormone or a distilled water control. The results expressed as μM NEFA released per 100 mg. adipose tissue per three hours were as follows: control, $+0.19 \pm 0.06$ (S. E.); oxycel ACTH, 1 μg., 1.17 ± 0.21, 5 μg. 2.37 ± 0.28. Samples of protein ACTH, Corticotropin A, American Cyanamid fractions of α_1-α_2 δ-1 and γ and a product of pepsin digestion of β-corticotropin were all active whereas a chymotrypsin fraction was not. Exposure to 0.1 N NaOH at 25° C. for 24 hours did not affect activity, but boiling in NaOH for 20 minutes abolished it as did oxidation with H_2O_2; however, in the latter case activity could be restored by incubation with cysteine. These inactivation-reactivation procedures influence adrenal ascorbic acid depleting potency and other extra-adrenal metabolic activities of ACTH *in vivo* in the same manner. Somatotropic hormone (1,000 μg.), thyroid stimulating hormone (100 μg.), β-melanocyte stimulating hormone (400 μg.), oxytocin (Pitocin®) (1 U) and vasopressin (Pitressin®) (2 U) were inactive, whereas adrenaline and noradrenaline (1 μg.) were active but could be shown not to be responsible for the ACTH effect.

These results strengthen the hypothesis that ACTH influences directly the intermediary metabolism of extra-adrenal tissues (Yale J. Biol. Med. 1957, **30**, 201.).

The Mechanism of the Protein-Sparing Effect of Glucose. JEAN D. WILSON and MARVIN D. SIPERSTEIN, Dallas, Texas. (Introduced by Gladys J. Fashena).

The mechanisms by which glucose administration during starvation spares protein are unknown. Previous studies in our laboratory demonstrated that glucose oxidation can control lipogenesis by generating rate-limiting cofactors, particularly during the operation of the hexosemonophosphate shunt. The present study was undertaken to investigate the relationship between protein synthesis and the cofactors generated during glycolysis—TPNH, DPNH, and ATP.

The influence of cofactors and substrates on protein synthesis from acetate-C^{14} was studied in cell-free homogenates of rat liver. Addition of TPN plus glucose-6-phosphate (G-6-P) to homogenates increased protein synthesis three- to sevenfold; addition of DPN plus G-6-P stimulated protein synthesis to a lesser extent. Addition of an ATP-generating system (creatine phosphate plus ATP) also accelerated protein synthesis markedly. However, when protein synthesis was maximally stimulated by the ATP-generating system alone, addition of TPN plus G-6-P causes further protein synthesis up to threefold; again, the effect of DPN plus G-6-P was usually less. Furthermore, to establish that pyridine nucleotides generated during glycolysis are, in fact, limiting in protein synthesis, valine-C^{14} incorporation was studied. Again, TPN and DPN were found to stimulate protein synthesis above levels attainable with maximal ATP generation alone.

It is noteworthy that pyridine nucleotides can regulate protein synthesis, since the only reaction in mammals resulting in net amino acid synthesis (α-ketoglutarate to glutamate) requires reduced pyridine nucleotide. Thus, the rate of amino acid synthesis is probably rate-limiting in protein synthesis. The conclusion may also be drawn that the generation during glycolysis of the cofactors—TPNH, DPNH, and ATP—may account for the protein-sparing effect of glucose. In addition, the finding that TPNH is more limiting than DPNH suggests that the glucose oxidized via the hexosemonophosphate shunt may be of particular importance in the sparing of protein by glycolysis.

Depression by Growth Hormone of the Phosphogluconate Oxidative Pathway in Adipose Tissue. ALBERT I. WINEGRAD, WALTER N. SHAW, and ALBERT E. RENOLD,* Boston, Mass., and Philadelphia, Pa.

