ABSTRACTS

Relationship between Resting Tension and Electrolyte Content in the Walls of Large and Small Arteries and Veins. FRANÇOIS M. ABBOUD AND JOHN W. ECKSTEIN,* Iowa City, Iowa.

These experiments were done to see if the electrolyte content differs in small and large arteries and veins. Segments of brachial arteries and veins, and small metacarpal arteries and veins were taken from forelegs of 11 anesthetized dogs. Pressures were recorded from the same vessels in the opposite foreleg. In the large artery, the water content, ± SE, averaged 70.9 ± 0.58%; sodium, potassium, and chloride concentrations averaged 36.6 ± 2.56, 9.5 ± 0.45, and 21.9 ± 0.83 mEq per 100 g of dried tissue, respectively. Corresponding values in the small artery were 77.9 ± 1.10, 42.1 ± 2.18, 20.6 ± 1.08, and 27.7 ± 2.13%; in the small vein, 76.6 ± 2.10, 51.4 ± 3.07, 7.50 ± 0.45, and 32.8 ± 3.56%; and in the large vein, 70.0 ± 1.10, 41.5 ± 2.28, 6.4 ± 0.54, and 24.3 ± 1.92%. Serum sodium, potassium, and chloride concentrations averaged 147.8 ± 2.53, 4.1 ± 0.21, and 114.2 ± 1.31 mEq per L, respectively. Similar values were obtained in an earlier group of 13 dogs by a different analytical technique. The greater potassium content of small arteries could not be attributed to a greater intracellular space, since measurement of inulin space averaged 36.8% of the large artery, 38.2% of the small artery, 49.5% of the small vein, and 39.5% of the large vein. Mean blood pressures averaged 125 mm Hg in the brachial artery, 87 mm in the small artery, 16 mm in the small vein, and 4 mm in the brachial vein. Since tension is directly proportional to pressure and radius, the resting tension in small arteries is lower than in large arteries. *In vitro* observations show that an increased ratio of intracellular to extracellular potassium in vascular strips is accompanied by decreased tension. The results reported here suggest that a similar relationship may exist *in vivo*.

The Physiology of Platelet Destruction in Humans and Animals. EDWARD ADELSON, RICHARD M. KAUFMAN, ARNOLD A. LEAR, AND JACK J. RHEINGOLD, Washington, D. C. (introduced by Theodore J. Abernethy).

We have devised techniques for obtaining populations of labeled young platelets of homogenous age (cohorts) in humans and dogs. We have studied the physiology of platelet destruction by comparing the survival of these cohorts transfused into normal recipients and into recipients receiving anticoagulants. In humans, platelets are randomly destroyed by a process that results in a half-life of 2.8 days. In dogs, the half-life is 1.5 days. The random process of platelet destruction is so active that in normal humans and dogs only a few platelets live long enough to die by senescence. Therefore aging plays no significant role in determining platelet survival. The random process of platelet destruction appears to be continuous *in vivo* coagulation, since it is slowed by anticoagulant therapy. In a human, heparin injections prolong the normal 2.8-day half-life to 7.0 days. In dogs, heparin prolongs the normal 1.5-day half-life to 6.0 days. In dogs, warfarin prolongs the half-life to 4.0 days. These changes in platelet half-life cannot be explained by any defect in the labeling technique, which is kept unchanged throughout. When the random process of platelet destruction is slowed by anticoagulants, death by platelet aging begins to show itself. Human platelets begin to die by aging on day 10 of life, and none live beyond 15 days. For canine platelets, death by aging begins on day 8, and none live beyond day 12. We conclude that platelet survival is an index of *in vivo* coagulation. Both warfarin and heparin slow *in vivo* coagulation. In dogs, heparin is the more effective of the two anticoagulants.

Hemodynamic Effects of Ouabain upon the Hypertrophied Left Ventricle in Aortic Stenosis. LAWRENCE N. ADLER, NICHOLAS A. YANKOPOULOS, ERNEST E. FEDERICI, AND WALTER H. ABELMANN,* Boston, Mass.

Previous clinical and physiologic observations have raised doubt as to the effect of the cardiac glycosides upon the hypertrophied left ventricle in aortic stenosis. To evaluate this problem, 7 patients with aortic stenosis (2 also had mild aortic insufficiency) in sinus rhythm with cardiomegaly, but clinically not in heart failure, were studied at rest before and after the iv administration of 0.4 to 0.75 mg of ouabain. Before ouabain, the cardiac index (indicator-dilution method) ranged from 3.2 to 4.3 with a mean 3.7 L per minute per m^2, and the left ventricular end-diastolic pressure (LV_{ED}) from 10 to 31 with a mean 19 mm Hg. Cardiac index rose in 5 patients and remained unchanged in 2, with a mean increase of 0.4 L per minute per m^2 (all mean changes refer to the entire group). Stroke volume increased with one exception; the change was small, from 69 ml before to 77 ml after ouabain. LV_{ED} pressure decreased uniformly by a mean of 5 mm Hg, but became normal in only one patient. While left ventricular mean systolic pressure increased only from 153 to 159 mm Hg, left ventricular ejection rate rose from 251 to 313 ml per second after ouabain. Left ventricular minute work increased in 6 patients from 7.7 to 9.0 kg-m per minute per m^2, and left ventricular stroke work in 5 from 80 to 96 g-m per beat per m^2. The peak effect of all parameters was achieved 30 minutes after ouabain, except in 2 patients who further lowered their LV_{ED} pressure at 60 minutes. Right atrial or caval venous pressure was measured in 6 patients and did not change after ouabain (6 mm Hg before and 6 mm Hg afterward).

* Member.

A definite positive inotropic effect by ouabain upon the overloaded hypertrophied left ventricle in aortic stenosis has been demonstrated. The improvement in myocardial contractility, clearly shown by studies of left ventricular dynamics, was not evident from measurements of right ventricular filling pressure and systemic blood flow alone.

Characteristics of the Antibodies against Gamma Globulin Found in Patients with Multiple Transfusions. JAMES C. ALLEN AND HENRY G. KUNKEL,* New York, N. Y.

An examination of the blood of 52 subjects who received multiple transfusions has revealed agglutinating antibodies in 35 against genetic γ-globulin factors absent in the individual's serum γ-globulin. The majority of these were anti-Gm(a) where the incidence among Gm(a−) subjects was 85%. Anti-Gm(b) and anti-Gm(x) agglutinators were also found. Agglutinating titers up to 1/2,560 were encountered, but no precipitating antibodies. Each of these sera proved useful as typing reagents to distinguish the genetic types of γ-globulin in the Gm system. Studies of the antibodies by tests of sensitivity to 2-mercaptoethanol and by density gradient ultracentrifugation indicated that the great majority were of the 19S class, but 7S antibodies were clearly identified in one serum. In addition, a different type of antibody was encountered in certain of the sera. This failed to react with specific types of γ-globulin, but reacted with antigen-antibody complexes and aggregated γ-globulins. No correlation was observed between the occurrence of this type and the specific anti-Gm antibodies described above. A close similarity to the usual types of rheumatoid factors was observed. It appeared likely that this group of antibodies arose as a response to antigen-antibody complexes formed through the multiple transfusions, whereas the first group arose directly in response to the foreign genetic types of γ-globulin.

Neonatal Unconjugated Hyperbilirubinemia Associated with Breast-Feeding and a Factor in Milk That Inhibits Glucuronide Formation In Vitro. IRWIN M. ARIAS, LAWRENCE M. GARTNER, SAM SEIFTER, AND MATHILDA FURMAN, New York, N. Y. (introduced by M. Henry Williams, Jr.).

Four unrelated, 2-week-old, breast-fed infants who were clinically well had serum unconjugated bilirubin concentrations of 15.0 to 24.3 mg per 100 ml not due to known causes. The hyperbilirubinemia disappeared within 5 days after cessation of breast-feeding in three infants. The fourth infant alternated breast and artificial feeding and became anicteric 12 days later at 4 weeks of age. The mothers had breast-fed four previous children, three of whom had unexplained and prolonged jaundice. Breast milk obtained from these women strongly inhibited the formation of *o*-aminophenol glucuronide and direct-reacting bilirubin by rat, guinea pig, and rabbit liver homogenates and microsomes *in vitro*. Eighty-one of 84 samples of milk obtained from 62 unselected mothers from day 2 to 20 postpartum had negligible inhibitory activity. The inhibitory activity in the milk of each of the four mothers was nondialyzable, heat-stable, and present in the ether extract of the non-saponifiable solid fraction. This fraction was partially purified by chromatography and zone elution. The final zone eluate was dissolved in aqueous methanol, and crystallization occurred as methanol was slowly evaporated. After repeated crystallization, approximately 100 μg of crystals was obtained from 55 ml of milk. The crystals melted at 264 to 266° C and accounted for 65% of the inhibitory activity of the milk.

Gluconeogenesis as a Determinant of Hepatic Ketone Formation. R. A. ARKY, S. J. BLEICHER, AND N. FREINKEL,* Boston, Mass.

Although the precise determinants of ketogenesis are poorly understood, current concepts attribute pathogenetic significance to impaired intrahepatic disposition of glucose, or excessive intrahepatic availability of 2-carbon products of fat mobilization, or both. A more immediate link to gluconeogenesis from noncarbohydrate precursors has not been formulated. The recent demonstration from this laboratory that ethanol can directly interrupt hepatic gluconeogenesis has been confirmed with human liver slices, and has provided a pharmacological tool for re-examining the metabolic consequences of such interruption. Normal subjects, fasted for 12 to 72 hours, and juvenile diabetics, fasted for 12 hours and deprived of insulin for 8 to 24 hours, were infused with ethanol (2 ml per minute; 15% vol/vol in saline). The ethanol effects were contrasted with findings obtained under similar conditions, in the same subjects, during control infusions of saline. Among the multiple measurements that were secured, plasma glucose, ketones, and FFA were employed as indexes of intrahepatic gluconeogenesis, ketogenesis, and availability of endogenously derived 2-carbon donors, respectively. In normal subjects, fasted 3 days, and in diabetics, despite progressive decompensation, ethanol reduced blood sugar or attenuated the rising hyperglycemia. Concomitantly, serum ketones were reduced or the rising ketonemia was arrested. Although FFA usually declined in parallel, the ketone changes occurred even in those instances in which FFA rose, and despite the intrahepatic availability of 2-carbon fragments derived from alcohol. Contrariwise, in the normal subjects fasted overnight, where alcohol did not reduce blood sugar, serum ketones were unaffected, or rose despite the usual decrements in FFA. In all situations, alcohol induced a rise in lactic acid; in the diabetics, this was sufficient to sustain the acidosis despite the reduced ketonemia. The findings suggest that some aspect of gluconeogenetic renewal from noncarbohydrate precursors has a pivotal role in ketogenesis.

Influence of Body Temperature on Lethal Action of Bacterial Endotoxin. ROGER P. ATWOOD AND EDWARD H. KASS,* Boston, Mass.

The role of fever in resistance to infection has not been clearly defined. Recent studies have shown that fever, induced by elevation of ambient temperature, greatly increases susceptibility of mice, rats, and guinea pigs to the lethal action of endotoxin. These animal species could represent a special case, however, for they usually exhibit hypothermia in response to endotoxin or to infections. Accordingly, rabbits that usually become febrile in response to endotoxin were studied to explore further the relationship of body temperature to endotoxin lethality. Hyperthermia 1.5 to 2.0° C above the usual body temperatures of rabbits was induced in one group by exposure to 37° C and in another by pyrogenic doses (50 μg per kg) of lysergic acid diethylamide (LSD). The hyperthermic rabbits exhibited an augmented febrile response to endotoxin with acceleration of the rate of appearance of endotoxin effects, including lethality. The LD_{50} of endotoxin in both groups of hyperthermic animals was between 2 and 9 μg per kg compared with 236 μg per kg for control animals kept at 23° C. Shearing of body fur lowered the body temperature of the rabbits at room temperature by 0.5 to 1.0° C and abolished the pyrogenic response to LSD as a result of increased heat loss. In shorn rabbits, the response to endotoxin was hypothermic rather than hyperthermic, and the LD_{50} of endotoxin for shorn rabbits given endotoxin alone was increased almost 8 times to 1,700 μg per kg. The LD_{50} for shorn rabbits given LSD and then endotoxin was 420 μg per kg. These data demonstrate that a 25- to 100-fold *increase* in susceptibility to lethality of endotoxin occurs in rabbits with increased fever, and that a 2- to 7-fold *decrease* in susceptibility occurs in rabbits prevented from developing fever. The adverse effect of fever on susceptibility to endotoxin appears to be a general one and may have useful therapeutic implications.

Quantitative Determination of the Osmotic Threshold for Vasopressin Release in the Human. RICHARD H. AUBRY, HOWARD R. NANKIN, ARNOLD M. MOSES, AND DAVID H. P. STREETEN, Syracuse, N. Y. (introduced by Eugene L. Lozner).

A quantitative method has been devised to determine the level of plasma osmolality at which release of vasopressin is initiated in human subjects. Six fasted, normal subjects with indwelling venous catheters were maintained at steady maximal diuresis. After several control periods, a constant infusion of 5% saline (0.05 ml per kg per minute) was begun and continued until antidiuresis occurred. Osmolality was determined on 13 to 20 plasma samples obtained in each study. The osmolal threshold for vasopressin release was defined as the plasma osmolality at the inception of the observed abrupt fall in free water clearance. The osmolal threshold averaged 290.9 mOsm per kg and was remarkably constant (SEM ± 0.5). Because of the known antagonism between adrenocortical and neurohypophyseal hormones with regard to their effects on water excretion, the method devised was used to assess the influence of hydrocortisone on osmotically induced vasopressin release. The study was repeated in the subjects above after the oral administration of hydrocortisone (250 mg in divided doses) over 2 days. Under the influence of hydrocortisone, the osmolal threshold was increased in every subject. The mean threshold after hydrocortisone was 294.6 ± 0.6 mOsm per kg ($p < .001$). At the threshold, there was no significant fall in creatinine excretion or osmolal clearance. There was no difference in osmolality between arterial and venous plasma during the infusion of 5% saline, either before or during the administration of hydrocortisone, indicating that the osmolality in venous plasma accurately reflected that in the arterial blood flowing to the head. Studies by other investigators have shown that renal responsiveness to the concentrating action of submaximal amounts of vasopressin is not impaired by hydrocortisone. The present findings indicate that hydrocortisone may alter neurohypophyseal release of vasopressin by increasing the level of plasma osmolality at which the osmoreceptors are first stimulated.

Effects of Pancreozymin, Secretin, Insulin, Epinephrine, ACTH, and Hydrocortisone on Adipose Tissue from Various Species. D. ANNE AUTOR AND WILLIAM S. LYNN, JR.,* Durham, N. C.

In an effort to understand obesity, the hormonal response of various adipose tissues removed from rabbits and cholesterol-fed rabbits (perirenal), obese and nonobese human females (buttocks), and rats (epidermal) have been studied *in vitro* by the assay system of Renold. These studies show that three major effects of hormones are exerted on adipose tissue: *1*) glucose uptake stimulation with production of either lactate or fatty acid, *2*) lipolysis, and *3*) glucose uptake inhibition. Except for the third, these effects vary enormously among species. Any one hormone can exert either one or more of the effects above. Under our conditions, hydrocortisone, the only hormone showing glucose uptake inhibition, is very specific, inhibiting all species. Pancreozymin (Boots), 600 μg per ml, and secretin (Vitrium), 30 μg per ml, stimulate glucose uptake in all species. Lactate only is produced in all species but the rat, in which fatty acid is also produced. These hormones are not lipolytic in rabbit or obese human tissues, but are lipolytic in normal human tissue. Pancreozymin, but not secretin, is lipolytic in rat tissue. Insulin has the same effects as pancreozymin, except that insulin in rabbit tissue stimulates lipolysis. ACTH greatly stimulates lipolysis in rabbit tissue, whereas epinephrine is only weakly lipolytic. In rat and human tissues, epinephrine is the more powerful lipolytic agent. Epinephrine stimulates glucose uptake in all species, but to a much smaller extent than insulin. ACTH stimulates glucose uptake only in rat and normal human tissues,

although ACTH is the most effective lipolytic agent in rabbit tissue. Hydrocortisone, added as a crystalline powder, has only one effect, that of depressing glucose uptake in control and in hormone-stimulated tissues without altering lipolysis, except, perhaps, in normal human tissue.

Digital Computer Compartmental Analysis of Mg^{28} Kinetics in Normal Subjects, Paget's Disease, and Thyroid Disease. LOUIS V. AVIOLI, THEODORE N. LYNCH, AND MONES BERMAN, Jersey City, N. J., and Bethesda, Md. (introduced by Philip H. Henneman).

Ten normal subjects, 5 patients with Paget's disease, 3 hypothyroid, and 5 hyperthyroid patients were given iv Mg^{28} of high specific activity (200 μc per mEq) during balance studies on constant intakes. Specific activities of plasma, urine, and feces were measured for 3 to 6 days, and the data subjected to compartmental analysis using a digital computer and assuming a parallel, 3-compartment, open system. In normal subjects, total exchangeable Mg was 12 to 14% of reported whole-body Mg. The extracellular fluid (plasma) compartment contained 0.31 mEq exchangeable Mg per kg body weight and approximated extracellular fluid in volume. A second extravascular compartment contained 0.21 mEq exchangeable Mg per kg body weight and was tentatively identified as "bone" in that this compartment was selectively increased 2 to 3 times in Paget's disease and it alone presented increased Mg flux rates. Only 2% of estimated bone Mg was exchangeable in the normal subjects. A third extravascular compartment, assumed to be primarily muscle on the basis of reported tissue distribution of Mg, contained 2.51 mEq exchangeable Mg per kg body weight. Thus, 35% of "muscle" Mg was exchangeable in normal subjects. In hypothyroidism, exchangeable Mg was decreased in "extracellular fluid," "muscle," and "bone," and the rate of exchange between the vascular and "bone" compartments was increased 3 to 10 times. In hyperthyroidism, exchangeable Mg was markedly decreased in "muscle," with corresponding two- to threefold increments in flux rates. In one patient, propylthiouracil therapy produced a fourfold increment in "muscle" exchangeable Mg and a threefold decrease in flux rates. Thus, compartmental analysis of Mg^{28} kinetics by digital computer techniques identifies and quantitates selectively increased exchangeable Mg in bone in Paget's disease, selectively decreased exchangeable Mg in "extracellular fluid," "muscle," and "bone" in hypothyroidism, and selectively decreased exchangeable Mg in "muscle" in hyperthyroidism.

The Renal Circulation in Cirrhosis: Studies Based on Catheterization of the Renal Vein. W. P. BALDUS, W. H. J. SUMMERSKILL, J. C. HUNT, AND F. T. MAHER, Rochester, Minn. (introduced by H. R. Butt).

Previous observations suggest that impairment of renal function and subsequent azotemia arising spontaneously during the course of hepatic failure are due to diminished renal perfusion. To confirm this, and to investigate relevant pathophysiological mechanisms, studies of the renal circulation involving catheterization of the right renal vein were made in 18 patients with cirrhosis and variable impairments of hepatic and renal function. Simultaneous measurements included inulin and para-aminohippurate (PAH) clearances, PAH extraction ratios, arterial blood pressures, renal vein pressures, renal A-V oxygen differences, cardiac outputs, systemic vascular resistances, and renal vascular resistances. Tests of renal function yielded a significant separation ($p < .01$) between patients without ascites, or with ascites responding promptly to treatment (group I), and those with ascites relatively resistant to treatment (group II); the latter all had severe impairment of renal function, and some were azotemic. Extraction ratios were normal or only slightly reduced ($> .81$) in both groups, and there were no significant differences in arterial blood pressure, cardiac output, renal vein pressure, systemic vascular resistance, or renal A-V oxygen differences. The renal fraction of the cardiac output, however, was significantly reduced ($p < .02$), and the renal vascular resistance was significantly raised ($p < .01$) in patients with impaired renal function (group II) compared with those of group I. A linear relationship existed between increasing renal vascular resistance and decreasing inulin clearance. No change in clearances of inulin or PAH resulted from removal of the ascitic fluid, or from infusions of metaraminol (elevating systemic blood pressure) or mannitol. These studies confirm reduction in total renal blood flow as a major factor in the spontaneous impairment of renal function in patients with cirrhosis. Impairment of renal function occurs independently of changes in arterial blood pressure, renal venous pressure, cardiac output, or intrarenal shunting of blood, but is associated with an increased total renal resistance.

Provocative Test for Vitamin B_{12} Deficiency. LEWIS BARNESS, DIANA YOUNG, ROBERT NOCHO, AND BENHAM KAHN, Philadelphia, Pa. (introduced by A. M. Bongiovanni).

A sensitive test for vitamin B_{12} deficiency has been found to be urinary excretion of methylmalonate. The test seems to be equally effective in man and in rats. The excretion of methylmalonate depends on the biochemical reactions *1)* propionyl CoA + $CO_2 \rightleftharpoons$ methylmalonyl CoA and 2) methylmalonyl CoA $\rightleftharpoons$ succinyl CoA. Reaction 1 is biotin-dependent and Reaction 2 is vitamin B_{12}-dependent. It was therefore believed that minimal B_{12} deficiency could be proved by feeding propionate and measuring methylmalonate excretion. Male weanling Sprague-Dawley rats were fed a vitamin B_{12}-deficient diet for 2 weeks. After they were regularly excreting methylmalonate, their diet was supplemented with 1% sodium propionate. Excretion of methylmalonate increased markedly. After the oral administration of 10 μg of vitamin B_{12}, a dose known to eliminate methylmalonate excretion in 5 days, methylmalonate excretion slowly decreased during 2 weeks. Methylmalo-

nate excretion is a sensitive test for vitamin B_{12} deficiency. Proprionate ingestion, moreover, apparently increases the sensitivity of this test.

Effects of Angiotensin on Hepatic Circulation in Dogs. F. A. BASHOUR, R. A. TAHA, AND D. P. SELLERS, Dallas, Tex. (introduced by Carleton B. Chapman).