Paired preparations of rat epididymal adipose tissue have been adapted for the study of hormonal effects *in vitro*. Studies concerned with production of CO_2 and synthesis of long-chain fatty acids from glucose-1-C^{14} and glucose-6-C^{14} by adipose tissue from normal, fed animals suggested approximately equal participation of glycolytic and nonglycolytic pathways of glucose utilization. The presence of the phosphogluconate oxidative (PGO) pathway in this tissue was further supported by the demonstration of glucose-6-phosphate and 6-phosphogluconate dehydrogenase activities. Insulin added *in vitro* consistently stimulated glucose oxidation to CO_2 and fatty acid synthesis from glucose by *both* pathways. The effect of insulin in stimulating fatty acid synthesis from acetate or pyruvate was dependent upon concomitant stimulation of glucose utilization.

Bovine growth hormone added *in vitro* (0.2 to 1 mg. per ml.) consistently stimulated glucose uptake and oxidation to CO_2, but fatty acid synthesis was not increased. Furthermore, in the presence of growth hormone the ratio of carbon 1 to carbon 6 metabolized to CO_2 tended towards unity, strongly suggesting depressed glucose utilization by the PGO pathway. Failure to observe increased fatty acid synthesis concomitant with increased glucose uptake and oxidation in the presence of growth hormone and the observed depression in PGO pathway activity support the view that glucose utilization by the PGO pathway is essential for synthesis of fatty acids in adipose tissue. The different patterns of glucose utilization produced by growth hormone and insulin *in vitro* make untenable the view that this effect of growth hormone is dependent upon the activation of insulin bound to tissues. The relevance of these observations to the physiological action of growth hormone is yet to be ascertained in view of the high concentrations of growth hormone required.

Mechanisms of Body Fluid Enzyme Alterations in the Absence of Tissue Necrosis. FELIX WRÓBLEWSKI, ALICE E. MOORE, CARLOS MANSO, and KANEMATSU SUGUIRA, New York, N. Y. (Introduced by Olof H. Pearson).

In the absence of cellular necrosis, intracellular enzymes may leave the intact cells and find access into the body fluids. Using the technique of tissue culture, it has been

shown that growing cells with intact cellular boundaries contribute lactic dehydrogenase activity to the bathing fluid medium; other intracellular enzymes simultaneously measured are not lost from the intracellular confines. Experimentally produced malignant tumors in rodents are associated with increased serum glutathione reductase and serum lactic dehydrogenase activity at a time when the malignant tumor shows decreasing enzyme activity of the former, increasing the activity of the latter enzyme and no tissue necrosis. Other tissue enzymes measured are simultaneously unaffected by neoplastic tissue growth. Inflammation of liver tissue results in the loss of intracellular transaminating enzymes in amounts independent of the hepatocellular enzyme content and not proportional to tissue necrosis. Plasma phosphatase enzymes from osseous and prostatic tissue sources are independent of tissue concentration and of cellular necrosis.

In vitro and *in vivo* studies indicate that major alterations of body fluid enzymes may occur in the absence of tissue necrosis, and that intracellular enzymes of tissues involved by neoplastic and inflammatory pathologic states may find access to plasma, serous effusion, and cerebrospinal fluid in amounts relatively independent of tissue enzyme concentrations and in the absence of cellular necrosis. The clinical parallelisms and implications are noteworthy.

On the Regulation of Purine Nucleotide Synthesis and Uric Acid Production in Gout. JAMES B. WYNGAARDEN and HAROLD R. SILBERMAN, Durham, N. C. (Introduced by R. Wayne Rundles).