The effects of angiotensin were studied in 21 dogs. Angiotensin, 0.5 μg per minute per kg, was infused at a constant rate in 12 dogs for 30 minutes (group I) and in 9 dogs for 120 minutes (group II). Hepatic blood flow was measured by the extraction-and-clearance method of Bradley using I^{131}-Rose Bengal. Flow was estimated at 10-minute intervals for 3 consecutive periods before and after angiotensin infusion, and during angiotensin at 10-minute intervals in group I and at 30, 60, and 120 minutes in group II. Continuous measurements of systemic arterial and hepatic wedge pressures were made. Systemic arterial and hepatic venous blood oxygen contents and total body oxygen consumption were determined before and at the end of angiotensin infusion. Both systemic arterial and hepatic wedge pressures increased 1.5 to 2 times during angiotensin in groups I and II. Hepatic blood flow averaged 19 ml per kg per minute. During angiotensin, the flow decreased to 75, 79, and 107% of the control level in group I and 69, 52, and 49% in group II. The hepatic oxygen consumption revealed no consistent change during angiotension infusion in group I, but decreased significantly in group II, in spite of a moderate increase in total body O_2 consumption. The total splanchnic resistance increased 2.5 times during angiotensin infusion in both groups, and the increase was maintained throughout the infusion. Angiotensin increases both hepatic wedge pressure and splanchnic resistance, and decreases hepatic blood flow and O_2 consumption. Also, these studies suggest that portal venous inflow decreases during angiotensin infusion.

The Effect of Diabetes on the Synthesis of Unsaturated Fatty Acids in Adipose Tissue. WILLIAM BENJAMIN AND ALFRED GELLHORN,* New York, N. Y.

Earlier studies of lipid metabolism during aging in the rat revealed a decrease in the biosynthesis of monounsaturated fatty acids in adipose tissue. This change could be reversed either by the administration of insulin or by feeding the animals a fat-free diet. In the present investigation, the fatty acid composition and biosynthesis of individual fatty acids was determined by gas-liquid chromatography and radioisotope technics in alloxan-induced diabetic rats. From the onset of the diabetes, there was a progressive decline in the proportions of the monounsaturated 16-carbon palmitoleic and 18-carbon oleic acids of the epididymal fat, with a concomitant increase in the proportion of the saturated 18-carbon stearic acid, compared to age or weight controls. Incubation of the adipose tissue *in vitro* with acetate-1-C^{14} demonstrated that the over-all decrease in lipid biosynthesis in the diabetic state involved a striking depression in the incorporation of radioactivity into palmitoleic and oleic acids (trace and 1.0% compared to 10 and 15% in controls) and an increase in the C^{14} incorporation into stearic acid (13% in the diabetic rats and 7% in the controls). This pattern of isotopic labeling was returned to normal by the administration of insulin to the diabetic rats, but not by the addition of insulin to the incubation flasks. When diabetic rats were fed a fat-free diet, the disturbance in monounsaturated fatty acid metabolism was minimal, compared to normal animals. The present experiments demonstrate that one of the metabolic consequences of diabetes in the rat is a modification in the biosynthesis of specific fatty acids. The fact that a qualitatively similar defect is present in old and in diabetic rats suggests a similarity in mechanism between natural aging and the accelerated aging seen in diabetes.

The Use of Sodium Fluoride in Metabolic Bone Disease. DANIEL S. BERNSTEIN, CHARLES GURI, PHIN COHEN, JOHN J. COLLINS, AND SPYROS TAMVAKOPOULOS, Boston, Mass. (introduced by Francis D. Moore).

The effect of sodium fluoride in the treatment of various types of bone disease was studied. Patients with postmenopausal (senile) osteoporosis, Paget's disease, urticaria pigmentosa with osteoporosis, and idiopathic osteoporosis have had metabolic balance periods before and after fluoride administration (50 to 200 mg per day NaF) that have shown that fluoride initiates a grossly positive calcium retention mainly through increased gastrointestinal absorption of calcium. Coincident fluoride balances have shown a gross positive retention of fluoride. Serial bone biopsies have been performed and demonstrate increased mineralization after fluoride therapy. Ca^{47} studies have indicated a tendency toward an increased bone accretion rate produced by fluoride administration. Clinically, almost all of the patients studied have had a favorable response in relief of their bone pain. It appears that sodium fluoride may be an important bone nutrient in those diseases where there is increased resorption or defective mineralization of bone.

Unilateral Urine Sampling Utilizing External Ureteral Compression. LIONEL M. BERNSTEIN AND WILLIAM HAMBY, Chicago and Hines, Ill. (introduced by Malcolm M. Stanley).

Present techniques used for diagnosis of unilateral renal disease are excretory urography, retrograde pyelography, isotope renograms, renal biopsy, aortography, and the "Howard" test and its modifications. The discomforts and hazards of cystoscopy and ureteral catheterization decrease the usefulness of the "Howard" test. A simple, safe, clinically practical technique for unilateral urine sampling is used whereby one ureter is obstructed by external compression; urine is collected from the opposite kidney by bladder catheterization. With the patient on a flat table, an abdominal compression band is applied firmly. A block of wood (triangular in cross-section) is placed in such fashion that its base fits

directly under the abdominal compression band, and its apex exerts pressure on the anterior abdominal wall. The apex is applied on an angle extending from the anterior-superior iliac spine toward L-4. A sphygomomanometer cuff attached to the base of the wooden block is then inflated, causing occlusion of one ureter between the depressed anterior abdominal wall and the brim of the pelvis. The iliac artery is simultaneously compressed, and decrease in femoral pulsation is used as an index of correct placing of the unit. The pressure applied is then lessened so that the femoral pulsations are normal. Such compression does not cause significant discomfort when applied for periods up to 20 minutes. That this technique successfully occludes one ureter has been shown by intravenous pyelography and by unilateral function studies. Using the unilateral urine sampling technique in 25 patients, early studies have revealed significant unilateral renal disease in three patients, unilateral hematuria in one patient with sickle cell disease, and unilateral pyuria and bacteriuria in one patient with nephrolithiasis.

An Unusual Group of Fat Particles in Plasma of Hyperlipemic Subjects Maintained on High-Carbohydrate, Low-Fat Diets. EDWIN L. BIERMAN, Seattle, Wash. (introduced by Robert S. Evans).

Previous studies have demonstrated that two distinct groups of fat particles circulate in plasma during alimentary lipemia in normal subjects: large, "primary" particles that appear to be identical with fat particles in lymph, and smaller "secondary" particles presumably derived from the liver. Characterization of particulate fat in postabsorptive plasma of hyperlipemic subjects maintained on diets containing 70 to 85% carbohydrate and 0 to 15% fat has revealed a third type of fat particle that differs in electrophoretic properties and lipid composition from normal. Although all types of particle float readily in saline ($d < 1.006$), they can be separated by zone electrophoresis on a starch granule medium. The particles in plasma of these hyperlipemic subjects migrate in an intermediate zone (0.46 ± 0.10; $n = 6$; origin = 0.00; albumin = 1.00) compared with primary particles (0.60 ± 0.10; $n = 21$; $p < .05$) and secondary particles (0.23 ± 0.08; $n = 23$; $p < .001$) formed during alimentary lipemia in normal subjects. These "intermediate" particles cannot be distinguished from secondary particles on 0 to 5% PVP density gradients. They are, however, unusually rich in cholesterol ($24\% \pm 5\%$ by weight) and contain smaller proportions of triglyceride ($< 65\%$) when contrasted to "normal" particles (cholesterol $< 10\%$; triclyceride $> 85\%$). When fat is included in the diet, an additional group of particles resembling primary particles can be identified; however, the intermediate particles persist, and their triglyceride fatty acid pattern remains markedly different from that of the dietary fat. On the other hand, plasma from patients with fat-induced lipemia contains only primary particles, while plasma from patients with lipemia associated with other diseases (nephrosis, myxedema) contains only secondary particles. These findings indicate that one type of hyperlipemia, dependent on a large intake of carbohydrate, results from the production of an unusual cholesterol-rich fat particle.

Hyperdesoxycorticosteronism with Hypokalemic Alkalosis and Edema. EDWARD G. BIGLIERI, San Francisco, Calif. (introduced by Peter H. Forsham).

A 39-year-old white woman with hypokalemic alkalosis, low blood pressure, edema, and slight melanine pigmentation was studied. High potassium intake decreased edema and corrected the hypokalemic alkalosis, which was again accentuated by restricting potassium. Blood volume, measured by Cr^{51}-labeled red cells, was found to be 54 ml per kg, or 19% below normal. Estimation of aldosterone by the double-isotope method revealed normal urinary aldosterone excretion (9 to 25 μg per day) and adrenal secretion (109 μg per day). The use of spironolactone, however, known to block the renal effects of both aldosterone and desoxycorticosterone (DOC), led to prompt and consistent correction of the hypokalemic alkalosis and to avid potassium retention on four separate occasions. By suitable paper chromatography and double-isotope derivative techniques, an increased amount of DOC (45 to 290 μg per day) and tetrahydro-DOC (300 to 600 μg per day) were identified in the urine. Various electrolyte manipulations increased the production of DOC without affecting aldosterone. A similar case has since been seen by the author. After the removal of two, nodular, hyperplastic adrenal glands with an aggregate weight of 32 g in this patient, the edema disappeared and serum potassium rose to normal levels. A concentration of 0.18 μg per ml of DOC was found in the adrenal veins at surgery, compared to nearly undetectable levels in other subjects. The adrenal tissue removed contained three times more DOC than control tissue from 10 other subjects (0.81 mg per g against 0.27 μg per g). Persistent hypovolemia may well have served as an abortive stimulus to aldosterone secretion, which apparently could not occur because of an intra-adrenal biosynthetic defect resulting in significant DOC secretion instead. Although DOC has been found consistently in adrenal tissue, the syndrome of hypokalemic alkalosis, low blood pressure, hypovolemia, and edema associate with secondary increase in DOC secretion has not heretofore been described.

Micropuncture Study of the Roles of the Proximal Tubule, Distal Tubule, and Collecting Duct in the Acidification of Urine. H. ALLEN BLOOMER, FLOYD C. RECTOR, JR.,* AND DONALD W. SELDIN,* Dallas, Tex.

Experiments were performed to determine the ability of each segment of the nephron to generate pH gradients between tubular fluid and plasma and to form titratable acid (TA). Maximum pH gradients were studied in rats given NH_4Cl and infused with sodium sulfate. TA formation was determined during sodium phosphate infusion in normal rats, rats with NH_4Cl acidosis, and rats given acetazolamide. Tubular fluid pH was meas-

ured with quinhydrone microelectrodes, and blood and urine pH were measured with a glass electrode. Contributions of proximal tubule (PT), distal tubule (DT), and collecting duct (CD) to total TA formation were calculated from pH changes along the nephron and rates of phosphate excretion. In PT the maximal gradient was 0.8 pH units in both normal and acidotic rats infused with phosphate or sulfate. In DT the maximal gradient was 1.5 in normal rats and increased significantly to 1.8 in acidotic rats. A further increase in gradient (to 2.4 units) occurred in CD only in sulfate-infused rats. TA formation by PT accounted for 60 to 70% of total TA excretion, was not increased by NH_4Cl acidosis, but was markedly reduced by acetazolamide. The DT formed 25 to 40% of total TA and increased its rate of TA formation during acidosis. TA formation by CD was minimal under all conditions, contributing less than 5% at high rates of phosphate excretion. It is concluded that PT can establish a maximal gradient of 0.8 pH units that is not increased by metabolic acidosis and has the greatest capacity for TA formation. The DT can generate steeper gradients and is the primary site for augmented TA formation during metabolic acidosis. The greatest gradient can be established by the CD. Its capacity to secrete acid, however, is limited so that little change in pH occurs when buffer is present.

The Effect of "Friends" or "Strangers" upon the Individual's Physiological Responses while Performing a Challenging Task. MORTON D. BOGDONOFF,* THOMAS C. HOOD, MARY L. BREHM, AND KURT BACK, Durham, N. C.

Patients frequently report that the performance of a challenging task produces symptoms and that when other people are working together with them the symptoms are accentuated. The purpose of these studies was to measure the physiological responses that occur when a challenging task is undertaken in two specific situations: *a*) when the individual works with strangers and *b*) when he works with people he already knows. Twelve groups of four subjects each (men 20 to 25 years old) were studied during a difficult visual identification task. A panel of lights before each subject enabled him to see what decisions the other members of the group were making. Six groups were made up of subjects who knew one another during their daily living patterns ("friends") and six groups were made up of subjects who did not know one another ("strangers") and who were individually recruited for the study. Serial plasma FFA concentrations were measured as an index of the subject's neurohumoral response. All subjects demonstrated a significant rise in FFA levels during the initial phases of the study. In the latter phase of the study, however, FFA levels continued to rise in the "friends," whereas they fell in the "strangers." In addition, the degree to which the subjects conformed to one another's decisions was significantly greater in the "friends" than in the "strangers." These studies are interpreted to demonstrate that how well a person knows those with whom he works is an important variable affecting both the independence of his own decisions and the neurohumoral responses accompanying the performance of the meaningful task. Awareness of these factors may be useful in planning patient management.

The Failure of Transfused Isologous Granulocytes to Move Normally from the Blood into Inflammatory Exudates. D. R. BOGGS, O. P. HAAB, S. O. RAAB, AND J. W. ATHENS,* Salt Lake City, Utah.

This study was designed to determine if transfused, isologous, neutrophilic, granulocytic leukocytes migrate from blood to inflammatory exudates as readily as the recipient's own granulocytes. Blood granulocytes were labeled *in vitro* with diisopropylfluorophosphate (DFP^{32}), infused into normal human subjects, and their rate of disappearance from the blood was determined. Inflammatory exudates, produced by adding heat-killed *Staphylococci* to cantharidin-induced skin blisters, were begun as the labeled cells were infused. Six exudates in 6 subjects were studied after the infusion of labeled *autologous* granulocytes, and 12 exudates in 4 subjects after the infusion of labeled *isologous* granulocytes. The proportions of labeled isologous and autologous granulocytes in 4-hour-old exudates were then compared. The mean specific activity of exudate granulocytes (expressed as percentage of the initial blood specific activity) was 78% after infusion of autologous granulocytes (range 52 to 87%) compared to only 15% (range 4 to 28%) after infusion of isologous granulocytes. Isologous granulocytes tended to disappear more rapidly from the blood than did autologous granulocytes; thus, 4 hours after isologous granulocyte transfusion, the mean blood granulocyte specific activity was 15% (range 7 to 29%) of the initial blood specific activity compared to 36% (range 22 to 27%) after autologous granulocyte transfusion. This difference, however, was not great enough to explain the difference in exudate granulocyte specific activity between the two groups. After correction of exudate specific activity for the rate of disappearance of granulocytes from the blood, it was calculated that the subjects' own granulocytes were at least 3 times more likely to migrate into the inflammatory exudate than were isologous granulocytes. These studies are of some interest in regard to the use of isologous granulocytes in the treatment of infected, granulocytopenic patients.

Antihypertensive Renal Factor. E. BOOTH, J. W. HINMAN, E. G. DANIELS, M. KOSINSKI, AND E. E. MUIRHEAD,* Detroit and Kalamazoo, Mich.

As previously demonstrated, extracts of renal medulla prevent renoprival hypertension, whereas crude extracts of renal cortex, lung, erythrocytes, and muscle do not. Refined medullorenal extracts act against canine renoprival hypertension in doses of 25 to 300 μg per kg per

day. The active principle (mol wt < 1,000) does not appear to be a presently known depressor agent. The concept of an antihypertensive medullorenal factor was recently supported by Lee, Hickler, Saravis, and Thorn. Utilizing animals subjected to pentobarbital plus pentolinium and vagotomy, a depressor agent of low molecular weight was derived from renal medulla by technics similar to our earlier ones. In the present studies, renal extracts were further refined and tested in three canine systems: renoprival; vagotomized, pentolinium-treated; and renal hypertensive. Solvent extraction, adsorption, and thin-layer chromatography yielded a medullorenal productive active against renoprival hypertension in doses of 2 to 3 μg per kg per day. Thin-layer chromatography indicated near purity, so the term "antihypertensive renal factor" (AHRF) appears appropriate. AHRF has been derived from normal human, porcine, and canine kidneys. Doses of porcine AHRF that usually do not lower arterial pressure of intact dogs cause a sustained depressor effect in vagotomized, pentolinium-treated dogs. The same preparation of AHRF prevents renoprival hypertension, depresses the arterial pressure of vagotomized, pentolinium-treated dogs, and lowers significantly the arterial pressure of renal hypertensive dogs. Eight separate preparations of AHRF active against renoprival hypertension were tested in vagotomized, pentolinium-treated dogs; seven caused prolonged depressor effects. It would appear that the medullorenal depressor agent of Lee and associates and AHRF, as we have derived it, are similar agents. AHRF is a naturally occurring, antihypertensive agent of multispecies origin, including human. Effectiveness of AHRF in renoprival and renal hypertension and its depressor effect in vagotomized, pentolinium-treated dogs serve to unify renal roles in the pathogenesis of hypertension.

The Clot-promoting Effect of Soaps of Long-Chain Saturated Fatty Acids. ROBERT E. BOTTI AND OSCAR D. RATNOFF,* Cleveland, Ohio.

The existence of a "hypercoagulable" state in thrombotic disease has been sought with little success. Since disturbed lipid metabolism has been associated with such disorders, efforts have been made to relate alterations in plasma lipids to the pathogenesis of thrombosis. Previous investigations have demonstrated that the sodium soaps of long-chain saturated fatty acids dramatically accelerate the recalcified clotting time of whole blood. Connor proposed that this effect is related to the activation of Hageman factor. The clot-promoting action of the sodium soaps of long-chain saturated fatty acids was confirmed by measuring their effect on the recalcified clotting time of normal plasma. The mechanism through which soaps accelerated clotting was studied with a purified preparation of activated Hageman factor and partially purified preparations of inactive and active plasma thromboplastin antecedent (PTA). These experiments showed that soaps acted not upon Hageman factor, but upon PTA. Soaps appeared to accelerate the activation of PTA occurring either in the presence of Hageman factor or "spontaneously." No activity was detectable, however, when soaps were added to "native" plasma that had not been decalcified. Moreover, the procoagulant properties were reduced when calcium was added to the mixture of activated Hageman factor and PTA before the addition of soaps. These experiments, then, confirm the clot-promoting properties of the sodium soaps of long-chain saturated fatty acids *in vitro* and localize their effect to the activation of PTA. The failure of soaps to accelerate the clotting of native plasma makes doubtful the importance of the free fatty acids of human plasma in the development of intravascular thrombi, but the possibility is by no means excluded. These experiments provide further evidence that PTA may be activated in the absence of Hageman factor, hinting at a possible explanation for the asymptomatic character of Hageman trait.

A Shorter Method for Isolation of Human Angiotensin. ROGER BOUCHER, MARC LAUNAY, JACQUES DE CHAMPLAIN, ROBERT VEYRAT, AND JACQUES GENEST, Montreal, Canada (introduced by J. S. L. Browne).

Our previous procedure for isolation and determination of human blood angiotensin, despite advantages of high recovery, sensitivity, and specificity, is laborious and time-consuming. An improved method is proposed: *1*) Arterial blood drawn under vacuum is rapidly cooled (0 to 5° C) through a silicone-treated copper coil immersed in crushed ice to prevent angiotensinase activity. *2*) After centrifugation at 0 to 5° C, citrated plasma is directly placed on a Dowex 50 W-X2 (NH_4^+) resin column (10 × 1 cm). The fraction containing angiotensin is eluted with 0.1 N diethylamine followed by 0.2 N ammonium hydroxide and *3*) subsequently fractionated by ascending chromatography on Whatman 2 paper in a system of *n*-butanol: acetic acid: water (45: 10: 50, vol/vol) for 10 hours at room temperature (R_f angiotensin, 0.27). *4*) Concentration of angiotensin is determined by a rat pressor assay. Mean recovery of 12 experiments is 83% (range, 72 to 116%). Detection limit is 0.002 μg of angiotensin. The isolated pressor material has the same characteristics as synthetic valine-5 angiotensin II, aspartic β-amide: *a*) identical R_f value in two paper chromatographic systems of *n*-butanol: acetic acid: water (45: 10: 50, vol/vol) (R_f 0.27) and of 2-butanol: isopropanol: water: phosphate buffer, pH 8 (7: 7: 5: 2, vol/vol) (R_f 0.55); *b*) identical migration in one paper electrophoretic system, at 0.05 M sodium borate buffer, pH 8.6; *c*) identical rat pressor response curve; and *d*) inactivation by trypsin. Six determinations can easily be done in 3 days by the same person. Levels in 25 normotensive subjects vary between 0 to 30 mμg per 100 ml. Twenty patients with various types of hypertension and 22 patients with edema of different origins were studied. Results obtained in hypertensive patients agree with findings reported using the first procedure and indicate that high levels are often found in normotensive patients with generalized edema.

Minimal Glomerular Filtration Pressure. JOHN W. BOYLAN AND W. G. SCHENCK, Buffalo, N. Y. (introduced by David K. Miller).

To form final urine, glomerular capillary pressure must overcome tubular resistance, plasma oncotic pressure, and glomerular membrane resistance. The fact that dogs do not form final urine at arterial pressures below 60 to 75 mm Hg has been interpreted to indicate zero glomerular filtration below these pressures and, by implication, a glomerular membrane resistance requiring 35 to 50 mm Hg (plasma oncotic pressure averaging 25 mm Hg). In studying the effect of mannitol infusion on renal function in hemorrhagic hypotension, however, we have found that mannitol-infused dogs form urine at mean arterial pressures of 10 to 17 mm Hg (average minimal pressure in 5 dogs = 14.7 ± 2.8 mm Hg). This approached the calculated plasma oncotic pressure, which (because of repeated hemorrhage and infusion) averaged 10 mm Hg. Glomerular filtration rate (inulin clearance) falls linearly with decrements of arterial pressure below 60 to 75 mm Hg. Winton noted that cyanide or cold also lowered the minimal arterial pressure necessary to produce urine in the perfused kidney. We interpret our findings and Winton's observations as follows: *1*) Normally, at arterial pressures below 60 to 75 mm Hg, filtration continues, but reabsorption is complete and no urine appears. *2*) The presence of an unabsorbable solute, mannitol (or cold, or cyanide), prevents total reabsorption, and urine is produced as long as filtration continues. *3*) The pressure drop due to tubular resistance and glomerular membrane resistance becomes negligible at small filtration rates, and filtration pressure approaches plasma oncotic pressure as a limit.

On the Electrogenic Nature of Active Sodium Transport across the Isolated Frog Skin. NEAL S. BRICKER* AND SAULO KLAHR, St. Louis, Mo.

For many cell types, active transport of Na^+ is believed to be linked 1:1 with K^+ transport, and hence to occur nonelectrogenically (i.e., without charge transfer). The usual schema depicts a carrier crossing the plasma membrane with a sodium ion and returning with a potassium ion. Evidence for this linkage, however, and for nonelectrogenicity is largely circumstantial. Recent studies involving exposure of the internal surface of isolated frog skin to K^+ concentrations $\geq$ estimated intracellular K^+ values suggested that active sodium transport accounts for charge transfer. The experimental conditions minimized any contribution of K^+ diffusion (from cell water to internal medium) to the observed short-circuit current (SCC) and spontaneous potential difference (PD). While electrogenic Na^+ transport appeared to explain the SCC, a significant chloride current could not be excluded. The present studies were performed with chloride-free bathing media. Hypotonic or isotonic K_2SO_4-Ringer's (K = 110 or 170 mM) comprised the internal and Na_2SO_4-Ringer's (Na = 110 or 170 mM) the external solution. SCC and PD values were high (comparable to Na-Ringer control values), and net transcellular sodium transport approximated the SCC. Na flux ratios (Na^{22}-influx/Na^{24}-efflux) exceeded sodium concentration ratios (external/internal solutions) during complete short-circuiting, thereby documenting active transport. With strophanthin (1.4×10^{-5} M), net sodium transport fell and flux ratios approached concentration ratios. Transcellular sulfate movements, determined isotopically, were small, and net transcellular potassium movement occurred down the electrochemical gradient (from internal to external solutions). Since these studies were performed with an impermeant anion and potassium concentrations at the internal border of transporting epithelial cells exceeding estimated intracellular K^+ concentrations, neither anionic nor potassium diffusion currents can account for the PD and SCC. The only ion that can account for the electrical phenomena is Na^+. It is concluded that Na^+ transport was not linked 1:1 to K^+ transport and that the sodium pump operated electrogenically.

Determination of Sweat Gland Precursor Fluid Osmolality by Direct Cryoscopy. SAUL W. BRUSILOW, Baltimore, Md. (introduced by Robert E. Cooke).

While abundant information is available on the electrolyte composition of externally delivered sweat, little is known about the osmolality of sweat as it is formed in the secretory segment of the gland. Because of the inaccessability of both cat and human sweat glands to micropuncture, direct cryoscopy of the gland was attempted by a modification of the method described by Wirz, Hargitay, and Kuhn in demonstrating renal medullary hypertonicity. Skin (sweating in response to pilocarpine) was biopsied and frozen in liquid nitrogen, cut in 40-μ sections at $-10°$ C, and placed in a calibrated cryoscope (a modified Ramsey-Brown melting-point apparatus). Whereas Wirz used as his melting point the last crystal to disappear in a cortical slice or in the renal papilla, in the present experiments attention was confined only to ice crystals within the lumen of the secretory segment. Sixty-two melting-point determinations were made in biopsies from seven cats. Milliosmolalities calculated from these determinations revealed a range from 301 to 450, with 3% of the values between 301 and 325, 24% between 326 and 350, 27% between 351 and 375, 39% between 376 and 400, 11% between 401 and 425, and 5% between 426 and 450. In view of the fact that externally delivered sweat from the foot pad of the cat has average sodium and potassium concentrations of 171 and 23 mEq per L, respectively, the data suggest there is little modification of the precursor fluid from its formation to its external delivery. Studies done on sweat glands from healthy adult males (secreting sweat with chloride concentrations below 40 mEq per L) also revealed precursor fluid to be hypertonic, with 63% of the values falling between 301 and 400 mOsm per kg. These results demonstrate the previously postulated alterations in sweat from formation to delivery. Limita-

tions of this method to be discussed include possible heterogeneous distribution of solute, ice-crystal size, and our inability to determine osmolalities below isotonic levels.

The Effects of Hypertrophy and Dilatation on Left Ventricular Performance. IVAN L. BUNNELL, COLIN GRANT, AND DAVID G. GREENE, Buffalo, N. Y. (introduced by Evan Calkins).

In 24 patients with valvular and ischemic heart disease, left ventricular volumes and pressures were measured by biplane angiocardiography and simultaneous left ventricular catheterization by techniques previously described. Calculations were made of left ventricular stroke work, maximal left ventricular power, and an index of maximal tension developed in the left ventricular wall. The left ventricular hypertrophy of aortic stenosis allows up to three times normal stroke work and peak power development at normal end-diastolic volumes. In the presence of dilatation, as in mitral regurgitation or aortic regurgitation, stroke work and maximal power are linearly related to end-diastolic volume. Ventricles with a twofold increase in end-diastolic volume over normal can develop comparable increases in stroke work and power. With or without dilatation, maximal tension varied directly with stroke work and with peak power. Ventricles doing twice normal stroke work and producing twice normal peak power develop 50% more tension in their walls. The ischemic ventricle cannot proportionately increase stroke work or power even with increased end-diastolic volume, and develops a disproportionate increase in tension in producing the small amounts of stroke work and power of which it is capable.

Lack of Coupling between the Active Efflux of Sodium and the Influx of Potassium in Rabbit Renal Tubules. MAURICE B. BURG, EVELYN F. GROLLMAN, AND JACK ORLOFF,* Bethesda, Md.

It is generally assumed that sodium is actively transported out of renal tubule cells across the peritubular border by a mechanism involving a 1:1 exchange for potassium. In order to test this hypothesis, sodium and potassium fluxes were measured in tubules separated from rabbit renal cortex by means of collagenase, and the coupling ratio between sodium and potassium was determined. Kinetic analysis revealed the presence of at least two intracellular sodium compartments. Addition of cardiotonic steroids (strophanthidin or ouabain), which interfere with active transport, decreased the rate constant for sodium efflux by approximately 65% without significantly affecting sodium influx. Since the observed rise in tissue sodium was proportional to the decrease in the sodium efflux rate constant, it is probable that these drugs do not alter the passive permeability of the membrane to sodium and that there is no significant sodium exchange diffusion in the preparation. The active sodium efflux was calculated to be at least 376 mEq per kg dry weight per minute under control conditions, whereas the potassium influx was 41 mEq per kg dry weight per minute. On the basis of these observations, it is apparent that a large fraction of sodium transport in the proximal tubule is not directly coupled to potassium transport. Sodium transport, however, is dependent upon the presence of potassium in the external solution, since reduction of medium potassium to less than 0.3 mEq per L resulted in a 50% fall in the rate constant for sodium efflux without a significant change in sodium influx.

Protein Synthesis in Human Reticulocytes: A Defect in Thalassemia Major. EDWARD R. BURKA AND PAUL A. MARKS,* New York, N. Y.

This study was designed to give insight into the mechanism of the decreased hemoglobin A synthesis in thalassemia major. Investigations with rabbit reticulocytes have shown that ribosomes are the site of assembly of amino acids into protein and heavy ribosomes (>100 S) are primarily active in this synthesis. The role of ribosomes in protein synthesis in human reticulocytes has now been examined in cells from subjects with thalassemia, sickle cell anemia (SS), and acquired hemolytic anemia (AHA). The data indicate that *1*) only a small fraction of the total ribosomes is active in protein synthesis, corresponding in size to > 100 S, and *2*) reticulocyte ribosomes from thalassemic subjects have a decreased capacity for peptide bond formation in comparison with those of SS and AHA. Blood cell samples were obtained from 6 thalassemic, 5 SS, and 2 AHA subjects. After incubation at 37° C for 30 minutes in the presence of C^{14}-leucine, reticulocytes were shock-lysed without disrupting leukocytes. Ribosomes were isolated from the hemolysate and analyzed by sucrose gradient techniques to estimate molecular size (S) and specific radioactivity. In all conditions studied, while the major fraction of ribosomes sedimented as 80 S, the radioactivity was primarily associated with >100 S particles. The millimicromoles of leucine incorporated per milligram of ribosomes averaged 1.4 (range = 0.8 to 2.2) in thalassemia as compared with 4.2 (range = 3.5 to 5.0) for SS and 7.4 (range = 4.6 to 10.7) for AHA. These differences do not reflect variation in the degree of reticulocytosis, as the leucine incorporated per 10^6 reticulocytes averaged 0.11 mμmole in thalassemia, 0.19 in SS, and 0.57 in AHA. These data demonstrate that protein synthesis takes place on ribosomes of similar size in nonthalassemic and thalassemic cells. However, the level of peptide bond formation in the latter is consistently and significantly decreased. Thus, the genetically determined defect in hemoglobin synthesis of thalassemia may involve an altered functional capacity of the ribosomes.

Identification of a "Primitive" Bile Acid in Man as an Intermediate in the Transformation of Cholesterol to Cholic Acid; a Biochemical Sign of Human Evolution. JAMES B. CAREY, JR., Minneapolis, Minn. (introduced by C. J. Watson).

Studies in comparative biochemistry have shown that lower animals, such as certain amphibia and reptiles, do

not shorten the side chain of cholesterol to form bile acids, but simply oxidize the terminal carbon atom to form "primitive" C_{27} bile acids. Man and other animals, however, have "modern" C_{24} bile acids formed by oxidative cleavage of a 3-carbon fragment from the side chain. Crystallization of a naturally occurring "primitive" C_{27} bile acid (3α,7α,12α-trihydroxycoprostanic acid) from human fistula bile, identical with the chief bile acid isolated from alligator bile by Haslewood, led to the present experiment to determine if this compound was a normal intermediate in the conversion of cholesterol to cholic acid in man. After intravenous administration of 27 μc of cholesterol-4-C^{14}, 18.4 mg of radioactive trihydroxycoprostanic acid was isolated by column chromatography and crystallized to constant specific activity (1,012 cpm per mg). Identity was established by infrared spectra, paper chromatography, and melting point (153 to 156° C) of the methyl ester. The labeled acid was then given intravenously to a second patient with a bile fistula, and over 85% of the administered radioactivity was excreted within 5 hours as radioactive cholic acid, which was isolated from the bile and identified by the same procedures as were used for trihydroxycoprostanic acid (SA, 101 cpm per mg). This experiment demonstrates that trihydroxycoprostanic acid is a naturally occurring intermediate in the conversion of cholesterol to cholic acid in man. The fact that this acid is the major bile acid derived from cholesterol in certain lower vertebrates (alligator, crocodile, frog, toad), but an intermediate in the formation of "modern" C_{24} bile acids in man and other animals provides strong support for the hypothesis that the oxidation of cholesterol to cholic acid in man represents a biochemical recapitulation of human evolutionary history.

The Diagnosis of Myocardial Infarcts by Photoscanning after Administration of Cesium^{131}. EDWARD A. CARR, JR., BARBARA J. WALKER, AND JOHN BARTLETT, JR., Ann Arbor, Mich. (introduced by Fred M. Davenport).

Photoscanning offers the possibility of visualizing myocardial lesions directly. Sixteen dogs received 40 to 650 μc of Cs^{131} or Cs^{134} intravenously. In 4, myocardial infarcts had been created by coronary ligation 1 to 12 days previously; the thoracic incisions had been closed, and the dogs allowed to recover. Scans were performed 1¼ to 3 hours after isotope injection. The animals were then sacrificed. The excised blood-free hearts were rescanned and tissue Cs concentrations then determined. The myocardial [Cs]:blood [Cs] ratio increased linearly with time, reaching 20 by 2 hours after injection. This permitted myocardial scanning without interference from the cardiac blood pool. The [Cs] in infarcts was 7.9 to 42.0% (mean 19.6%) of that in normal myocardium. Scans *in vivo* showed the left ventricular muscle mass as a sharply outlined, even density. Infarcts were clearly delineated as "cold" areas of decreased uptake. Excised heart scans confirmed this. Compared to Cs^{134}, Cs^{131} gave better resolution. Its absence of β-emission decreases radiation hazard. A 60-year-old woman and a 52-year-old man each received 1.25 mc of $Cs^{131}Cl$ intravenously. Neither had a history or present evidence of heart disease. Scans 2¾ hours later clearly showed an even density corresponding to ventricular muscle. A woman, aged 66, who had sustained an anteroseptal infarct 3 days previously (confirmed by history, diagnostic electrocardiographic changes, and elevated serum glutamic oxalocetic transaminase concentration), was similarly studied. The scan showed a striking "cold" area in the region corresponding to the septum, extending irregularly to the circumference of the myocardium at one point. The scan of a man, aged 47, with a similarly documented posterior infarct, 10 days old, showed a 3-cm "cold" area at the diaphragmatic border. These findings indicate that Cs^{131} permits visualization of myocardial infarcts in dogs and suggest similar usefulness in man.

Metabolic Patterns of Hemolytic Susceptibility. PAUL E. CARSON AND GEORGE T. OKITA, Chicago, Ill. (introduced by Alf S. Alving).

Several genetic variations of human glucose 6-phosphate dehydrogenase (G6-PD) deficiency have already been defined both clinically and by electrophoretic analyses of the enzyme. Also, deficiency of glutathione reductase results in hemolytic susceptibility. The present data were obtained from G6-PD–deficient subjects including Negroes, one Caucasian of Sephardic origin and one with chronic nonspherocytic hemolytic anemia (CNSHA), and one Oriental. Data from two Caucasian subjects with GSSG reductase deficiency are also included. Both whole-body and hemolysate studies were done. The results suggest not only a common mechanism of hemolytic susceptibility, but also similar alteration of glucose metabolism in all these genetic variants. In the basal state, 2 hours after injection of glucose-1-C^{14} or glucose-6-C^{14}, the percentage of expired $C^{14}O_2$ is abnormally low. Normal values are $9.1 \pm 1.1\%$ ($n = 11$) and $8.6 \pm 1.1\%$ ($n = 8$), respectively. In one Caucasian (CNSHA) and six Negro G6-PD–deficient subjects, the average values were 4.9 and 4.1%, and in one subject with GSSG reductase deficiency, 5.7 and 4.3%. In patients with these enzyme deficiencies who were either nonfasting or ill, $C^{14}O_2$ from glucose-1-C^{14} tended to rise toward normal, but preliminary data suggest that the $C^{14}O_2$ from glucose-6-C^{14} does not. Hemolysate assays confirm the increased CSSG reductase in all cases of G6-PD deficiency. Study of stromal effects in normal hemolysates confirms the inactivation of G6-PD with concomitant increased activity of GSSG reductase. In G6-PD–deficient hemolysates, however, stromata fail to increase GSSG reductase activity. Thus, normal hemolysates can be changed to hemolysates like those found in G6-PD deficiency; this suggests that similar mechanisms may already have operated in G6-PD–deficient cells. GSSG reductase has a specific role as part of the oxidative portion of the pentose phosphate pathway.

The Treatment of Subacute Thyroiditis with Thyroid. CARL E. CASSIDY AND ALEXANDER S. ANDERSON, Boston, Mass. (introduced by E. B. Astwood).

Between January, 1956, and December, 1961, thyroid was prescribed in 74 cases of subacute thyroiditis. This report comprises the results of treatment in 46 patients observed throughout the course of the illness. Symptoms referable to subacute thyroiditis had antedated diagnosis for days or years; 17 patients had fever, 38 had tenderness of the thyroid, and all had goiter. Clinical manifestations were often more helpful than laboratory tests in making the diagnosis. The 24-hour accumulation of radioiodine by the thyroid was <15% in 18 patients, and the serum protein-bound iodine, >8.1 μg per 100 ml in 11 patients. The usual combination of a low radioiodine uptake and a serum protein-bound iodine above normal was found in only 8 patients. Remission of symptoms was attained in 36 patients during treatment with a single daily dose of 120 or 180 mg of thyroid U.S.P. Ten patients who experienced little or no improvement were then treated with corticosteroids. Treatment of thyroid was usually continued for 6 months or longer, and 30 patients were observed after the medication was discontinued. Eighteen of these experienced remissions lasting from 3 to 60 months, one for 1 month. Recurrence of symptoms within 2 months appeared in 9 patients, and within 6 months in two. Resumption of therapy again effected remission. This simple form of treatment effective in most cases of subacute thyroiditis acts, presumably, by inhibiting the secretion of thyrotropin.

Indirect Immune Hemolysis and Erythrophagocytosis Induced by Bacterial Antigens and "Natural" Antibody. PATRICIA CHARACHE AND COLIN M. MACLEOD,* Boston, Mass., and New York, N. Y.

Shortened red cell survival, occasionally associated with frank hemolysis or erythrophagocytosis, has been shown to occur in a wide variety of infectious disease. Human erythrocytes are agglutinated when they are coated with bacterial antigens and are exposed to antibacterial antibody. Human erythrocytes were coated with bacterial antigens by incubation for 1 hour at 37° C with boiled saline suspensions of bacteria. Fresh serum from 118 normal donors was added to *E. coli*-coated erythrocytes. After incubation at 37° C, lysis was read by eye as 0 to 4+. Serum from 69% of normal donors caused detectable hemolysis of coated autologous or compatible homologous cells. Most nonhemolytic sera contained anti-*E. coli* antibody, but were complement-deficient under test conditions. Hemolytic activity could be abolished by heating serum to 56° C for 20 minutes, or by removing antibody by preadsorption with antigen-coated erythrocytes. Activity could be restored by mixing heated, antibody-containing serum with adsorbed, complement-active serum; or by adding heated serum to serum from a person who lacked demonstrable antibody. Guinea pig serum was ineffective as a complement source. In high concentration, reconstituted, dried, guinea pig serum was found to inhibit the lysis caused by human serum. Antigens from *Proteus, Pseudomonas, Salmonella B, E. coli, Streptococcus,* and *Staphylococcus* were shown to cause this type of hemolysis. The hemolytic antibody present in serum of nonimmunized persons was shown to be cross-reactive, although some specificity could be demonstrated. The reaction was independent of blood group antigenic activity. When leukocytes were added to the preparations, erythrophagocytosis occurred. In some preparations, up to 80% of all monocytes and 20% of polymorphonuclear leukocytes contained one or more erythrocytes, giving a total phagocytic index of up to 30%. Occasional eosinophiles were phagocytic. In one patient, erythrophagocytosis occurred under conditions suggesting that this type of indirect immune reaction was operative.

Correlation between Clinical Manifestations and Rate of Sickling of Erythrocytes in Sickle Cell Disorders. SAMUEL CHARACHE, Baltimore, Md. (introduced by C. Lockard Conley).

Clinical differences between sickle cell anemia and heterozygous sickling disorders were related to differences in rheological behavior of blood during deoxygenation under controlled conditions. Relative viscosity of blood, measured in a falling-ball viscometer, was proportional to the product: (percentage of sickled cells) × (hematocrit value)^2. Serial measurements of viscosity at a hematocrit value of 30% were made during equilibration of blood at 37° with two gas mixtures, pO_2 of 0 mm Hg and pO_2 of 20 mm Hg (pCO_2, 35 mm Hg in each). In the hemoglobinopathies studied (S-S, S-C, S-D, S-F, S-Thal, J-S, A-S), severity of disease was related to the *rate* at which viscosity increased during the first 15 to 30 minutes of exposure of blood to a pO_2 of 20 mm Hg. Severity of disease was much better correlated with rate of increase in viscosity during partial deoxygenation than with the ultimate viscosity achieved. In the disorders studied, with the exception of sickle-thalassemia, differences in rates of sickling could be predicted from the rheological behavior of mixtures of the corresponding hemoglobins in solution. Sickling was enhanced in the presence of hemoglobin D_{Punjab}, correlating with severe sickle cell disease in an S-D heterozygote whose hemolysate contained only 42% hemoglobin S. There was little interaction between hemoglobins S and F, and S-F heterozygotes with as much as 70% hemoglobin S in their hemolysates were asymptomatic and not anemic. Interactions of hemoglobins S and $J_{Baltimore}$ were similar to those of S and A, and a J-S heterozyygote with 37% hemoglobin S had no manifestations of disease. Symptoms of sickle cell disease were abolished after exchange transfusions of normal blood; recurrence of symptoms correlated well with the rheological characteristics of blood as measured *in vitro*.

Production of Granulocytes by the Spleen in Chronic Granulocytic Leukemia (CGL). BAYARD CLARKSON, KAZUO OTA, ANNABEL O'CONNOR, AND DAVID A. KARNOFSKY,* New York, N. Y.

Since splenic irradiation causes remissions in CGL, the spleen may be the major source of leukemic cells. To study the kinetics of cellular proliferation in the spleen in CGL, tritiated thymidine (TdR·H^3) was infused for 4 days through a catheter inserted via the brachial artery into the splenic artery in two patients. Daily blood and periodic marrow and spleen samples were taken for radioautographs; the number of labeled cells and their grain counts were determined. Under the conditions employed, only cells originating in the spleen were labeled. In Patient 1, after 4 days, the percentages of labeled myeloblasts/myelocytes were: spleen, 80/80; blood, 70/80; and marrow, 70/59. The percentage of total leukocytes labeled at 4 days was 52%. Labeled metamyelocytes, bands, and polymorphonuclears first appeared in the blood 1, 3, and 5 days, respectively, after the start of the infusion and reached maximal values at 5, 8, and 10 days. Patient 2 was developing an acute transformation of CGL with 74% blasts in the marrow and 25% in the spleen. After the 4-day TdR·H^3 infusion, 83 to 85% of myelocytes were labeled in the spleen and blood, and 50% in the marrow; the subsequent labeling pattern of the more mature granulocytes was similar to that of Patient 1. The percentages of labeled blasts were much lower: spleen, 39; blood, 27; and marrow, 6%. These studies suggest that in these two patients with CGL, the spleen, as a result of an unknown stimulus, is producing most of the circulating immature granulocytes that are capable of division and differentiation. The marrow is seeded with these cells; most have a near-normal rate of maturation, although a few labeled myeloblasts and myelocytes persist in the blood for several weeks. When acute blastic transition occurred in Patient 2, the blasts underwent an unknown intrinsic alteration and became incapable of differentiation; they proliferated more widely in the spleen, marrow, and probably other tissues, but at a slower rate compared to normal myeloblasts.

Endogenous Carbon Monoxide Production in Patients with Hemolytic Anemia. R. F. COBURN, W. J. WILLIAMS,* S. B. KAHN, AND R. E. FORSTER,* Philadelphia, Pa.