Patients with primary gout show prompt overincorporation of tracer doses of glycine-1-C^{14} into urinary uric acid. All known pathways yielding uric acid involve purine nucleotide intermediates. Therefore, overproduction of uric acid in gout probably involves excessive synthesis and rapid cleavage of nucleotides. Factors controlling purine nucleotide synthesis have been investigated in pigeon liver homogenate supernatants (105,000 × G). *De novo* synthesis of inosinic acid from glycine-1-C^{14}, formate, glutamine, aspartate, bicarbonate, ribose-5-phosphate (R5P) and ATP was inhibited by a number of purine compounds. When glycine-1-C^{14} and R5P were replaced by glycineamide ribotide-1-C^{14} (GAR), none of these compounds was inhibitory; therefore, the inhibitory compounds could be studied in terms of their influence on reactions prior to GAR formation. Three potential feedback control mechanisms were defined, by which purine end-products might regulate *de novo* purine synthesis. Possibly the most important is one by which preformed purine bases compete for phosphoribosylpyrophosphate (PRPP) in the course of conversion to the corresponding 5'-nucleoside monophosphate, thus preventing utilization of PRPP for *de novo* purine synthesis. This suggested, as one possibility in gout, that a mechanism resulting in accumulation or overproduction of PRPP might permit, ultimately, excessive *de novo* synthesis of purine nucleotides. This possibility is being explored in partially purified extracts of erythrocyte acetone powders. In limited studies to date, however, enzyme preparations from gouty erythrocytes appear to produce PRPP from ATP and R5P at normal rates, and to utilize PRPP in conversion of hypoxanthine and guanine to nucleotide stages at normal rates. Furthermore, they also cleave inosine at normal rates. These preliminary observations on gouty erythrocytes have not disclosed a defect which might either overproduce or underutilize PRPP, nor one which might cleave nucleosides excessively. Observations on liver might be of more critical importance in relation to a possible biochemical lesion in gout.

Circulatory Dynamics of Aortic Stenosis. PAUL N. YU, FRANK W. LOVEJOY, JR., BERNARD F. SCHREINER, HEINZ WALTHER, ROBERT H. LEAHY, and C. ALPHEUS STANFIELD, Rochester, N. Y. (Introduced by Nolan L. Kaltreider).

Combined right heart catheterization and direct left ventricular puncture were performed in 14 patients with "pure" aortic stenosis.

These patients are classified into three hemodynamic categories: Group I–Little or no alteration of left ventricular function, clinically asymptomatic; Group II–Moderate alteration of left ventricular function, clinically symptomatic; and Group III–Left ventricular failure and pulmonary hypertension, clinically incapacitated.

In patients of the first two groups, aortic valve varied between 0.5 and 1.1 $cm.^2$, with normal or nearly normal cardiac index, and left ventricular end-diastolic pressure 15 mm. Hg or less. Systolic upstroke time was normal or slightly delayed. Pulmonary artery pressure and "left heart resistance" were normal or slightly elevated.

Patients in Group III had aortic valve areas of 0.4 $cm.^2$ or less with subnormal cardiac index and left ventricular end-diastolic pressure 25 mm. Hg or more. The systolic upstroke time was considerably delayed. Pulmonary artery pressure and "left heart resistance" were markedly elevated.

Left ventricular stroke work (LV_{SW}) was evaluated in relation to left ventricular filling pressure. In patients with a compensated left ventricle, the total LV_{SW} was markedly increased, and the effective LV_{SW} against pressure normal or increased. In those with a decompensated left ventricle the total LV_{SW} was normal or decreased and the effective LV_{SW} markedly decreased. In the latter patients, higher left ventricular filling pressure was required to perform the same work in comparison to the compensated patients.

Following intravenous injection of acetyl strophanthidin, distinct improvement in left ventricular function was observed in a patient of Group III, but no significant change was noted in three patients of Group I and II either at rest or during exercise.

Hyperpolarization of Muscle Membrane by Insulin. KENNETH L. ZIERLER,* Baltimore, Md.

There are several reasons for suspecting that insulin acts by association with muscle membranes and there is increasing evidence that insulin accelerates movement of

certain substances into and even out of muscle. Collapse of the membrane barrier to diffusion explains some insulin effects but fails to explain its role in potassium accumulation in muscle. This latter effect could be explained if insulin hyperpolarized muscle membranes. To test this hypothesis resting membrane potential of excised rat peroneus longus muscle was measured. Insulin, but not cysteine-inactivated insulin, hyperpolarized the membrane by about 8 per cent. There are two possible explanations for this hyperpolarization; it might be primary in the sense of a direct effect upon the membrane, or insulin might make potassium move into muscle by some other mechanism and the consequent shift in ratio of intra- to extracellular potassium would be responsible for the hyperpolarization. The latter possibility is less likely because the observed changes in potassium concentration were inadequate to account for the degree of hyperpolarization. It is proposed that insulin acts by distorting the membrane, simultaneously increasing its permeability to certain substances and increasing its resting potential. Potassium then moves to adjust its concentration ratio in response to the new membrane potential.