Previous studies performed in our laboratory have demonstrated that carbon monoxide (CO) is produced in normal resting humans at an average rate of 0.42 ± 0.07 ml per hour (0.019 mmoles per hour), apparently as a by-product of hemoglobin catabolism in a ratio of 1 mole CO to 1 mole heme. In the present study, we have measured the rate of production of CO ($\dot{V}_{CO}$) in eight patients with hemolytic anemia by preventing the loss of CO from the lungs and measuring the rate of increase of CO in the blood. Erythrocyte survival times were estimated by a radioactive chromate method, assuming random destruction and correcting for elution of Cr^{51}, and total body hemoglobin was measured by CO dilution. The average destruction rate of circulating hemoglobin ($\dot{V}_{Hb}$) was calculated from these data. $\dot{V}_{Hb}$ in these patients ranged from 0.3 to 1.47 g Hb per hour, or from 0.025 to 0.104 mmoles heme per hour. $\dot{V}_{CO}$ was markedly elevated in all of these patients, ranging from 0.70 to 3.44 ml per hour, or 0.031 to 0.154 mmoles per hour. $\dot{V}_{CO}$ and $\dot{V}_{Hb}$ (both in mmoles per hour) were linearly related ($r = 0.93$). The average ratio of $\dot{V}_{CO}$ to $\dot{V}_{Hb}$ was 1.31 ± 0.10 and was not significantly different from this ratio in normal subjects. Two additional patients have been studied (one with porphyria cutanea tarda, and the other with fever of unknown origin) with markedly elevated $\dot{V}_{CO}$ and $\dot{V}_{CO}/\dot{V}_{Hb}$, but with normal Cr^{51} survival times. In these patients, the production of CO was greater than could be explained by the degradation of circulating hemoglobin. It appears that the measurement of $\dot{V}_{CO}$ is useful in assessing the rate of circulating hemoglobin and erythrocyte destruction and might prove useful in quantitating hemoglobin or porphyrin catabolism from sources other than the peripheral blood, as in the state of "ineffective erythropoiesis."

Antagonism of the Contractile Effect of Digitalis by EDTA in the Normal Human Ventricle. SIDNEY COHEN, CLYDE D. SCHOENFELD, ARNOLD M. WEISSLER, AND JAMES V. WARREN,* Columbus, Ohio.

Animal studies have demonstrated reduced effectiveness of cardiac glycosides on myocardial contractility when ionized Ca^{++} is diminished. The present study was designed to assess this relationship in the human ventricle. The contractile effects of digitalis were assayed by means of the left ventricular ejection time (carotid artery) corrected for heart rate (ETI), and hypocalcemia was induced by infusion of Edathamil Disodium (EDTA). Deslanoside (Cedilanid-D) administration in normal subjects is associated with a dose-dependent shortening of the ETI which persists for 8 hours. In the present study, Deslanoside administration (1.6 mg iv) to 12 normal male subjects was attended by a prompt decrease in ETI that averaged 15 msec per beat ($p < .001$) below control levels during the 8-hour study period. In repeated studies in 7 of the above, EDTA administration (3 g per 30 minutes) begun at 2 hours after Deslanoside was attended by a prompt and consistent reversal of the digitalis-induced shortening of the ETI, accompanied by a decrease in the mean serum Ca^{++} from 9.6 to 7.4 mg per 100 ml ($p < .001$). The EDTA effect on ETI persisted 30 to 60 minutes and paralleled the changes in serum Ca^{++}. As serum Ca^{++} rose to pre-EDTA levels, the digitalis-induced shortening of the ETI reappeared. It would appear that specific antagonism of the contractile effects of digitalis on the human ventricle, as reflected in the duration of left ventricular ejection, is induced by diminished levels of ionized Ca^{++}.

The Enzymatic Synthesis of Dehydroepiandrosterone Sulfate by a Human Adrenal Cell-Free System. GEORGE L. COHN AND VERA C. DUNNE, New Haven, Conn. (introduced by Philip K. Bondy).

The enzymatic synthesis dehydroepiandrosterone sulfate (DHAS) *in vitro* has been accomplished previously with mammalian liver microsomal-free preparations and only recently with human adrenal carcinomatous homogenates and adenomatous microsomal-free supernatant fluid. An investigation was instituted to study human hydroxysteroid sulfokinase activity with a microsomal-free system of "normal" and hyperplastic adrenals obtained at operation from patients with prostatic carcinoma ("normal"), and adrenogenital and Cushing's syndrome (hyperplastic). Aerobic incubation of microsomal-free supernatant fluid was carried out for periods up to 2 hours at 37° C in a 0.1 M phosphate buffer at pH 6.5 containing 0.013 M $MgCl_2$, 0.008 M K_2SO_4, 0.014 M ATP, and 0.56 to 1.9×10^6 cpm (SA, 1.25 mc per μmole) H^3–DHA in a total volume of 0.5 to 1.0 ml. Control flasks included zero-time, boiled, and absent enzyme. The "free" steroid was separated from the sulfate by dichloromethane extraction of a 70% methanol: media mixture. Free and steroid sulfate radioactivities were measured after elution from appropriate paper chromatograms. The conversion of DHA to DHAS with "normal" adrenal cell-free systems ranged from 1.2 to 35% per 2 hours per mg of protein. A comparable conversion rate was observed with the hyperplastic cell-free systems. Ammonium sulfate fractionation produced a fourfold increase of sulfokinase activity after the addition of dialyzed adenosine-3′-phosphate-5′-phosphosulfate (PAPS) generating system and 0.005 M. neutralized cysteine. No loss of sulfokinase activity was observed after 3 months of frozen storage in 0.1 M phosphate buffer at pH 7.0 with 0.001 M Versene. The observations indicate that adrenal hydroxysteroid sulfokinase(s) transfers activated sulfate from PAPS to DHA in a fashion similar to the pathway described for mammalian liver.

Alterations in Hepatic Uptake and Excretion of Sulfobromophthalein Sodium (BSP) in Normal Pregnancy. BURTON COMBES,* HISAO SHIBATA, REUBEN H. ADAMS, BILLIE D. MITCHELL, AND VICTOR TRAMMELL, Dallas, Tex.

Hepatic BSP removal mechanisms from blood were appraised quantitatively during normal pregnancy and the first week postpartum by the method of Wheeler and associates. Values were obtained for *1*) the hepatic relative storage capacity for BSP, S, defined as the milligrams of BSP stored in the liver per mg per 100 ml of plasma concentration, and for *2*) the maximal rate of BSP excretion into bile, BSP Tm, in milligrams per minute. During the last half of pregnancy, S rose 122% ($p < .001$) above values obtained in control nonpregnant women, 96.5 ±22.6 (SD) compared to 43.4 ±12.3 (SD) mg per mg per 100 ml. Increased BSP removal from blood could not be accounted for by uptake by fetus or placenta, or by urinary excretion, suggesting, therefore, that the storage capacity of the liver for BSP increases markedly during the last half of pregnancy. Since the liver size does not change significantly during the course of human pregnancy, a rise in S appears to be due to increased storage capacity of each unit of liver tissue. After delivery, S tended to fall toward control values, although it still remained elevated in some patients as long as 1 week postpartum. BSP Tm decreased 27% ($p < .01$) in the last half of pregnancy from a control value of 8.5 ± 1.3 to 6.2 ± 1.3 (SD) mg per minute, then rapidly returned to control values after delivery. BSP Tm measured directly in bile duct-cannulated rats also decreased in the last half of pregnancy, then returned to control values by 6 days postpartum. Changes in S and Tm appear to reflect alterations in two separate hepatic mechanisms, and tentatively are considered to result from increased hormone levels in pregnancy.

Immunological Deficiency Disorders Associated with Multiple Myeloma and Chronic Lymphocytic Leukemia. LAWRENCE A. CONE AND JONATHAN W. UHR,* New York, N. Y.

It was the purpose of this study to define more precisely the previously reported immunological deficits that often accompany multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). The following parameters of immunologic capacity were tested in 14 patients with MM, 11 with CLL, 1 with macroglobulinemia, and 20 hospitalized "control" patients: *a*) primary antibody response to bacteriophage ΦX 174; *b*) secondary antibody response to diphtheria toxoid; *c*) primary delayed-type hypersensitivity to 2,4-dinitrofluorobenzene (DNFB); and *d*) delayed-type hypersensitivity to commonly encountered antigens (CEA) in which sensitization was presumed to have occurred before clinical disease. The results of the study can be summarized as follows. *1*) Patients with MM and 1 with macroglobulinemia had a relatively intact capacity for secondary antibody formation and expression of delayed-type hypersensitivity to CEA. Only one-half of these patients, however, developed primary delayed-type hypersensitivity and almost all had a moderately reduced primary antibody response. *2*) In contrast to those with MM, none of 11 patients with CLL displayed a secondary antibody response, only 1 developed primary delayed-type hypersensitivity, and the primary antibody response was severely depressed or absent in all. The capacity to express delayed-type hypersensitivity to CEA was relatively unimpaired. These results indicate that MM is associated with a different immunological deficiency disorder than is CLL; moreover, both these deficiency disorders are different from that described in Hodgkins disease. It is also clear that immunological memory or persistence of an immunological responsiveness, such as delayed-type hypersensitivity, can be present without the capacity for a detectable response to a new antigenic function.

Intestinal Mucosal Mechanisms Controlling Iron Absorption. MARCEL E. CONRAD AND WILLIAM H. CROSBY,* Washington, D. C.

Total body radioactivity was measured in humans with a whole-body liquid scintillation detector at intervals after oral administration of a test dose of Fe^{59}. Iron-deficient subjects absorbed over 29% of the test dose and achieved equilibrium in 3 to 7 days. Normal volunteers retained less than 10% of the Fe^{59} and most did not attain equilibrium until 6 to 15 days after the test dose. The cause for the delay in iron-replete subjects was investigated. One might expect a delay if iron were sequestered by the intestinal epithelial cells until they were desquamated from the villus at the end of their life cycle. If little iron were retained in epithelial cells, no delay in achieving iron equilibrium would be observed. Radioautographs of duodenum and jejunum obtained from normal, iron-replete rats at intervals after an oral dose of Fe^{59} showed loss of labeled columnar epithelial cells which progressed from the base to the tips of the villi over a period of about 40 hours. This approximates the turnover time of villus epithelium in the rat. Two to 8 hours after the test dose of Fe^{59}, most of the epithelium covering the villus was radioactive. At 12 to 24 hours, only the distal half of the villus was radioactive. At 36 to 40 hours, only the distal tips of the villi were radioactive. Sections from iron-depleted and iron-loaded rats showed little radioiron within epithelial cells at any time. This suggests the mechanism by which the small intestine controls the absorption of iron. The columnar epithelial cells absorb iron from the gut lumen. If there is no demand for this iron, it remains in the cells and is lost into the intestinal lumen when they are shed at the end of their life cycle. In iron deficiency, very little of the absorbed iron is retained in these cells because it passes into the animal's body; in iron-loaded animals, the mucosa accepts very little iron from the lumen.

A Role of Mitochondria in Iron Metabolism of Developing Erythrocytes. RICHARD G. COOPER, LESLIE T. WEBSTER, JR., AND JOHN W. HARRIS,* Cleveland, Ohio.

Mitochondria of developing erythrocytes have been shown by electron microscopy to contain abnormally increased amounts of iron in certain hematologic abnormalities. Consequently, a study was undertaken to demonstrate a possible physiologic role for mitochondria in the metabolism of intracellular iron. The uptake and release of Fe^{59} were investigated in mitochondria prepared from reticulocytes of rabbits treated with phenylhydrazine and from human reticulocytes. An *uptake* of iron by mitochondria was shown that was dependent on time, temperature, and the production of ATP by oxidative phosphorylation. Iron uptake was diminished when ADP was depleted, thereby limiting the production of ATP. Addition of optimal concentrations of ADP to the depleted mitochondria stimulated iron uptake and oxygen consumption, the latter assayed by the oxygen electrode. Excessive amounts of ADP or ATP retarded iron uptake and decreased oxygen utilization. The *release* of Fe^{59} against a concentration gradient was demonstrated for actively metabolizing mitochondria. Pyridoxal phosphate was necessary for this process. Two patients with anemias due to combined vitamin B_6 and folic acid abnormalities were studied. Iron-saturated mitochondria obtained from the reticulocytes produced by correcting the folic acid defect could not take up Fe^{59} until pyridoxal phosphate was added. However, mitochondria obtained during the subsequent reticulocytosis induced by vitamin B_6 administration showed a normal iron uptake *without* added pyridoxal phosphate. In this instance, vitamin B_6-dependent unloading of endogenous iron was a prerequisite for the uptake of Fe^{59}. These studies demonstrate that mitochondria from erythrocyte precursors play a role in intracellular iron metabolism that is energy dependent and that the hematologic abnormality in the pyridoxine-responsive anemias is due in part to a block in the release of iron from mitochondria.

The Effects of Methandrostenolone and Hepatic Disease on Thyroxine Metabolism. W. S. COPPAGE, JR., AND ANNIS E. COONER, Nashville, Tenn. (introduced by David E. Rogers).

A decrease in the protein-bound iodide (PBI) without hypothyroidism is produced by certain androgenic or "anabolic" steroids. Decreased protein binding of thyroxine has been demonstrated and might explain this change. Since bromsulphalein retention is a constant accompaniment of such PBI depression, a direct hepatotoxic effect may be involved. To elucidate this problem and add to our knowledge of thyroid function in liver disease, the dynamics of thyroxine metabolism were studied in normal subjects, patients with viral hepatitis or cirrhosis, and subjects receiving methandrostenolone. Tracer doses of I^{131}-labeled L-thyroxine were given intravenously, and plasma samples, complete urine collections, and epithyroid counts were obtained for 9 to 16 days. The extrathyroidal organic iodide pool (EOIP), turnover rate (k) of EOIP, half-time of plasma thyroxine disappearance ($t_{\frac{1}{2}}$), thyroxine disposal rate, fecal and urinary iodide excretion, rate of thyroid uptake of iodide released from hormonal deiodination, and tissue thyroxine degradation rate were calculated. Striking differences were noted in all groups. *1*) Patients with hepatic cirrhosis showed increased PBI, EOIP, k, thyroxine disposal rate, and tissue thyroxine degradation, while $t_{\frac{1}{2}}$ was decreased in comparison with normal subjects. *2*) Two patients in the recovery phase of viral hepatitis showed increased PBI, EOIP, $t_{\frac{1}{2}}$, thyroxine disposal rate, and decreased k and thyroidal iodide uptake, while tissue degradation remained normal. *3*) Subjects receiving methandrostenolone had decreased PBI, EOIP, and increased k, while thyroxine disposal and tissue degradation remained normal. Thus, changes in thyroxine metabolism in cirrhosis, hepatitis, and with methandrostenolone are distinctive. The action of methandrosteno-

lone is specific and compatible with interference with protein binding sites for thyroxine or inhibition of thyroxine binding protein synthesis.

Infection of Volunteers with Artificially Propagated Eaton Agent (Mycoplasma Pneumoniae): Implications for Development of Attenuated Vaccine for Cold Agglutinin Positive Pneumonia. R. B. COUCH, T. R. CATE, AND R. M. CHANOCK,* Bethesda, Md.

Epidemiologic studies have associated Eaton agent with cold agglutinin-positive pneumonia as well as milder forms of respiratory diseases. Volunteers given a tissue-culture-propagated strain developed bullous myringitis as well as pneumonia. Recently, it was shown that Eaton agent is not a virus, but a pleuropneumonia-like organism (*Mycoplasma*) that can be cultivated on a special cell-free agar medium. In order to determine the virulence, pattern of infection, and antigenicity of the artificially propagated organism, 10^5 to 10^6 colony-forming units of 2 strains of Eaton agent (*Mycoplasma pneumoniae*) were administered to 42 antibody-free volunteers. Febrile illness occurred in 8 of these volunteers. Significantly, none of the volunteers developed pneumonia or bullous myringitis. When throat swabs were streaked on PPLO agar plates, it was possible to recover the organism from 21 of 27 volunteers during weeks 2 to 4 after inoculation. Thirty-nine of the 42 volunteers developed fluorescent stainable antibody to *M. pneumoniae,* 32 a rise in cold agglutins, and 7 a rise in antibody to *Streptococcus MG.* These findings indicate that agar-grown *M. pneumoniae* can infect man and cause febrile respiratory disease. This disease, however, occurred less frequently and was less severe than that produced by a tissue-culture-propagated strain. This difference suggests that propagation on a cell-free agar medium resulted in a decrease in virulence of *M. pneumoniae* for man. This finding has implications for the development of attenuated strains of *M. pneumoniae* to be used for vaccination of man.

Production of Tracheobronchitis and Pneumonia with Submicron-Size Particles of Coxsackie A_{21} Aerosol. R. B. COUCH, P. J. GERONE, T. R. CATE, W. R. GRIFFITH, D. L. LANG, K. M. JOHNSON, AND VERNON KNIGHT,* Bethesda and Frederick, Md.

Evidence indicates that inhaled particles 1.0 μ in diameter or less will deposit in the lower respiratory tract. With a modified Henderson aerosol generator with a Collison atomizer, volunteers were exposed to an aerosol cloud of an enterovirus (Coxsackie A_{21}). The median particle diameter of this aerosol was 0.5 μ. Ten susceptible volunteers infected with this aerosol characteristically exhibited an acute febrile tracheobronchitis, a syndrome not seen in susceptible subjects inoculated in the *upper* respiratory tract. Two of them developed segmental pneumonia. Infection was produced with as few as 16 inhaled $TCID_{50}$, only 50% of which is estimated to be retained. The human ID_{50} under the conditions of this experiment was 28 $TCID_{50}$ (95% confidence limits, 13 to 63 $TCID_{50}$). Virus isolation was invariably associated with illness and significant rise in neutralizing antibody. Volunteers with pre-existing antibody did not develop lower respiratory tract illness and exhibited decreased frequency and duration of virus shedding. This study demonstrated the small quantity of virus required to infect man and the importance of the site of localization of virus in determining the nature of resultant respiratory illness. This method of aerosol inoculation, which permits precise control of virus dosage and site of deposition, provides a useful model for investigations in man of pathogenesis, antiviral therapy, vaccine evaluation, and other problems in human viral disease.

The Effect of Human Uremic Serum and Its Fractions on Rat Brain Glucose Oxidation In Vitro. NANCY B. CUMMINGS AND DEWITT STETTEN, JR., Bethesda, Md. (introduced by Wallace P. Rowe).

Central nervous system symptoms are among the cardinal early manifestations of uremia. This study was undertaken to find a bioassay for a circulating toxin(s). Control serum and pools of sera drawn from 10 to 30 acutely, or chronically uremic patients, or both, were used as media. Rat brain mince was incubated in Krebs-Ringer phosphate (KRP) buffered serum at various glucose concentrations—11.84, 20, 40, 80, and 120 μM per flask containing 3 ml medium—for various times—15, 30, 60, 90, and 120 minutes—and with glucose-U-C^{14}, -1-C^{14}, and -6-C^{14} as label. Production of $C^{14}O_2$ was measured. An *increased* glucose oxidation by the mince incubated in uremic serum over that in the control of 18 to 80% (mean 51%) was found that was neither concentration- nor time-dependent. The ratio of glucose-1/glucose-6 oxidation approximated unity. Both uremic and control sera were treated by dialysis, trypsinization, trichloracetic acid deproteinization, and ultrafiltration. Dialysis and trichloroacetic acid deproteinization diminished but did not abolish the augmentation of glucose oxidation by uremic serum. The activity apparently was increased in ultrafiltrate and was not destroyed by trypsin or heat. No activity could be recovered in eluates from Dowex 1 and Dowex 50 columns. Due to the recovery of large amounts of succinic acid in the dialysate from two patients treated by hemodialysis, sodium succinate was tested, and in KRP it was found to exhibit a stimulatory effect on glucose oxidation by brain mince. Phenylacetylglutamine had a depressant effect and 200 mg per 100 ml urea had no effect. The data show a metabolic variation in brain glucose oxidation produced by a component of uremic serum that provides a possible assay for uremic toxin.

Physiologic Studies of Antidiuretic Hormone (ADH) by Its Direct Measurement in Human Plasma. WALTER J. CZACZKES AND CHARLES R. KLEEMAN,* Los Angeles, Calif.

Using the water-loaded, ethanol-anesthetized rat with exteriorized urinary bladder (modified method of Heller), we have accurately measured as little as 0.25 μU

per ml antidiuretic hormone (ADH) in plasma. A reproducible dose-response curve was obtained. This method made it possible to measure directly physiologic plasma levels and to study the turnover of endogenous ADH and of exogenous arginine-vasopressin administered in physiological doses. ADH in normal human plasma correlated well with the state of hydration. Values ranged from 1.5 μU per ml 3 hours after breakfast to 22.0 μU after 3 days of hydropenia. After an acute water-load, these basal levels fell to zero. Disappearance curves of endogenous and exogenous ADH were studied after 12 to 72 hours of dehydration. All disappearance curves of endogenous ADH consisted of two exponentials. The slope of the first (m_1) directly correlated with the initial level of ADH, while that of the second (m_2) was identical in 5 normal subjects studied after 12 to 24 hours of water deprivation. The second curve represents the true turnover rate with a "half-life" of 16 minutes. The difference between m_2 and m_1 represents the temporary continued "release" of ADH after inhibition of the neurohypophysis by the acute water-load. The "half-life" of arginine-vasopressin, given during sustained water-load, after 12 to 24 hours of water deprivation, was 20 to 22 minutes. Dependence of turnover rate on the state of previous hydration was demonstrated. The "half-life" of arginine-vasopressin given after 3 days of overhydration was 40 to 44 minutes. That of endogenous ADH after 3 days of dehydration was only 10 minutes. The volume of distribution of exogenous ADH invariably approximated plasma volume. By anaerobic ultrafiltration, no binding to plasma proteins could be demonstrated. Peak water diuresis *coincided* with ADH plasma concentrations between 1.9 and 0 μU per ml.

Comparison of the Effect of Tilting on Physiologic Dead Space and Diffusing Capacity in Normal Subjects and in Patients with Chronic Pulmonary Congestion. WALTER J. DALY, LEROY KING, JOSEPH C. ROSS, HARVEY FEIGENBAUM, AND EDWARD STEINMETZ, Indianapolis, Ind. (introduced by John B. Hickam).

This study was designed to provide further information concerning the effects of chronic pulmonary congestion on ventilation perfusion relationships. Physiologic dead space (V_D) was measured, supine and tilted (60° head up), in 16 patients with mitral valvular disease and pulmonary hypertension (mean pulmonary artery pressure = 38 ± 13 mm Hg, mean left atrial pressure = 22 ± 5 mm Hg) and in 15 patients without pulmonary hypertension (mean pulmonary artery pressure < 20 mm Hg). In the group with normal pulmonary vascular pressures, V_D increased during tilting from 142 ± 40 to 201 ± 55 ml ($p < 0.001$). In those with pulmonary hypertension, although V_D increased slightly during tilting, 192 ± 81 to 208 ± 84 ml ($p = 0.05$), the change was much less ($p < 0.001$). This difference between the V_D responses of the two groups was independent of differences in the effect of tilting on functional residual capacity or tidal volume. Pulmonary diffusing capacity (D_{Lco}) was measured under comparable conditions in 7 normal subjects and 4 patients with mitral valvular disease and pulmonary hypertension. During tilting, D_{Lco} decreased from 34.2 ± 6.9 to 27.9 ± 5.5 ml per mm Hg per minute ($p < 0.001$) in normal subjects; in those with pulmonary hypertension, D_{Lco} (25.6 ± 6.3 ml per mm Hg per minute) was not affected. These observations suggest that *a*) the unevenness of distribution of lung perfusion in relation to ventilation and the decrease in diffusing capacity that appear in normal subjects in upright postures do not occur in patients with pulmonary congestion and that *b*) the evenness of distribution of perfusion in relation to ventilation and the maintenance of pulmonary capillary blood volume are in part dependent upon pulmonary vascular pressure.

Potassium Depletion—A Disorder of the Pasteur Effect. ROBERT P. DAVIS AND GLADYS F. RAND, New York, N. Y. (introduced by Quentin B. Deming).

Oxidative metabolism inhibits glycolysis (the Pasteur effect) by promoting conversion of ADP to ATP within mitochondria, thereby depleting the cytoplasm of ADP. The balance of oxidative and glycolytic metabolism can be utilized as a sensitive indicator of energy production in tissues. We have used this indicator to study the effect of potassium depletion on energy metabolism in the kidney. Oxygen consumption is normal in both renal cortex and medulla of potassium-deficient rats. The rate of anaerobic glycolysis in the normal renal medulla is 19 ± 2.2 mμmoles lactate per mg wet wt per hour and is significantly inhibited by oxygen to 11.7 ± 2.1 mμmoles lactate per mg wet wt per hour. In potassium depletion, despite increased levels of glycolytic enzymes, demonstrated in homogenates, anaerobic medullary glycolysis is unchanged, but the Pasteur effect is completely effaced since oxygen is no longer inhibitory. Cortical metabolism is unaffected by potassium depletion. We interpret the lack of a Pasteur effect in the potassium-depleted medulla as indicating a significant decrease in conversion of ADP to ATP within mitochondria despite normal respiration. This defect in ATP formation may account for changes in medullary functions that require energy, like renal concentrating capacity. Further, the increased medullary aerobic lactate formation may explain the cellular acidosis and paradoxical aciduria seen in potassium depletion.

Water Transport across the Isolated, Everted Rabbit Gallbladder. JOHN M. DIETSCHY AND FRANZ J. INGELFINGER,* Boston, Mass.

Characteristics of water transport across the gallbladder (GB) wall were studied in rabbit gallbladders that were excised, everted, secured to glass cannulas, and maintained in oxygenated Tyrode's solution at 37° C. The gallbladders were dried to constant weight, and the data expressed in terms of the average GB dry weight (20 mg). *1*) Average net mucosal to serosal water transport was 210 μL per hour per 20 mg GB. During this transfer, serosal sodium and chloride concentrations did not

change. The mucosal surface was 0.9 ± 0.8 mv negative to the serosal surface. *2*) Transport was stopped by iodoacetate, dinitrophenol, and strophanthin G as well as by anoxia and sulfate-for-chloride substitution in the mucosal solution. *3*) When the mucosal solution was made hyperosmolar by the addition of mannitol, water transport continued until the osmolarity of the mucosal solution exceeded that of the serosal solution by 80 mOsm per L. *4*) Substitutions of sulfate for chloride ions in the mucosal solution caused only a moderate drop in net water transport until the chloride concentration was below 30 mEq per L. Below this level, transport rapidly dropped to zero as the chloride concentration approached 0 mEq per L. *5*) Unidirectional water fluxes were approximately eight times the net mucosal to serosal transport. In summary, net water transport across the gallbladder was essentially in the form of an isotonic NaCl solution; this movement was inhibited by agents that blocked metabolic energy sources, or which stopped net sodium transport. Transport of water under isosmotic conditions is sometimes regarded as a phenomenon secondary to small osmotic gradients caused by primary solute transport, but this concept is not supported by our findings that water continued to move against osmotic gradients as high as 60 mOsm per L. Low chloride concentrations in the mucosal solution limited water transport in the everted gallbladder, as it may well do in the *in vivo* gallbladder during the process of bile concentration.

Protein Binding of Cortisol in Man. RICHARD P. DOE AND ULYSSES S. SEAL, Minneapolis, Minn. (introduced by Wendell H. Hall).

We have previously isolated in highly purified form the corticosteroid-binding globulin (CBG) present in the serum of patients receiving diethylstilbestrol for cancer of the prostate. More recently, we have isolated the CBG of normal human serum and found it to be identical to that from estrogen-treated patients in terms of change of association constant with change in temperature, carbohydrate content, molecular weight, and cross-reaction with rabbit antihuman CBG antibodies. The proteins are homogeneous on the analytical ultracentrifuge and to immunoelectrophoresis. Normal serum contains about 25 mg per L of CBG, estrogen-treated plasma about 75 mg per L. Recent studies using purified CBG "stripped" of all steroids have revealed an association constant for cortisol of 1×10^7; aldosterone, 6.5×10^6; progesterone, 9.5×10^7; corticosterone, 6.4×10^6; estradiol, 2×10^4; and testosterone, 1.4×10^6 at 37° C. Thyroxine does not bind to CBG in this system. The relatively strong binding by progesterone allows it to displace cortisol in an isolated system. Data obtained by Daughaday, however, indicate that it cannot do this as strongly in whole serum. The presence of a strong non-CBG binding system for progesterone is thereby implied. It is possible that the elevation in nonprotein-bound cortisol that we have previously reported in pregnancy may be caused in part by elevated progesterone levels. A definitive answer in this regard cannot be obtained without identification and isolation of the progesterone-binding systems of plasma.

Role of Glucose in Appetite Regulation. P. M. EDELMAN, I. L. SCHWARTZ,* E. P. CRONKITE,* AND G. BRECHER, Upton, N. Y., and Cincinnati, Ohio.

The hypothalamic regulation of appetite involves a continually active lateral feeding center (LAT) that induces a sustained, manifest urge to eat and a normally quiescent, ventromedial satiety center (VM) that, when activated, suppresses this urge. It has been suggested that the satiety signals originate as a result of the uptake of glucose at specific glucoreceptor sites in or on the cells of the VM center. This glucostatic theory of appetite regulation appeared to receive support from our previous studies which found that gold thioglucose (GSG) produced gold-containing lesions in the VM area of the hypothalamus followed by hyperphagia and obesity, presumably because the critical neurones are destroyed by the gold accompanying the specifically accumulated glucose moiety of the GSG molecule. In recent studies involving the induction in mice of hypothalamic hyperphagia by injections of gold thioglucose at various blood levels of glucose and mannose, we have found that hyperglycemia does not suppress, but in fact enhances hypothalamic accumulation of gold. The gold was measured by exposing the brain to a neutron flux (7.5×10^{12} neutrons per cm^2 per second) for 9.3 hours and then, after an appropriate interval, resolving the gamma emission spectrum of the hypothalamic area with a multichannel analyzer. Therefore, the primary event in a postulated glucostatic regulation of appetite would appear to be not the attachment or accumulation of glucose at glucoreceptor sites on satiety neurons, but rather a specific effect of glucose on the permeability of the blood-brain barrier in the area of the VM nucleus. If this effect on permeability applies to glucose as well as to GSG, hyperglycemia would then be self-amplifying with respect to the postulated function of glucose as the activator of the satiety center.

Steroid Inhibition of Pituitary-adrenal Secretion. RICHARD H. EGDAHL * AND RICHARD N. ANDERSEN, Richmond, Va.

The purposes of these studies were *1*) to determine whether steroids inhibit pituitary-adrenal activation by means of a direct action on the adrenal cortex or anterior pituitary and *2*) to measure the effectiveness of steroids in inhibiting pituitary-adrenal activation after a variety of different stimuli. Adrenal cortical function was determined in dogs with chronic adrenal venous cannulas by measurement of adrenal venous blood corticosteroid secretion using the method of Peterson. One group of 7 hypophysectomized animals was infused with 5 mg per hour of dexamethasone. ACTH sensitivity tests using threshold doses of ACTH (2 and 10 mU) were carried out on these dogs before and 2 hours after dexamethasone infusion. No decrease in adrenal cortical responsiveness to ACTH was observed as a result of steroid administration. In another group of experiments, total brain

removal down to the hind-brain, leaving an isolated pituitary, was carried out. We have previously shown that such animals exhibit adrenocortical hypersecretion in the postoperative period. The intravenous infusion of 5 mg per hour of dexamethasone failed to depress the elevated corticosteroid secretory status of 9 dogs with isolated pituitaries, although hypophysectomy led rapidly to basal secretion in these animals. The other series of experiments involved the use of animals with intact central nervous system and of stimuli that we have previously found to yield rapid and maximal adrenocortical activation. There were *1*) hemorrhage to blood pressure of 60 mm Hg, *2*) abdominal laparotomy, *3*) leg burn, and *4*) electrical stimulation of sciatic nerve. The day after adrenal venous cannulation, the animals were anesthetized and an intravenous infusion of 5 mg per hour of dexamethasone was administered. At this dosage, dexamethasone was effective in partially preventing the normal pituitary-adrenal activation after abdominal laparotomy in 10 dogs, and completely prevented it in 7. Smaller doses of dexamethasone were less effective in inhibiting the response to laparotomy, and therefore the larger dosage was used throughout in these experiments. Dexamethasone completely inhibited th response to burn in 3 animals. On the other hand, steroid administration failed to prevent the maximal adrenocortical response to hemorrhage in 6 animals and only partially modified it in 2, and had no effect on the response to nerve stimulation in 6 animals, with some modification in 4. These studies suggest that *1*) steroids do not act directly on the adrenal cortex or anterior pituitary in producing their inhibtory effect on pituitary-adrenal activation and that *2*) steroid administration results in variable inhibition of pituitary-adrenal activation, depending on the stimulus used and the dose of steroid.

Sodium Reabsorption and Oxygen Consumption in the Human Kidney. GILBERT M. EISNER, LAWRENCE M. SLOTKOFF, AND LAWRENCE S. LILIENFIELD,* Washington, D. C.

Several laboratories have reported a linear relationship between oxygen consumption and sodium reabsorption in the dog kidney. The present study was undertaken to explore this relationship in the kidney of man. Renal vein catheterizations were performed via retrograde percutaneous femoral vein puncture. Determinations were then made of arterial and renal venous oxygen content. Inulin clearance, para-aminohippurate clearance and extraction, and sodium excretion were also measured. To date, 15 acceptable determinations in 5 subjects over a wide range of sodium reabsorptions are available for analysis. When sodium reabsorption per 100 ml of basal glomerular filtration rate is plotted against oxygen consumption per 100 ml of basal glomerular filtration rate, a linear relationship is observed. The slope of the line has a value of 17 mEq sodium reabsorbed per 1 mmole of oxygen consumed. In contrast to the reported animal results, this line has a positive intercept with the sodium reabsorption axis at zero oxygen consumption. The data are interpreted to indicate a stoichiometric relation between renal oxygen consumption and sodium reabsorption. In addition, however, there appears to be a significant amount of sodium reabsorbed without the utilization of oxygen by the human kidney.

Molecular Localization of the Immunochemical Properties of Gamma Globulins. JOHN L. FAHEY,* Bethesda, Md.

The three major serum γ-gobulin classes (6.6 S γ, β_{2A}, and γ_{1M}) share a relationship having *1*) distinctive immunochemical properties, specific for each globulin class, and *2*) common features shared by all γ-globulin classes. The present studies were undertaken to localize the class-specific properties and the common properties on subunits of the γ-globulin molecules. Normal γ-globulins, γ- and β_{2A}-myeloma proteins, and γ_1-macroglobulins were reduced and alkylated to obtain "H" and "L" polypeptide chains. These subunits represent approximately 75 and 25%, respectively, of the original molecule and differ in antigenic composition. The "H" polypeptide chains were found in three forms that determine the class-specific properties (γ, β_{2A}, γ_{1M}) of γ-globulin molecules. "L" chains obtained from all three γ-globulin classes had similar antigenic determinants, and the InV genetic factors common to all classes of γ-globulin were found on the "L" polypeptide chains. Two antigenically distinct types (I and II) of "L" polypeptide chains were identified. Observations in 125 sera indicate that the normal plasma cell system has the capacity to form three types of "H" chain and two types of "L" chain. Although individual molecules have only one type of "H" and one type of "L" polypeptide chains, six different combinations of "H" and "L" chains are represented among the normal γ-globulins. Malignant plasma cells are restricted to the synthesis of only one type of "L" and "H" polypeptides, and for this reason, myeloma proteins and macroglobulins appear to be antigenically deficient when compared to normal γ-globulin preparations.

Rate of Plasma Triglyceride Synthesis in Carbohydrate-induced Lipemia. JOHN W. FARQUHAR, GERALD M. REAVEN, RICHARD GROSS, AND ROGER WAGNER, Palo Alto, Calif. (introduced by Herbert Hultgren).

Lipemia (an increased plasma D < 1.006 lipid, present 12 hours postprandially) was present in 3 hypercholesteremic males during 5 weeks of ingestion of high carbohydrate formula diets and absent during isocaloric high fat diets, supporting the contention of Ahrens and associates that most patients with hyperlipemia are "carbohydrate-induced." Glycerol-H^3 was administered to 2 patients during both dietary periods when plasma triglycerides (TG) were stable, and was chosen in preference to fatty acid-albumin complexes (FFA) as a precursor of plasma D < 1.006 TG, since glycerol is not utilized by adipose tissue—it is incorporated into hepatic and plasma triglyceride in rats—and because of the known extensive recycling of FFA. After intravenous

injection of glycerol-H^3 12 hours postprandially, peak radioactivity appeared in 1 to 2 hours in plasma D < 1.006 TG, and remained first order in its decline until <5% of peak activity was present. In contrast, published data on plasma D < 1.006 TG activities after C^{14}-FFA are more curvilinear (beginning at approximately 25% of peak radioactivity). Increased plasma TG after high carbohydrate feeding were accompanied by prolongation of D < 1.006 TG $t_{\frac{1}{2}}$ in Subject A from 1.8 to 2.7 hours and in Subject B from 3.4 to 8.6 hours, and increases in $t_{\frac{1}{2}}$ correlated well with changes in D < 1.006 TG pool size (Subject A, 237 to 490 mg per 100 ml and Subject B, 432 to 790 mg per 100 ml). Turnover rates were also increased (0.84 to 1.24 g per hour in Subject A, 0.64 to 1.37 g per hour in Subject B) after periods of high carbohydrate intake. Our data is consistent with the hypothesis that carbohydrate-induced lipemia results from increased synthesis of D < 1.006 TG; net D < 1.006 TG removal rates increasing insufficiently to prevent expansion of pool size. In conclusion, *1*) carbohydrate-induced lipemia is associated with increased triglyceride synthesis, and *2*) glycerol-H^3 can be used as a precursor for plasma D < 1.006 TG and allows more valid measurements of triglyceride synthesis rates than does FFA.

Boolean Algebra and the Clinical Spectrum of a Human Disease. ALVAN R. FEINSTEIN, New Haven, Conn. (introduced by Stuart C. Finch).

A major problem of statistical evaluations in modern medicine is the need for dividing human illness into subgroups of patients similar enough to justify their comparison in therapeutic, epidemiologic, and experimental studies. The problem is not adequately managed by current techniques that divide patients into groups with a common diagnosis, common laboratory data (in tissues, fluids, excreta, X rays, etc.), and common personal properties (age, race, sex, etc.). Such techniques fail to classify signs, symptoms, and mode of discovery of each instance of the disease. The unclassified clinical data act as unrecognized sources of heterogeneity in any apparently homogeneous subgroup. The clinical manifestations of a particular disease can be classified by set theory, the Venn diagrams of symbolic logic, and elementary Boolean algebra. Although each disease has its own specific clinical patterns, a general spectrum suitable for all diseases can be constructed. The general spectrum, showing the clinical stage of each patient at the time his disease is found, has seven subsets of patients, defined according to the presence or absence, singly or in combination, of primary clinical features, secondary features (or complications), and specific complaints due to these features. The Venn diagrams of these intersecting sets can be augmented by the subsets of disease discovered first at autopsy or not found at all. The ultimate diagram shows the analytic synthesis of the spectrum of a human disease, with overlapping clinical complexities that can be identified and organized to bring new precision to numerical analyses of data. Individual spectrum patterns, subordinate to the general spectrum, have been constructed for rheumatic fever and carcinoma of the lung. These constructions demonstrate how the new classification technique improves understanding of mechanisms in pathogenesis and accuracy of prediction in prognosis.

Fibrinogenolysis In Vivo: Identification, Occurrence, and Characterization in Man. SHARON FISCHER, ANTHONY P. FLETCHER,* NORMA ALKJAERSIG, AND SOL SHERRY, St. Louis, Mo.

In vivo, fibrinogen and fibrin proteolysis is mediated through the enzymatic actions of the plasminogen plasmin system, which digests these substrates into fragments of widely varying molecular weight. The role of one such fragment (5.27 $S_{20,w}$) in the pathogenesis of a unique coagulation disorder, defective fibrin polymerization, has recently been established, but hitherto identification and characterization of these fibrinogen fragments in plasmas has been accomplished. Analytical ultracentrifugal studies of fibrinogen digestion by plasmin were correlated with an immunoelectrophoretic procedure employing acrylamide gel (30A pore size for molecular sieving action and to exclude native fibrinogen) for initial separation and agar gel for immunodiffusion (rabbit antihuman fibrinogen sera). A total of four fibrinogen fragments characterized by ultracentrifugation, with respective S values of 5.6 (uncorrected), 5.27, 3, and 1.4 (the latter are extrapolated values) were also identified by immunoelectrophoresis using dual electrophoretic and immunologic criteria. The two larger fragments (5.6 and 5.27 S) were also demonstrated to inhibit fibrin polymerization. Plasma proteolysis was induced *in vivo* by infusing the plasminogen activator urokinase (100 to 600,000 U per hour) or nicotinic acid; in mild proteolytic states, only the 5.6 S and sometimes the 5.27 S components were identified in plasma, but in more intense plasma proteolytic states, also components of lower sedimentation constant. Comparable *in vitro* experiments yielded similar results. Immunoelectrophoresis performed on patients undergoing cardiac bypass procedures, suffering from coagulation disorders associated with hepatic cirrhosis, acute leukemia, prostatic carcinoma, and obstetric complications yielded variable patterns with predominant 5.6 and 5.27 S components. These patterns correlated well with other biochemical evidence of plasma proteolytic or thrombolytic activity. These observations define the reaction sequence by which *in vivo* fibrinogen proteolysis proceeds and suggest that defective fibrin polymerization may be of wider significance than has been hitherto appreciated.

A Mechanism of Viruria. C. LARKIN FLANAGAN AND IRWIN SCHULTZ, Chicago, Ill. (introduced by David P. Earle).

Viruria is a common phenomenon, but little is known about the mode of entry of viruses into the urine. Coxsackie B_1, one of the small enteroviruses, was administered in a single injection containing 10^7 to 10^9

tissue-culture infective doses ($TCID_{50}$) intravenously or directly into one renal artery of 15 anesthetized dogs. Virus appeared in ureteral urine from *both* kidneys after injection by *either* route whenever blood levels exceeded 10^3 $TCID_{50}$ per ml. Urine levels as high as $10^{2.5}$ $TCID_{50}$ per ml resulted when viremia of at least $10^{4.5}$ $TCID_{50}$ per ml persisted for 30 minutes. During diuresis induced by hypertonic mannitol, virus appeared in the urine within 3 minutes, slightly before the peak urinary concentration of simultaneously administered I^{131}-labeled *o*-iodohippuric acid (I^{131}-Hippuran), a substance excreted primarily by proximal tubules. Viruria also was measured under conditions of stop-flow analysis during which I^{131}-Hippuran and ferrocyanide were used as proximal tubular and glomerular markers, respectively. Virus was injected 2 minutes before the end of an 8-minute period of ureteral obstruction. Upon resumption of urine flow, virus appeared before the markers and near the region of maximal sodium reabsorption by the distal tubule. These data suggest that Coxsackie B_1 virus enters the urine in a region relatively far down the dog nephron, presumably either in distal tubule or loop of Henle. The experimental design does not permit conclusions about whether this site is the sole region of entry or only the most distal one. The results may have important implications, however, not only for viruria, but also with regard to the mechanisms of renal excretion of macromolecular substances in general.

Micropuncture Study of Experimental Tubular Necrosis. WILLIAM J. FLANIGAN, RAJA N. KHURI, AND DONALD E. OKEN, Boston, Mass. (introduced by Kendall Emerson, Jr.).

Acute anuria was induced in rats with 12 mg per kg $HgCl_2$ im. Satisfactory fluid intake was assured by injection of 0.45 M NaCl as indicated by fluid ingestion. Arterial blood pressure was measured either directly, or with a tail microphone. Intratubular pressure and tubule flow were assessed up to 26 hours after injection. Sections of kidney were taken for histologic correlation, and the appearance of the kidney and renal blood flow noted *in vivo*. Renal circulation did not appear grossly impaired in most animals, even those with total anuria. Normal intratubular pressure measured with a water manometric system in 59 control experiments on 23 rats was 15.6 ± 0.3 (SE) cm H_2O. Four to 6 hours after $HgCl_2$, mean intratubular pressure in 52 experiments on 9 rats was 16.2 ± 0.6 cm H_2O, a value not statistically different from controls. Animals at this time were frequently diuretic, and the tubules *in vivo* appeared grossly normal. At 9 to 13 hours, mean tubule pressure in 79 experiments on 14 animals was reduced to 11.3 ± 0.9 cm H_2O ($p < 0.001$). Pressure was further reduced to 8.5 ± 0.5 cm in 128 measurements on 24 animals 18 to 26 hours after $HgCl_2$. These animals showed pallor and frank desquamation of cells into the lumen. Tubule flow was totally lacking in most nephrons, pressures being lowest in frankly obstructed tubules and unrelated to systemic blood pressure. If anuria were the result solely of tubular obstruction with debris or of interstitial edema, proximal tubule pressures should be high. That pressures are low before evidence of obstruction suggests that reduced glomerular filtration plays a significant role in the development of anuria in experimental tubular necrosis.

Inhibition by Sugars of Renal Amino Acid and Phosphate Transport. MAURICE FOX, SAMUEL THIER, LEON ROSENBERG, AND STANTON SEGAL,* Bethesda, Md.

Mellituria in diabetes, galactosemia, fructose intolerance, and the Fanconi syndrome is frequently associated with amino aciduria and hyperphosphaturia. In an effort to explain these phenomena, experiments were undertaken to ascertain the effect of sugars on amino acid transport by rat kidney cortex slices. The intracellular accumulation of neutral amino acids was inhibited by 3 mg per ml of D-glucose, D-galactose, and D-fructose, but not by D-xylose, D-ribose, D-3-O-methylglucose, D-2-deoxyglucose, D-3-deoxyglucose, sucrose, or raffinose. Michaelis-Menten analysis indicated that D-fructose reduced the V_{max} for amino acid transport, but did not alter the K_m, indicating noncompetitive inhibition. Slices preincubated in glucose or fructose and then transferred to flasks containing amino acid but no sugar still showed impaired ability to transport amino acids. In addition, the inhibition of amino acid uptake by sugar was not evident before 30 minutes incubation. These data suggest that a metabolite of glucose, galactose, and fructose is responsible for the observed inhibition. The relevance of these findings in man was confirmed as follows. Clearance studies were performed in five normal subjects before and after infusions of 10% solutions of various sugars. During the infusion of glucose, the urine flow rate increased 17% and the glomerular filtration rate (GFR) 10%, while the average excretion ratios of phosphate (C_{PO_4}/C_{inulin}) and α-amino nitrogen ($C_{\alpha AN}/C_{inulin}$) increased 89% (.127 to .240) and 166% (.029 to .077). The infusion of xylose at the same rate increased the urine flow rate 38% with no significant change in GFR, but reduced the excretion ratios of phosphate and α-amino nitrogen. Column chromatography revealed that glucose induced a generalized amino aciduria. The excretion ratios of phosphate and α-amino nitrogen could not be correlated with the plasma glucose concentration or the rate of glucose excretion.

Renal Clearance of Aldosterone and Its Major Metabolites. GEORGE W. FRIMPTER, WALTER SIEGENTHALER, AND RALPH E. PETERSON,* New York, N. Y.

The renal plasma clearances of free aldosterone, its 3-oxo conjugate, and tetrahydroaldosterone glucuronide were determined in 3 normal subjects. Glomerular filtration rate was measured by inulin clearance. In 2 subjects, tracer amounts of 1,2-H^3-aldosterone were infused continuously, and in the third subject, nonradioactive aldosterone only was infused continuously at various dose levels. The concentrations of plasma and urine

free aldosterone, aldosterone released after pH 1 incubation, and tetrahydroaldosterone released after incubation with β-glucuronidase were determined by a double-isotope assay (after H^3-aldosterone) using aldosterone-4-C^{14}, or by a double-isotope dilution derivative assay (after nonradioactive aldosterone) using C^{14}-acetic anhydride. The fractions of aldosterone and its metabolites not bound to protein were determined by equilibrium dialysis. The renal clearances were calculated on the basis of the unbound fractions. Free aldosterone was cleared at a rate of 3 to 6%, the 3-oxo conjugate at 250 to 300%, and tetrahydroaldosterone glucuronide at 70 to 100% of the inulin clearance. After administration of very large doses of aldosterone, renal excretion of free aldosterone increased to 22% of the amount filtered. The clearance of the 3-oxo conjugate was markedly decreased by administration of *p*-aminohippurate. These studies indicate that 94 to 97% of the filtered free aldosterone, but only a small percentage of the filtered tetrahydroaldosterone glucuronide, is reabsorbed by the renal tubule. The 3-oxo conjugate, however, is secreted by the renal tubule. These data explain the low levels of urinary free aldosterone (0.05 to 0.5 μg per day), the prompt appearance of significant quantities of the urinary 3-oxo conjugate after iv administration of aldosterone, and the delayed excretion of tetrahydroaldosterone glucuronide in renal disease.

Acidmucopolysaccharides (APS) in Human Cirrhosis. JOHN T. GALAMBOS, Atlanta, Ga. (introduced by John R. K. Preedy).

It has been shown that during regeneration and repair of connective tissue, the accumulation of APS precedes and influences the deposition of collagen fibriles and fibrous organization. The present study indicates that this same sequence occurs in human hepatic fibrogenesis. Livers from 132 biopsies and 30 autopsies were examined histochemically for APS by the alcian blue-PAS, colloidal iron-Van Gieson, and toluidine blue stains. The histochemical grading of APS correlated closely with the hexosamine concentration of APS fraction of the autopsied livers. In the normal liver, APS content is always low and is demonstrable only in bile ducts and vessel walls. APS appears promptly and in large amounts only after certain types of hepatic injuries. The accumulation of APS is not so commonly associated with centrolobular lesions or viral hepatitis as it is with portal or periportal injury. The sequence of change was followed from the alcoholic fatty liver without fibrosis, but with large amounts of APS present in broad, bandlike areas across the lobule, to the cirrhotic stage where collagenous septi are demonstrated. In some cirrhotic livers, APS surrounds proliferating ductules, or is seen between parenchymal cells and fibrous septi. In early biliary cirrhosis, APS is confined to the portal areas. The data suggest that in the human liver, histochemically demonstrable APS outside the bile ducts and vessel walls means increased activity of hepatic connective tissue elements and may be the first and still reversible phase of hepatic fibrosis. Accumulation of APS in fibrotic or cirrhotic livers means reactivation or continued activity in the fibrotic process, and its presence seems essential for active fibrogenesis in the human liver. Classification of cirrhosis based on the activity of the fibrotic process is of great clinical value.

Thyroid Hormone-Catecholamine Interrelationships. VALERIE ANNE GALTON, Hanover, N. H. (introduced by S. M. Tenney).

Considerable evidence exists to indicate a mutually potentiating action between thyroxine and the catecholamines, but the nature of this relationship is unknown. In view of the suggested association between the metabolism of thyroxine and its physiological action, the effect of catecholamines on the deiodination of this hormone was investigated. Deiodination was studied in thyroidectomized rats (100 g) maintained with I^{131}-labeled thyroxine (5 μg per day) by measuring the daily urinary excretion of I^{131}-iodide. When isotopic equilibrium was obtained, approximately 45% of the daily dose of I^{131} was excreted in the urine. Urinary I^{131} was increased after an injection of adrenaline (100 μg) and was decreased by reserpine (50 μg). These effects were maintained for at least 24 hours. The effect of catecholamine administration *in vivo* on the deiodinating activity of tissues studied *in vitro* was also assessed in mice. Homogenate systems (1 g tissue per 10 ml Krebs-Ringer phosphate buffer) were incubated with I^{131}-labeled thyroxine, and the radioactive products were analyzed by standard chromatographic and scanning techniques. Both adrenaline and noradrenaline, when administered for 3 days or more (20 μg per day), induced a significant increase in the deiodinating activity of mouse liver homogenate. The effect was not solely due to the hyperglycemic or hypermetabolic actions of the catecholamines, since the increase was observed after administration of glucose or 2,4-dinitrophenol. Further deiodinating activity was greatly decreased in tissues obtained from mice pretreated with reserpine. Others have indicated that thyroxine may augment the action of adrenaline by retarding its metabolic breakdown. The present studies indicate that the catecholamines enhance the deiodination of thyroxine, a process postulated to be linked to hormonal action. It is therefore suggested that the mutually potentiating effects of the two hormones define a "closed-loop" type of control system in which each hormone influences the degradation of the other.

Surface Polysaccharides as Recognition Sites for Cellular Interactions of Lymphocytes. BERTRAM M. GESNER AND VICTOR GINSBURG, Bethesda, Md. (introduced by J. E. Rall).

The well-established fact that lymphocytes are concentrated in the spleen and other lymphoid tissues after intravenous injection raises the question of what factors cause a specific cell to associate with a specific tissue. In order to investigate this process, P^{32}-labeled lympho-

cytes obtained from the thoracic duct were injected intravenously into rats of the same stock. The recipients were sacrified 30 minutes after injection, and estimation of radioactivity in several organs confirmed the finding that lymphocytes are markedly concentrated in the spleen at this time. Prior incubation of lymphocytes *in vitro* with a partially purified glycosidase preparation derived from *Clostridium perfringens* prevented the accumulation of lymphocytes in the spleen. Treatment with the enzyme preparation did not appear to alter the viability of the cell population. The specific effect of the enzyme preparation could be prevented by the presence of L-fucose or N-acetyl-D-galactosamine in the incubation mixture. D-Glucose, D-galactose, D-mannose, or N-acetyl-D-glucosamine were without effect. Morgan and Watkins have found that destruction of blood group specificity by a similar enzyme preparation could be prevented by specific sugars that act by inhibiting their respective glycosidases. Such a mechanism would appear to be responsible for the protective effect of L-fucose and N-acetyl-D-galactosamine reported here. Thus, it may be proposed that selective accumulation of lymphocytes in lymphoid tissue is dependent in part on the recognition of specific polysaccharide surface structures on lymphocytes. Alteration of the lymphocyte surface by enzymic removal of monosaccharide components apparently destroys specific surface configurations that are normally recognized by complementary structures in lymphoid tissue.

On the Role of the Adrenergic Nervous System in Sodium Metabolism. JOHN R. GILL, JR., DEAN T. MASON, AND FREDERIC C. BARTTER,* Bethesda, Md. (introduced by James H. Baxter).

Sodium excretion ($U_{Na}V$) and retention have been studied in patients with normal cardiac function before and during depletion of catecholamines and blockade of sympathetic reflexes. Initially, maximal $U_{Na}V$ with a sodium load (normal saline 2 L over 2 hours) ranged from 248 to 552 μEq per minute. With guanethidine, 20 to 90 mg per day, $U_{Na}V$ was increased 2 to 4 times to reach 969 to 1,275 μEq per minute, with no change or a slight rise in the clearance of inulin and para-aminohippurate, while potassium excretion was less than without guanethidine. The ability of guanethidine to increase Na excretion markedly suggested that it might prevent Na retention. The subjects were given a high Na intake (250 mEq Na per day) and a Na-retaining steroid desoxycorticosterone acetate, Doca (20 mg per day). They retained 674 to 972 mEq of Na and gained 3 to 4.6 kg in body weight before Na excretion rose to equal Na intake, and weight gain ceased (i.e., "escape" occurred). With guanethidine, they retained much less Na (187 to 536 mEq) and gained much less weight (0.7 to 2.2 kg). Guanethidine produced this "escape" from Doca at a lower creatinine clearance and lower mean brachial arterial pressure, and without a greater K excretion. Thus, normal subjects with sympathetic blockade behave similarly to those with untreated hypertension in their response to Na-loading and Na-retaining steroids. The sympathetic nervous system probably produces alterations in Na retention and excretion by its effect on the renal vasculature. Adrenergic mechanisms appear to contribute to the regulation of Na metabolism in health as well as in disease.

The Site of Intestinal Absorption of Vitamin B_{12} in Man. D. GIORDANO, A. DOWELL, S. STOUDER, AND P. A. CHRISTIANSEN, Indianapolis, Ind. (introduced by Paul J. Fouts).

Application of the reference substance technique has allowed more physiologic measurement of *in vivo* intestinal absorption in man. This method was employed to study the site of absorption of vitamin B_{12}, which has not so far been conclusively shown. A nonabsorbed reference substance, polyethylene glycol (PEG), was fed with Co^{60}-vitamin B_{12} (range 0.42 to 0.76 μg), and their relative concentration was compared in samples at various loci in the intestine. Collections were made by a small-diameter, double-lumen polyethylene tube whose aspiration holes were 100 cm apart. Samples were divided into five 30-minute periods starting 30 minutes after the vitamin B_{12} was given. Absorption of vitamin B_{12} was indicated when its relative concentration to PEG decreased. Calculations were based on change in ratio, relative concentration in micrograms, and tubes treated singly and paired. Ten normal subjects were studied with sampling from sites varying from 50 to 305 cm from the nose. No absorption could be demonstrated in the proximal 100 cm of intestine. Significant absorption was demonstrated distal to 150 cm, $p > 0.025$. An analysis of time in relation to absorption revealed that no significant absorption occurred during the first hour after ingestion, regardless of site, but there was significant absorption in the succeeding 30-minute periods, when $p > 0.05$, 0.025, 0.01, and 0.05, respectively. The findings of this study indicate that vitamin B_{12} absorption occurs over a wide range of the small intestine from the distal half of the jejunum and beyond, and that time probably is an important factor. Vitamin B_{12} appears to be an exception to the usual site of absorption of most other natural substances in that it is absorbed in the distal two-thirds of the small intestine, in contrast to the proximal third for others.

Norepinephrine Metabolism in Essential Hypertension. STANLEY GITLOW, MILTON MENDLOWITZ,* ELIZABETH KRUK, SHERWIN WILK, ROBERT WOLF, AND NOSRAT NAFTCHI, New York, N. Y.

In an effort to evaluate the metabolism of norepinephrine (NE) in essential hypertension, 3 normal subjects and 3 patients with essential hypertension were infused for 1 hour with 0.05 μg per kg per minute of *dl*-β-H^3-NE of high specific activity. Blood samples were drawn at the end of the infusion and at varying intervals for up to 3 days after administration of the H^3-NE. Each plasma sample was separated into an alumina-adsorbable

fraction, the eluate, consisting predominantly of H^3-NE, and a remaining effluent, consisting largely of H^3-normetanephrine and H^3-vanillylmandelic acid. A sample of each eluate and effluent was assayed in a dioxane-naphthalene phosphor in a liquid scintillation counter, and then the H^3 activity was plotted against time. In both groups of patients, the effluent H^3 activity reached its peak minutes after the end of the infusion, declined minimally, and remained elevated for as long as blood samples were taken. After the H^3-NE infusion was stopped, on the other hand, the eluate H^3 activity fell precipitously during the first 5 minutes and then more slowly, until, about 3 hours later, the decline became rectilinear on a semilogarithmic scale. In the normal subjects, the half-life of the plasma H^3-NE during this portion of the disappearance curve was 230, 480, and 580 minutes. Half-life values of the patients with essential hypertension were only 123, 192, and 210 minutes. Extrapolation of these slopes to their respective ordinates showed that the intercept points of the normal subjects were 7,800, 2,400, and 2,200 cpm per ml of plasma, respectively, whereas they were as high as 11,500, 11,500, and 6,500 cpm per ml of plasma in the hypertensive patients. If further studies confirm these preliminary investigations, it would appear that essential hypertension is associated with a defect in the dynamics of NE metabolism.

Measurement of Ventricular Dimensions in Assessing Drug Action on the Human Heart. GERALD GLICK, DONALD C. HARRISON, ALAN GOLDBLATT, AND EUGENE BRAUNWALD,* Bethesda, Md.

Although knowledge of ventricular dimensions is of fundamental importance in the understanding of myocardial function, it has so far been impossible to determine the effects of drugs on ventricular dimensions in intact human subjects. Two vasoactive drugs, each serving as a prototype of a number of agents, were studied. The drugs selected were isoproterenol, which has a direct positive inotropic action and dilates the arteriolar bed, and methoxamine, which constricts the arteriolar bed, but has no direct cardiac action. Radiopaque silver clips were sutured to the surface of either or both ventricles of 14 patients undergoing cardiac operations. After recovery, cineradiograms were exposed at 30 frames per second, and distances between clips were measured. When isoproterenol was administered intravenously, at an average dose of 2.5 μg per minute, to 12 patients, end-systolic dimensions decreased in all patients, falling an average of 6.7% of control values in the left ventricle and 8.5% in the right ventricle. End-diastolic dimensions declined in 10 of 12 patients by an average of 6.1% of control values, and remained unchanged in the other 2. In contrast, methoxamine, given to 8 patients in doses that raised systolic arterial pressure by an average of 26 mm Hg, always augmented ventricular dimensions. Left ventricular end-systolic dimensions increased an average of 3.9% and end-diastolic dimensions by 3.0% of control. Changes in right ventricular dimensions were of similar direction and magnitude. Measurements of ventricular dimensions, when combined with the standard determinations of intravascular pressures and flow rates, allow a more complete analysis of the mechanism of action of these and of other drugs acting on the circulatory system of man.

Hypoglycemia: Potent Stimulus to Growth Hormone Secretion. SEYMOUR M. GLICK, JESSE ROTH, ROSALYN S. YALOW, AND SOLOMON A. BERSON,* Bronx, N. Y.

By use of a new I^{131}-immunoassay method, capable of detecting 0.25 mμg per ml of human growth hormone (HGH) in unextracted plasma, acute changes in endogenous plasma HGH have been demonstrated for the first time. In all normal subjects, insulin-induced hypoglycemia was followed by an increase of several times in plasma HGH concentration from fasting levels (0 to 5 mμg per ml) to values characteristic of acromegaly ($>$ 10 mμg per ml). Plasma HGH rose shortly after onset of hypoglycemia, reached a peak 30 minutes later, and remained elevated for several hours. If insulin hypoglycemia was prevented or quickly terminated by glucose administration, no rise in HGH occurred. Epinephrine and glucagon failed to cause a rise in plasma HGH. Two patients with mesenchymal tumors associated with hypoglycemia, but negligible endogenous plasma insulin (immunoassay) had plasma HGH levels of 30 to 50 mμg per ml. In a totally hypophysectomized patient, plasma HGH was unmeasurable after insulin hypoglycemia. Prolonged fasting in a normal subject was accompanied by a progressive rise in plasma HGH, a slight fall in blood glucose and very low plasma insulin levels. Feeding was followed by a rapid rise in blood glucose and plasma insulin and a sharp fall in plasma HGH. Hypoglycemia and fasting stimulate HGH release. Glucose may act to depress HGH release. Measurement of plasma HGH following hypoglycemia appears to be a specific and sensitive test of pituitary somatotropic function.

The Role of Lipoproteins in the Transferase-induced Plasma Cholesterol Esterification Reaction. JOHN A. GLOMSET, Seattle, Wash. (introduced by Robert H. Williams).

Although the cholesterol esters of fasting plasma are generally believed to be synthesized in the liver, we have recently obtained evidence that appreciable esterification may occur in the plasma itself as a result of an enzyme that we have designated "plasma fatty acid transferase." The reaction catalyzed by this enzyme chiefly involves the transesterification to cholesterol of fatty acids from the β-position of plasma lecithin, resulting in the formation of unsaturated cholesterol esters plus lysolecithin. In order to elucidate further the mechanism of the reaction, we have now investigated the roles played by the major plasma lipoprotein fractions. Two types of experiment were performed. In the first, plasma was incubated for 24 hours at 37° C, and the lipoproteins were

subsequently fractionated by preparative ultracentrifugation. Free and total cholesterol, lecithin, and lysolecithin were then measured in each fraction, and the results were compared with similar data from nonincubated control plasma. In the second type of experiment, the plasma lipoproteins were first fractionated and then incubated, both separately and in various combinations. The results indicate that although the greatest increment in esterified cholesterol is associated with the low-density lipoprotein fraction, the high-density fraction is of particular importance as a source of lecithin and, as such, limits the extent of cholesterol esterification on prolonged incubation. Moreover, most of the lysolecithin formed as a result of the transferase reaction is associated with the > 1.21 g per ml density fraction, suggesting that an important factor favoring the net formation of cholesterol esters at the expense of the plasma lecithin may be the dissociation of the resulting lysolecithin from the original site of transesterification.

In Vitro Studies of Platelet Damage by Immune Reactions. DAVID J. GOCKE AND ABRAHAM G. OSLER, Baltimore, Md. (introduced by W. Barry Wood, Jr.).

Relatively little is known about the nature and number of events initiated by the interaction of antigen with antibody and culminating in cell or tissue damage. This report describes a study of the mechanisms of cell damage by immune reactions in a well-defined *in vitro* system, with the hope of eventually understanding the process in chemical terms. The system employed involves the fluorometric measurement of histamine release from a standardized suspension of washed rabbit platelets by various rabbit antibodies and the homologous antigens. Some of the general characteristics of this reaction are that 50% of the histamine release is obtained with antibody concentrations of 0.5 to 1.0 μg nitrogen per ml, and that optimal release occurs with ratios of antibody to antigen in antibody excess in relation to precipitin data. Horse antibody is equally effective with rabbit antibody on a molar basis, but pepsin digests of rabbit antihuman serum albumin are ineffective. The implications of these findings in relation to the manner in which the immune complex acts as a trigger mechanism in the platelet-damaging process will be discussed. The reaction requires the presence of fresh rabbit plasma, and this plasma activity is heat-labile, present in low titer, and absent in guinea pig plasma. The time course of the reaction, the effect of varying platelet concentration and reaction volume, and the calcium and magnesium requirements will be described. In regard to the mechanism of the reaction, observations will be presented which suggest that the reaction begins in the fluid phase. No evidence of fixation of antibody to the platelet could be found. The histamine-releasing activity generated by the antigen-antibody reaction is unstable—half the activity is lost in 7 to 8 minutes at 37° C. The plasma appears to contribute at least two components to this activity. The primary effect of the reaction on the platelet appears to be lysis, rather than agglutination.

Intracellular pH in the Regulation of Ventilation. R. M. GOLDRING, H. O. HEINEMAN, P. J. CANNON, R. B. MELLINS, R. P. AMES, J. H. LARAGH,* AND A. P. FISHMAN,* New York, N. Y.

The regulation of ventilation is modified by changes in acid-base balance. The present study was designed to test the hypothesis that the regulation of ventilation is related to changes in intracellular rather than extracellular hydrogen ion concentration. For this purpose, two different types of extracellular alkalosis were induced in normal subjects under conditions of controlled metabolic balance: *1*) extracellular alkalosis with negative H^+ balance and, by inference, intracellular alkalosis; produced by the daily administration of $NaHCO_3$, Tris, or a phenoxyacetic acid diuretic; and *2*) extracellular alkalosis with unchanged or positive H^+ balance; produced by chlorthiazide or aldosterone. Elevated arterial blood pH with unchanged or positive H^+ balance was used as evidence for shift of H^+ into cells. Fifty studies of ventilation and gas exchange were obtained on 6 normal subjects before, during, and after the induction of alkalosis. K^+, Na^+, and Cl^- balances and urinary excretion of H^+ (pH, NH_4^+, TA, HCO_3^-) were determined daily. Results indicate that when extracellular alkalosis (blood pH 7.44 to 7.53) is associated with negative H^+ balance, there is: *1*) decrease in alveolar ventilation and alveolar P_{O_2}, *2*) elevation of arterial P_{CO_2}, *3*) decrease in arterial P_{O_2}, and *4*) decrease in ventilatory response to 5% CO_2 breathing. In contrast, when similar elevations of blood pH are produced without net H^+ loss, there is: *1*) no change in alveolar ventilation or alveolar P_{O_2}, *2*) no increase in arterial P_{CO_2}, *3*) no decrease in arterial P_{O_2}, and *4*) an unchanged or increased ventilatory response to 5% CO_2 breathing. The data indicate that alveolar hypoventilation in extracellular alkalosis occurs only when there is a net loss of H^+ from the body, a situation in which the intracellular H^+ concentration is presumably reduced. These observations emphasize the predominant role of the intracellular H^+ concentration in the regulation of ventilation.

Distinguishing Characteristics of Reovirus and Its RNA. PETER J. GOMATOS AND IGOR TAMM,* New York, N. Y.

Reoviruses, formerly known as ECHO 10 virus, occur widely in the respiratory and enteric tracts of man and animals, but little is known about their relation to disease. Reoviruses contain RNA. The reproductive process of reovirus 3 is characterized by three features that distinguish it from other RNA viruses: *1*) the multiplication of reovirus is relatively slow; *2*) the inclusion body developing in reovirus-infected cells stains greenish-yellow with acridine orange, as if it contained DNA, yet it contains RNA; and *3*) the multiplication of reovirus is inhibited by actinomycin D. Further evidence of the unusual characteristics of reovirus was obtained in studies of the synthesis of DNA, RNA, and proteins in infected and control cells. During the viral growth cycle, cellular DNA synthesis became specifically

inhibited, whereas RNA and protein synthesis continued at normal levels. All of these results suggested that reovirus RNA may possess distinguishing structural features. The anomalous staining with acridine orange raised the possibility that reovirus RNA, unlike any other known RNA, may be double-stranded. Results of physical-chemical studies have provided strong evidence in support of this view. Purified reovirus contains 14.7% RNA, but no DNA. The sedimentation coefficient of the virus, $s_{20} = 630$ S, is consistent with a minimal particle weight of 70×10^6 and a minimal complement of RNA of 10×10^6 per particle. The base ratios are approximately complementary. The mole percentage of G + C is 40.7. Reovirus RNA melts sharply in a narrow temperature range and reacts minimally with formaldehyde at 30° C. Thus, reovirus RNA appears to have a secondary structure similar to that of DNA. Both in this respect and also with respect to the large amount of genetic material in the virus particle, reovirus is unique among RNA viruses.

Correlation between Pathology and Function in Experimental Pyelonephritis: Studies on Concentrating Ability. H. C. GONICK, R. Y. FOOS, M. E. RUBINI, AND L. B. GUZE,* Los Angeles, Calif.

The early appearance of a concentrating defect is a distinguishing feature of human pyelonephritis. Current concepts suggest the following possible mechanisms: *1)* alteration of the tonicity of the medullary interstitium (by changes in medullary blood flow, distal reabsorption of sodium and urea, or increased deposition of collagenous protein); *2)* diminished responsiveness of the collecting ducts to circulating ADH; and *3)* increased solute flow per nephron occasioned by a reduction in total nephron mass. The development of an experimental model of pyelonephritis in the rat has permitted a systematic study of the progression of histological and chemical changes in the kidney as they relate to urinary concentration. Concentrating ability was evaluated by maximal U_{osm} and $T^c_{H_2O}$ (over a wide range of solute flow) at weekly intervals after the single injection of 10^8 *Enterococci* and at 12 weeks after repetitive injections. Reduction in maximal U_{osm} was seen as early as 1 week after infection ($1{,}369 \pm 448$ against $2{,}338 \pm 358$ mOsm per L); a further significant reduction was noted in the 12-week repetitive group (800 ± 146 mOsm per L), Diminution in $T^c_{H_2O}$, however, was not apparent until 12 weeks. Creatinine and para-aminohippurate clearances in the 12-week group did not differ significantly from normal controls. Analysis of papillae from the repetitively infected group showed no change in tissue water content or urea content as compared with normal (85.5 ± 3.1 against 85.0 ± 4.4% total wt and $.35 \pm .05$ against $.36 \pm .10$ moles per kg H_2O, respectively). Thus, a reduction in nephron mass could not account for the concentrating abnormality; decreased interstitial tonicity appears unlikely. The defect is most directly attributable to a diminished responsiveness of the abnormal collecting duct to antidiuretic hormone. This conclusion is supported by the pathological findings of structural alterations in collecting ducts without significant deposition of interstitial collagen or loss of glomeruli.

Substrate Balance in Fasting: The Response of Plasma Free Fatty Acid to Minute Changes in Plasma Glucose. CHARLES J. GOODNER AND J. THOMAS DOWLING, Seattle, Wash. (introduced by Robert G. Petersdorf).

It is well known that glucose and insulin inhibit the release of FFA from adipose tissue. The unexpected finding that in fasting normal subjects a single iv injection of 500 mg glucose is followed by prompt and sustained lowering of FFA (−20% in 10 minutes, −30% in 20 minutes, −14% in 40 minutes, and −10% in 50 minutes) suggested that the plasma concentration of glucose critically controlled release of FFA. The "catalytic" nature of this effect is apparent from the estimates that 500 mg glucose (2 Kcal) equals only 1 to 2 minutes of basal caloric requirement, or 3 minutes of substitution for hepatic glucose production, whereas FFA metabolism was altered for over 50 minutes. Several observations suggest that this effect is not mediated through acute secretion of insulin. *1)* The increment in arterial glucose (+2.8 mg per 100 ml at 10 minutes, +1.5 mg per 100 ml at 30 minutes, and 1.0 mg per 100 ml at 60 minutes) was probably too small to stimulate insulin release. *2)* With constant infusion of glucose at low rate (8 to 100 mg per minute), while FFA fell promptly, plasma glucose rose progressively during the first 60 to 80 minutes. Compensation, characterized by a simultaneous fall in glucose and rise in FFA back to control levels, was delayed until the second hour of infusion. Such a sequence suggests that insulin did not initiate or perpetuate the depression of FFA. *3)* Both treated and untreated adult diabetic subjects responded to 500 mg glucose regardless of the fasting glucose level (110 to 250 mg per 100 ml). *4)* Fructose (500 mg) produced the same response in normal subjects. The marked sensitivity of FFA metabolism to small increments in plasma glucose suggests that the fasting level of FFA is directly set by the availability of hexose at some responsive site. Whether this site is the fat cell per se, or a central locus controlling sympathetic tone or release of lipolytic hormones remains to be determined.

Cardiac Manifestations of Disturbed Intermediary Metabolism in Cholera. ROBERT S. GORDON, JR.,* WILLIAM B. GREENOUGH III, ABRAM S. BENENSON, KAMALUDDIN AHMAD, AND IRWIN H. ROSENBERG, Dacca, East Pakistan.

Certain cases of cholera show left heart failure, and develop pulmonary edema, remaining hypotensive, when fluid is replaced. Two cases with intractable failure died and were autopsied. Electrocardiographic changes, evolving toward normal during convalescence, are seen in nearly all cases. Flattening of T waves with normal QRS voltage is common; ST segment deviation occurs

in the more severe cases. Restoration of blood volume, replacement of calcium, potassium, and magnesium, and correction of acidosis fail to restore the electrocardiogram to normal. Administration of glucose exaggerates the ST segment deviation, and produces nausea, mental depression, and abnormal elevation of blood pyruvate. It is postulated that toxic products of *V. cholerae* give rise to a general metabolic disorder that is not merely a consequence of loss of fluid and electrolytes. The addition of tetracycline and water-soluble vitamins to the usual fluid replacement regimen in the treatment of cholera is beneficial. Although neurological examination, erythrocyte transketolase studies, and urinary thiamine excretion data have failed to demonstrate preexisting beriberi, thiamine is felt to be important in therapy on the basis of cardiac findings and elevation of blood pyruvate. Subclinical ascorbic acid deficiency exists in the area during the season of peak cholera incidence, and low blood levels of this vitamin, but no frank scurvy, have been found in cholera patients. Electrocardiographic improvement has followed treatment with large doses of ascorbic acid alone. Further studies are being undertaken that are designed to elucidate the role of nutritional factors in the etiology and pathologic physiology of cholera.

Kinetic Analysis of the Initial Distribution and Rate of Uptake of Sulfobromophthalein (BSP) in the Liver. C. A. GORESKY, Montreal, Canada (introduced by Francis P. Chinard).

Multiple-indicator dilution curves have been obtained after the simultaneous injection of labeled red cells, labeled albumin, and BSP into the portal circulation of the dog. Recoveries are expressed as fractions of the amount injected per milliliter of hepatic venous blood. The albumin curve is displaced relative to the red cell curve, and shows a lower peak concentration and a delayed transit. The dilution curve for BSP, bound to albumin, exhibits an upstroke that parallels the labeled albumin curve, a slightly lower peak, and a more rapid exponential decay. The difference between the albumin and BSP curves is largest for small doses, smallest for large doses. The data have been analyzed by means of a flow-limited, linear, two-compartment model system in which the rate of BSP removal from the second compartment is assumed proportional to the product of the concentration and the volume of the extravascular space. Theoretical consideration of this model leads to a method of graphical analysis of the group of indicator dilution curves that permits the *direct* estimation of both the volume of extravascular space accessible to BSP and the rate constant for removal. In this instance, the BSP is found to be distributed into an extravascular space equivalent in volume to that accessible to the diffusible reference substance, albumin. The rate constant for BSP removal diminishes with increasing dose, indicating saturation of transport at larger doses. From the initial rates of removal (rate constant × dose) as a function of dose, an affinity constant and maximal transport capacity were calculated for the system transporting BSP into the parenchymal cells. These studies show this maximal transport capacity to be many times the steady-state maximum for transport from blood to bile. This excess transport capacity results in relatively rapid equilibration between cellular contents and plasma.

Inferential Evidence for the Fenn Effect in the Human Heart. RICHARD GORLIN,* PETER M. YURCHAK, ELLIS L. ROLETT, WILLIAM C. ELLIOTT, AND LAWRENCE S. COHEN, Boston, Mass.

A correlation has been shown in skeletal muscle, not only between energy cost (qO_2) and tension, but also between energy and fiber shortening distance (Fenn). Although cardiac tension is a function of both radius and pressure, the pressure-time component alone usually correlates with qO_2. This may be fortuitous because other mechanical factors, i.e., radius and fiber shortening, change quantitatively so much less than pressure-time. The catecholamines that change both heart size and fiber shortening have permitted examination of energy cost coincident with fiber shortening. Seven subjects were studied by left ventricular (LV) and coronary sinus catheterization, before and during norepinephrine and isoproterenol infusion, with measurement of qO_2 per 100 g per beat and LV volumes (thermal dilution), and calculation of force-time and circumferential shortening per beat. In three subjects, qO_2 with catechols directly paralleled force-time; shortening was unchanged. In the others, with increased shortening "excess qO_2" could be calculated above that equatable to force-time. Supporting evidence was obtained in 30 subjects with no volume studies. In 10 in whom stroke volume did not change and LV end-diastolic pressure rose or remained unchanged, qO_2 rose *pari passu* with increase in pressure-time. In the other 20, a correlation of 0.59 ± 0.22 existed between change in stroke volume and qO_2 in excess of that predicted from pressure-time. End-diastolic pressure decreased in 13 of 19 observations. The combination of end-diastolic volume decrease and stroke volume increase represents even greater increase in shortening distance. Thus, in this larger series, a portion of energy again appeared related to shortening. These studies suggest that extensive fiber shortening can have an energy cost of its own. Thus, during catechol exhibition, cardiac qO_2 increased out of proportion to force only when fiber shortening was increased. In no instance was qO_2 "wasted" by catechols, and energy could be related to the various induced changes in mechanical performance.

Circulatory Consequences of Changes in Cardiac Rhythm Produced in Patients by Transthoracic Direct-Current Shock. JOHN S. GRAETTINGER, RICHARD A. CARLETON, AND JOSEPH J. MUENSTER, Chicago, Ill. (introduced by Theodore B. Schwartz).

Patients with ectopic atrial rhythms have been studied before and after conversion to sinus rhythm by means of direct-current shock across the closed chest. Cardiac out-

put by direct Fick and indicator dilution techniques and intravascular presssures were measured at rest and during exercise. Dilution outputs were determined during light pentothal anesthesia immediately before and after shock. Continuous electrocardiographic and intravascular pressure recordings were made during shock. The clinical data in 21 patients are similar to those reported by Lown. Sinus rhythm resulted in each study; in 3 instances it persisted less than 10 seconds, in 9 less than 13 days, and in 8 it has continued. Persistence has been unrelated to duration of fibrillation, previous cardiac surgery, or interval after surgery. No patient with predominant mitral regurgitation maintained sinus rhythm. After conversion, stroke volume at rest increased with a decrease in heart rate. Cardiac output did not increase significantly, with one exception, unless the ventricular rate had been over 100 at rest. The response of cardiac output to exercise and the changes in arterial, right atrial, and pulmonary arterial pressures, and total systemic resistances before and after conversion, at rest, and during exercise were not significant. The "a" waves that developed were surprisingly small. Preliminary studies were made during heavy exercise with additional measurements in the left atrium, ventricle, and ascending aorta. In one patient without apparent heart disease, a striking increase in left ventricular compliance ($\Delta V/\Delta P$) and stroke work occurred at rest and during exercise; in another patient with heart disease, a decrease in ventricular compliance accompanied a stroke work increase at rest and during exercise. The volume augmentation resulting from atrial systole would seem to be of less importance than changes in ventricular function in patients after conversion from ectopic atrial to normal sinus rhythm.

Prevention of Trachoma Eye Infections in Monkeys with Oil Adjuvant Trachoma Vaccines. J. THOMAS GRAYSTON * AND SAN-PIN WANG, Seattle, Wash., and Taipei, Taiwan.

Trachoma virus is related to the psittacosis-LGV group, and the natural history of trachoma, a long-lasting chronic disease, suggests that like psittacosis, it may be difficult to prevent by immunization. Among laboratory animals, only primates develop trachoma eye infection. We have produced in Taiwan monkeys (*Macaca cyclopis*) conjunctival and corneal disease typical of human trachoma. Alum or fluid egg yolk sac trachoma virus vaccines usually failed to protect monkeys and often caused hypersensitivity reactions with more frequent and more severe infections than in controls. When virus suspensions were emulsified with mineral oil adjuvant, a vaccine resulted that regularly protected monkeys against infection. Highly purified virus suspensions (Genetron and gradient sedimentation) have been prepared that protect as well as crude suspensions. Five experiments with four trachoma (Bour, TW-1, TW-2, and TW-3) and one inclusion conjunctivitis strain (IC-CAL-3) will be reported. Homologous strain protection was obtained in 75 to 80% of 74 immunized monkeys, but there was no protection from heterologous strains. Among the five strains tested, cross-protection was found only between TW-2 and TW-3, which belong to the same group in the mouse toxin prevention test. During 306 monkey trachoma infections, 17 monkeys have developed pannus. Pannus has always been associated with relatively severe infections and appears to be a hypersensitivity reaction, since it always occurred in previously sensitized monkeys. No pannus developed among 89 infections in monkeys that had not received vaccine or undergone previous trachoma infection. Pannus developed in 10 to 20% of infections with Bour and IC-CAL-3 strains in monkeys that had previous homologous or heterologous infection or vaccine. Pannus after IC-CAL-3 infection is potentially of great importance in understanding the relation of trachoma and inclusion conjunctivitis, and suggests that the differences in the disease may not be due to the organisms.

Glucocorticoid-induced Disappearance of the Long-acting Thyroid Stimulator in Graves's Ophthalmopathy. DONALD E. GREEN, NORTON J. SNYDER, AND DAVID H. SOLOMON, Los Angeles, Calif. (introduced by William N. Valentine).

Pharmacologic doses of adrenal glucocorticoid often lead to remission of the ophthalmopathy of Graves's disease, by mechanisms as yet unknown. Since severe ophthalmopathy is frequently accompanied by very high serum levels of long-acting thyroid stimulator (LATS), we examined the effect of glucocorticoid therapy on serum LATS. LATS was assayed by the method of McKenzie; titers are expressed as 100 times the difference between the 8-hour/10-hour ratio for the serum and the same ratio for saline controls. Two subjects were studied. A 47-year-old diabetic woman exhibited severe ophthalmopathy, localized pretibial myxedema, and a very high LATS titer (1,190). Her thyroid had been essentially ablated by prior I^{131}, and she was euthyroid on thyroid medication. Prednisone induced striking remission of ophthalmopathy. Four and a half months later, LATS was undetectable. Within 10 days after discontinuance of prednisone, LATS became detectable. Average titer of three subsequent assays during 10 weeks without treatment was 271 (p for each assay $< .001$). Concomitantly, the eyes worsened; prednisone was reinstituted, and the eyes again improved. Weekly assays of LATS revealed a constant high level for 3 weeks (average titer 263) and an abrupt drop to undetectable levels during the fourth week and in three subsequent determinations. The second patient, a 31-year-old man, had hyperthyroidism, severe ophthalmopathy, and an LATS titer of 499. He was treated with I^{131} and propylthiouracil, but remained hyperthyroid throughout the study. Prednisone therapy again caused improvement of the eye disease and disappearance of detectable LATS (assayed at 7, 10, and 20 weeks). These observations indicate that the changes in LATS titer induced by glucocorticoid parallel the eye condition rather than the state of thyroid function. They further suggest that the response of Graves's ophthalmopathy to

glucocorticoid is not limited to an anti-inflammatory effect.

Suppression of Endotoxin Tolerance during Typhoid Fever and Tularemia in Man. SHELDON E. GREISMAN,* HENRY N. WAGNER, JR.,* MASAHIRO IIO, RICHARD B. HORNICK, AND THEODORE E. WOODWARD, Baltimore, Md.

Whenever gram-negative bacterial endotoxins are administered intravenously to healthy subjects, tolerance is acquired rapidly to its toxic and pyrogenic activities. Some investigators have therefore suggested that circulating endoxtin cannot play a major role in those infections in which fever is sustained. Nevertheless, since many pathophysiologic alterations during gram-negative bacterial infections resemble those induced by endotoxin, it is essential to determine whether tolerance remains effective during infection before dismissing endotoxin participation. Healthy male volunteers were rendered tolerant to endotoxin by daily intravenous injections. Three subjects were given 0.25 μg *Salmonella typhosa* endotoxin for 27 days and then challenged with viable *Pasteurella tularense*. With the onset of clinically overt tularemia, tolerance was completely suppressed. Tolerance gradually reappeared during convalescence and was restored by the fourth afebrile day. Six subjects were given *Pseudomonas* endotoxin (25 μg increasing to 250 μg) for 30 days and then challenged with viable *S. typhosa*. Tolerance was completely suppressed 1 to 3 days before clinically overt typhoid fever and gradually reappeared during convalescence, with restoration by the sixth afebrile day. In contrast, four control subjects rendered tolerant to *S. typhosa* endotoxin and given sandfly fever retained full tolerance during overt illness. To determine whether suppression of tolerance was caused by depression of the reticuloendothelial system (RES), phagocytic capacity was measured with I^{131}-aggregated human serum albumin. No increase in RES activity developed during the acquisition of tolerance; with suppression of tolerance during overt illness, RES activity actually increased. Although the mechanism of suppression of tolerance in persons infected with typhoid fever and tularemia remains uncertain, our results lead to the conclusion that endotoxin can contribute significantly to the pathogenesis of these diseases.

Complement Fixation by Muscle Nucleoprotein and Serum of Patients with Myasthenia Gravis and Other Diseases. DAVID GROB* AND TATSUJI NAMBA, Brooklyn, N. Y.

A ribonucleoprotein was isolated from normal skeletal muscle that combined strongly and competitively with *d*-tubocurarine and acetylcholine. This protein fixed complement to a significant degree with the serum of 29 of 51 patients with myasthenia gravis, 1 of 3 with muscular dystrophy, 2 of 3 with polyarteritis, 1 of 3 with myositis, 1 of 4 with scleroderma, 1 of 9 with lupus erythematosus, and 2 of 3 with ulcerative colitis. Significant complement fixation occurred in none of 201 patients with other diseases, nor in 75 normal subjects. The highest titers occurred in patients with myasthenia gravis, but there was no correlation with the degree of muscle weakness in this or other diseases. The antibody to muscle ribonucleoprotein is a 7 S γ-globulin. There was no correlation between the presence of antibodies to muscle ribonucleoprotein and to thymic desoxyribonucleoprotein. Of 30 myasthenic patients whose serum contained the former, only 3 contained the latter, and of 10 patients with lupus erythematosus whose serum contained the latter, only 1 contained the former. The microsome and to a lesser extent, the mitochondrial fractions of muscle, which contain most ribonucleoprotein, fixed much more complement in the presence of antibody than the myofibril and soluble fractions. The fraction of saline extract precipitated by 1.2 to 2.4 M ammonium sulfate had most complement-fixing and *d*-tubocurarine-binding activity, and most ribonucleoprotein. The data do not indicate whether the antibody to muscle ribonucleoprotein in the serum of myasthenic patients is the cause of impaired muscle function, or the result of degenerative changes occurring in the muscle end-plates of some patients. The presence of the antibody in other diseases of skeletal or smooth muscle suggests the latter.

Autoantigenicity of Connective Tissue. PAUL HELLER AND VINCENT J. YAKULIS, Chicago, Ill. (introduced by H. J. Zimmerman).

Saline extracts from rabbit tendon have previously been shown to elicit antibodies in guinea pigs, which also reacted with the recipient's tissue, as shown by the presence of circulating complement-fixing antibodies and of fixed γ-globulin in the interstitial spaces and along basement membranes of several organs. In the present experiments, tendon extracts from rabbits were used in an attempt to determine their capacity of isologous and autologous antibody production. Pooled extracts were combined with Freund's adjuvant and used for the stimulation of isologous antibodies. Twelve rabbits underwent amputation of one hind leg, from which tendon extracts were prepared without pooling and reinjected into the donor animal. Amputated and nonamputated animals served as controls. They received injections of serum in adjuvant. Specific antibody response occurred in all animals injected with connective tissue extract and in none of the serum-injected control animals. The titers of complement-fixing isologous and autologous antibodies ranged from 32 to 128. The growth of the animals injected with the autologous antigen was markedly stunted. Fluorescent antibodies were demonstrated in the synovia, heart, liver, lung, spleen, adrenal gland, intestinal mucosa, kidney, and subcutaneous tissue. The antigenic component of the connective tissue extract is not yet known with certainty. Collagen prepared from tendon reacted only in low titer (<8) with the antisera. The results of these experiments suggest that adult animals may produce anti-

bodies to certain "forbidden" antigens. This antigenicity may depend on minor physical alterations of tissue proteins and on their displacement from their normal anatomic site.

Iodine-containing Compounds of Extrathyroidal Tissues as Determined by I^{125}. R. W. HENINGER, F. C. LARSON, AND E. C. ALBRIGHT,* Madison, Wis.

Characterization and quantitation of iodine-containing compounds in tissues under physiologic conditions have been impossible previously because of technical difficulties in measuring the minute amounts of iodine. With I^{125} (60-day half-life), it is possible to apply a highly sensitive method using isotope equilibrium. With this procedure, it is possible to measure the concentration of total iodine, iodide, thyroxine, triiodothyronine, and some unidentified iodine-containing substances in plasma, tissues, and excreta of the rat. Rats were fed an I^{125}-labeled diet of known total iodine content until equilibrium was established between the specific activities of the diet and the iodine-containing pools of the animals. At equilibrium, the iodine content of any compound may be inferred from its I^{125} activity. The compounds were isolated from plasma, urine, feces, and homogenized tissues by differential solubility and chromatography. Radioactivity of the compounds was determined, and the amount of iodine-containing compound was derived. Since very little increase in total radioactivity in tissues, plasma, and excreta occurred between days 23 and 30 of the labeled diet, rats after this time were considered to be in equilibrium with their diet. Radiochromatograms of plasma, feces, and tissues revealed iodide, thyroxine, and triiodothyronine. The feces, in addition, contained small amounts of tri- and tetraiodothyroacetic acid. Urine contained essentially only iodide. While most of the radioactivity was associated with these known compounds, several unknown peaks of varying mobility were observed. The largest of these remained at the origin. Concentration of thyroxine iodine in tissues ranged from 1 mμg per g in muscle and brain to 11 mμg per g in liver. Plasma contained 22 mμg per ml. Concentration of triiodothyronine iodine in tissues ranged from 0.4 mμg per g in testis and muscle to 5 mμg per g in kidney. Plasma contained 0.7 mμg per ml. Concentration of total iodine, iodide, and origin material varied with total iodine content of the diet.

Steroid-dependent Increase of Nerve Conduction Velocity in Adrenal Insufficiency. R. I. HENKIN, J. R. GILL, JR., J. R. WARMOLTS, A. A. CARR, AND F. C. BARTTER,* Bethesda, Md.

Patients with adrenal insufficiency exhibit taste and smell thresholds 100 to 10,000 times more sensitive than those of normal subjects. Carbohydrate-active steroids (CAS) return this heightened sensitivity to normal. The relationship of the adrenal cortex to nerve function was further explored by determining the effect of adrenal steroids on conduction rates in peripheral nerves. Ulnar nerve conduction velocities (NCV) were measured in normal subjects and in patients with adrenal insufficiency. They were studied untreated, treated with prednisolone (Δ_1F), 20 mg per day, and treated with desoxycorticosterone (DOC), 20 mg per day. The ulnar nerve was stimulated supramaximally at the ulnar groove and at the proximal volar carpal crease. The time (latency) from stimulation until onset of motor response of the abductor digiti quinti was recorded. NCV was calculated by dividing the distance between stimulation points by the time difference between latencies. Without treatment, mean NCV in 11 patients was 70.8±2.8 m per second (SEM). DOC alone did not significantly alter NCV (65.5 ± 1.6 m per second); Δ_1F lowered it by a mean figure of 16.4 m second (54.4 ± 1.1 m per second) to a value not significantly different from that of normal subjects (57.8 ± 2.5 m per second). DOC or Δ_1F did not affect NCV in normal subjects. In the patients, NCV increased to abnormal levels within 4 days after withdrawal of Δ_1F and returned to normal levels within 24 hours after Δ_1F was restored. These periods required for changes in NCV are similar to those required for corresponding changes in taste. Thus, profound changes in nerve function occur with adrenal insufficiency. These changes are unaffected by Na,K-active steroids, but return to normal with CAS. The results suggest an integral role for CAS in regulation of nervous system activity.

Serum Creatine Phosphokinase (CPK) Activity in Diseases of Muscular Tissue. J. W. HESS, R. P. MACDONALD, J. B. DALTON, R. N. JONES, AND J. NEELY, Detroit, Mich. (introduced by R. J. Bing).

Creatine phosphokinase (CPK) is an enzyme catalyzing the reversible reaction, $ATP + \text{creatine} \overset{CPK}{\rightleftharpoons} ADP +$ creatine phosphate (CrP). Workers in Europe and Japan have demonstrated that serum CPK elevations do occur after myocardial infarction and in skeletal myopathy, but to date the clinical usefulness of this enzyme has received little attention from workers in this country. Human and canine tissue and human serum CPK activities were assayed according to the method of Tanzer and Gilvarg that uses the following coupled reactions: *1)* $Cr + ATP \overset{CPK}{\rightleftharpoons} CrP + ADP$, *2)* $ADP + PEP \overset{PK}{\rightleftharpoons} ATP +$ pyruvate, and *3)* $\text{pyruvate} + DPNH \overset{LDH}{\rightleftharpoons} \text{lactate} + DPN$. The rate of oxidation of DPNH after creatine addition was measured on a Beckman DU spectrophotometer at 340 mμ and is proportional to the amount of CPK in the assay sample. The tissue analyses show that high CPK activity is present in skeletal muscle and myocardium, moderate activity in brain, uterus, and gallbladder, and low activity in pylorus, adrenal, thyroid, and lung. No detectable activity was demonstrated in kidney, liver, or pancreas. In the clinical disease states, highest values were found in diseases of skeletal muscle, with values up to 400

times normal recorded in childhood progressive muscular dystrophy. Elevations lasting from 1 to 4 days and occasionally up to 7 days were observed after acute myocardial infarction. The magnitude of elevation was usually much greater than that of LDH or SGOT. GPK was not elevated in diseases of the liver, hemolytic states, cancer, uremia, and acute and chronic pulmonary disease. The identification of CPK as an enzyme whose serum activity increases almost exclusively as a result of damage to muscular tissue represents a significant advance in "biochemical biopsy" techniques.

Renomedullary Vasodepressor Factor. ROGER B. HICKLER, CALVIN A. SARAVIS, JAMES F. MOWBRAY, DAVID P. LAULER, ANTHONY I. VAGNUCCI, AND GEORGE W. THORN,* Boston, Mass.

Protein-free ultrafiltrate of saline homogenate of rabbit medulla produces a prolonged reduction in the mean arterial pressure of the bioassay rat preparation (5 to 10 minutes) and anesthetized dog (30 minutes) on intravenous administration. The dose-response curve is linear up to a limiting maximum, beyond which increase in dose fails to elicit a further depression in pressure. Tachyphylaxis or untoward reaction is not observed on repeated administration. Deproteinized extract of rabbit renal cortex produces only short-duration depression of pressure, probably associated with its nucleotide content. Interarterial injection of medullary extract in the isolated rat hind limbs perfused with Ringer's solution is vasoconstrictor, as are ATP, bradykinin, and angiotensin. Histamine is vasodilator. In the isolated rat kidneys perfused with Ringer's solution, interarterial injection of medullary extract has a potent vasodilator effect, the response being proportional to dose, whereas ATP and bradykinin are clearly vasoconstrictor in the same preparation. Further purification of the protein-free ultrafiltrate has been achieved by electrophoresis at pH 8.6 on Pevikon, by elution from Dowex resin 50W-X2, and by paper chromatography in a solvent system of isopropanol and 2-butanol. The vasoactive substance is ethanol soluble and chloroform insoluble. While its exact chemical nature remains to be elucidated, the properties cited above are strongly against its being protein, nucleotide, or bradykinin. Sephadex chromatography indicates a molecular weight of less than 4,700. The substance retains its vasodepressor activity after treatment with carboxypeptidase, ribonuclease, phosphatase, and Panprotease (Worthington). Activity is retained after diazotization with nitrous acid, but is destroyed at pH 11 after incubation with 1.0 N sodium hydroxide for 1 hour at room temperature. There is a strong possibility that it is a small peptide.

The Replication Time and Pattern of the Liver Cell in the Growing Rat. JOSEPH HOFFMAN, CHEN-YA HUANG, AND JOSEPH POST, New York, N. Y.

Three-week-old male rats of the Wistar strain were injected intraperitoneally with 1 μc per g of tritiated thymidine (H^3TDR), and at intervals of 0.25 to 72 hours later, 2 to 6 animals were killed. Radioautographs of 5-μ liver sections, previously stained by the Feulgen technique, were studied for labeling of interphase and mitotic nuclei. From the time-phase changes in the labeling of prophase nuclei, estimates have been made of the times required for new cell formation, and its component intervals. The results show that after the administration of H^3TDR, "flash" labeling of interphase nuclei occurs. The respective time intervals are approximately as follows: generation time, 21.5 hours; DNA synthesis, 9 hours; post-DNA synthesis time (G_2), 0.5 hour; prophase, 1.3 hours; metaphase, 1.0 hours; anaphase, 0.4 hour; telophase, 0.3 hour; and postmitotic time (G_1), 9.0 hours. About 4% of the liver cells are dividing at any time in a wavelike pattern. These cells alternate with others in cell division. It is estimated that only about 10% of the liver cell population is engaged in new cell formation. One group of cells has been recorded in at least 3 successive, orderly replication cycles. The data do not reveal the total number of division cycles these cells pass through.

The Distinctive Detergent Properties of Conjugated Bile Salts and Their Relation to the Role of Bile Salts in Fat Digestion. ALAN F. HOFMANN AND BENGT BORGSTRÖM, New York, N. Y., and Lund, Sweden (introduced by Jules Hirsch).

Conjugated bile salts may be regarded as anionic detergents. Like all detergents, they form reversible, polymolecular aggregates called micelles. All micellar solutions dissolve water-insoluble materials to some extent, for the inside of the micelle can be regarded as a separate phase of liquid hydrocarbon. We postulated that intestinal content during fat digestion contained bile salts in micellar solution and that at least some of its lipids were in micellar form. Bile salt solutions showed distinctive detergent properties when they were compared with solutions of a household detergent, lauryl sulfate. Most remarkable of these properties was the extraordinary solvent power of bile salt solutions for the polar lipid 1-monoolein. Confirmatory results were subsequently obtained when the solubility of straight-chain fatty acid, monoglyceride, diglyceride, and triglyceride in bile salt solution was examined. Under appropriate conditions, fatty acid and monoglyceride had a high solubility in bile salt solution, whereas the solubility of diglyceride and triglyceride was negligible. These results suggested that if a micellar phase occurred in intestinal content during fat digestion, its lipids should be limited to fatty acid and monoglyceride. Intestinal content was obtained by intubation from 8 patients (29 samples) during active fat absorption and was separated by ultracentrifugation at 37° C into a lower, aqueous, transparent, micellar phase and an upper oil phase. The micellar phase of a sample contained almost exclusively fatty acid and monoglyceride; the oil phase contained almost all of the diglyceride and triglyceride present, plus some fatty acid and monoglyceride.

Thus, bile salts possess unusual detergent properties. The solvent properties of their solutions correlate with the proposed function of bile salts in fat absorption, namely, that of bringing the end products of pancreatic lipolysis into an isotropic, micellar solution, from which lipid is absorbed.

Studies of Lipoprotein Metabolism in Atherosclerotic Tissue. WILLIAM HOLLANDER AND RICHARD KAPLAN, Boston, Mass. (introduced by Charles P. Emerson).

The present study was undertaken because previous studies indicated that cholesterol in the nonprotein-bound form accumulated in arterial plaques and was nontransportable and inflammatory in contrast to cholesterol in the lipoprotein form. Fresh atherosclerotic tissue was removed at surgery for vascular disease and incubated with C^{14}-acetate. After incubation for 4 to 12 hours, the tissue was analyzed radiochemically for lipids and for lipoproteins, which were extracted in saline and separated ultracentrifugally. The C^{14}-lipids and C^{14}-sterols synthesized from C^{14}-acetate by the arterial tissue were recovered in the low-density ($D < 1.019$, D 1.019 to 1.063) and in the high-density (D 1.063 to 1.21) lipoprotein fractions. However, 6% of the total C^{14}-lipid activity and 20% of the C^{14}-sterol activity and of the total cholesterol contained in the tissues were recovered in the nonprotein-bound form. The low-density lipoproteins were extracted in greater amounts from the diseased tissues and contained five times more lipid per milligram of protein than the high-density lipoproteins. Over 85% of the lipids, including cholesterol, triglycerides, and phospholipids, and 65% of the lipid and sterol radioactivity contained in the extracted lipoproteins were recovered in the low-density lipoprotein fractions, especially the D 1.019 to 1.063 fraction. The specific activity of the lipids and sterols in the high-density lipoprotein fractions were higher than those in the low-density lipoprotein fractions. In conclusion, the lipids contained in and synthesized by atherosclerotic tissue were recovered mainly as low-density lipoproteins. A failure of the diseased artery to complex all its synthesized lipids to lipoproteins may account for the nonprotein-bound lipid recovered in the tissue.

The Role of Adipose Tissue in Altered Glucose Tolerance after Starvation in Normal Diabetic Humans. GUY HOLLIFIELD,* JOHN A. OWEN, JR., AND RICHARD W. LINDSAY, Charlottesville, Va.

Severe carbohydrate restriction is known to impair glucose tolerance in normal humans, and caloric restriction severe enough to produce significant weight loss is known to improve glucose tolerance in most "maturity onset" diabetics. Much of the available evidence has suggested that these changes in glucose tolerance are related to pancreatic insulin production or release. We have measured glucose tolerance, serum insulin-like activity (ILA) (method of Martin and Renold), and glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconic dehydrogenase (6PGD) activities in adipose tissue homogenates in five obese patients with normal glucose tolerance and eight maturity onset diabetics before and after 5-day fasts. The G6PD and 6PGD activities were determined in subcutaneous adipose tissue biopsies taken 1 hour after challenge with 100 g of glucose orally before and after 5-day fasts. In the nondiabetic obese patients, glucose tolerance was impaired after fasting, mean serum ILA rose 32% above prefast levels, and the mean G6PD and 6PGD activities fell 36% and 65%, respectively. In the diabetic subjects, glucose tolerance did not change significantly. The mean serum ILA rose 99% above prefast levels, and the mean G6PD and 6PGD activities were 80% and 3%, respectively, above prefast levels. These findings suggest that the deterioration of glucose tolerance with fasting in normal subjects is not the result of decreased insulin release, but rather a decrease in the lipogenetic capacity of adipose tissue. In obese diabetic subjects, serum ILA activity does increase after fasting, but glucose tolerance seems more clearly related to an increase in the enzymatic activity in the hexose monophosphate shunt pathway.

The Ability of Different Donor Cell Constituents to Shorten or Prolong Homograft Survival. HALSTED HOLMAN,* ALEXANDER FEFER, AND WILLIAM C. DAVIS, Palo Alto, Calif.

When injected into adult recipients before homografting, cytoplasmic and nuclear constituents of donor spleen cells differ in their ability to cause accelerated graft rejection, to prolong graft survival, and to provoke formation of circulating hemagglutinins. Strains of mice differing at histocompatibility loci and strains sharing the major locus (H-2) were employed. Hemagglutinin formation was studied in pairs of the former type, and accelerated skin homograft rejection in pairs of both types. Prolongation of graft survival was studied in adult animals sharing H-2 locus, with the recipient receiving sublethal radiation 24 hours before injection of the cell fraction. Some cell fractions (e.g., cytoplasmic mitochondria and microsomes) cause accelerated graft rejection and hemagglutinin formation, others (e.g., a lipoprotein of cell membranes) cause hemagglutinin formation without graft rejection, and still others (e.g., a soluble nuclear extract and perhaps nuclear "membranes") appear to cause only prolongation. Lyophilized fractions retain activity. Further characterization of active cell fractions has been possible through chemical, physical, and enzymatic studies. Thus *1*) isolated cell components have different antigenic activities in the transplantation system, *2*) certain subcellular fractions can prolong homograft survival, and *3*) the host response to grafts appears to be conditioned by the types of donor cell constituents previously encountered. It may therefore become possible to achieve a specific host tolerance of donor tissue despite major genetic differences through

appropriate preparation of the recipient with selected donor cell constituents. Persistence of activity in lyophylized cell fractions may facilitate such preparation. Identification of cell fractions with these different activities may assist in clarification of the influence of different histocompatibility gene loci on cell structure and function.

Measurement of Steroid Response in Progressive Liver Disease: An Appraisal. FRANK L. IBER, Baltimore, Md. (introduced by Palmer H. Futcher).

Adrenalcorticosteroids have been advocated in the treatment of chronic active hepatitis. Nitrogen balance and maximal sulfobromophthalein (BSP) excretory rate (Wheeler) were measured before and during treatment with prednisone (30 to 60 mg per day) of 12 patients on a constant diet containing 1.0 to 1.4 g protein per kg body weight. All patients had chronic hepatitis and had negative nitrogen balance (0.4 to 2.0 g per day). During 16 days on prednisone, the negative nitrogen balance increased in 10 patients. In the other two, urinary nitrogen losses decreased until a positive nitrogen balance of 1.0 and 1.8 g per day was reached. Maximal BSP excretion increased in these two patients, whereas no change was seen in the others. With prednisone treatment, elevated serum alkaline phosphatase, bilirubin, and globulin fell in all patients. Albumin and prothrombin levels and 45-minute BSP retention were unchanged in the 10 patients who continued to have a negative nitrogen balance. The liver disease of one of the patients reaching positive nitrogen balance became inactive after 12 months of steroid treatment and remained inactive after cessation of steroid therapy. The liver disease of the second patient progressed rapidly to death after steroids were discontinued. Three of the ten patients who continued to have negative nitrogen balance on steroids were maintained on this therapy (average 8 months). The course and present status of their liver disease are no better than those of the seven patients who received no further steroid. Long-term steroid therapy is of little value in those patients with active liver disease whose continuous negative nitrogen balance or BSP excretion fails to improve with a trial of steroids.

Cellular Permeability in the Pathogenesis of Hereditary Spherocytosis. HARRY S. JACOB AND JAMES H. JANDL,* Boston, Mass.

Although hereditary spherocytosis (HS) appears to involve an inborn error of red cells, no specific metabolic defect has been defined. It is known that erythrostasis *in vitro* rapidly damages HS cells and lowers their ATP levels. In the present study, effects of erythrostasis on the metabolism of normal and HS red cells were correlated with effects on their viability when transfused into normal subjects. Surprisingly, red cells of splenectomized HS patients consumed more glucose than normal even as the cells lost viability. The only change correlating with their abnormally rapid destruction *in vivo* was that HS cells accumulated sodium. Sodium flux in HS cells was found increased, confirming earlier work by others. When the ATPase-dependent sodium transport mechanism was inhibited by ouabain, sodium accumulation and cell damage accelerated markedly in HS, but not in normal, cells. Whereas in normal cells ouabain had no effect on glucose consumption, in HS cells glucose consumption was inhibited. Normal cells swollen by hypotonic suspension manifested increased glycolysis reversible by ouabain. Shrinkage of HS cells by addition of nonpermeable osmotic agents prevented the sodium accumulation, increase in osmotic fragility, and autohemolysis, and preserved the subsequent viability of such cells *in vivo*. These findings indicate that hemolysis of HS cells during erythrostasis is not due to deficient energy metabolism, but to "overwork." The following sequence appears to occur: *1*) HS red cells are inherently more permeable to sodium; *2*) this heightened influx of sodium accelerates active transport by arousing ATPase; *3*) thereby ATP breaks down more rapidly to ADP, which in turn acts as a stimulus to glycolysis. Under optimal conditions, as in the general circulation, this compensatory mechanism suffices for HS cells, but during metabolic stress, as in the spleen, sodium accumulates intracellularly, and irreversible changes in membrane permeability and then hemolysis ensue.

Renal Site and Mode of Action of Glucocorticoid and Aldosterone in Cirrhosis. HERSHEL JICK, JULIAN SYNDER, AND EDWARD W. MOORE, Boston, Mass. (introduced by Thomas C. Chalmers).

Three groups of hydropenic cirrhotics were maintained with 10% mannitol in a steady state of osmotic diuresis. Group I (7 patients) received 40 mg of methyl prednisolone intravenously. A significant decrease in sodium excretion occurred within 2 hours [mean difference = 11.2 ± 3.0 μEq per ml per minute (1 SE)] associated with a significant increase in $T^c_{H_2O}$ (0.6 ± 0.07 ml per minute) and no significant change in potassium excretion (-1.5 ± 0.9 μEq per ml per minute). C_{man} and C_{osm} remained relatively constant. Group II (5 patients) received 0.5 mg of D-aldosterone intravenously and showed a similar decrease in sodium excretion (10 ± 3.2 μEq per minute) associated with a significant increase in potassium excretion (1.9 ± 0.4 μEq per ml per minute), but no significant change in $T^c_{H_2O}$ ($+0.1 \pm 0.08$ ml per minute), C_{man}, or C_{osm}. Group III (3 patients) received mannitol alone and showed no significant change in urine composition. The data suggest that methyl prednisolone acts primarily in the ascending loop of Henle to increase sodium reabsorption and thus contributes to increased $T^c_{H_2O}$ formation. Aldosterone appears to increase sodium reabsorption at other sites, at least partially in the distal tubule where exchange for potassium takes place. The paradoxical potentiation of diuretics by a sodium-retaining hormone (glucocorticoid) may be explained as follows. A sodium-retaining action in the loop of Henle could result in increased free water

excretion in patients with fluid retention who are not excreting sodium and who do not have maximal antidiuretic hormone stimulation. Such an action would promote delivery of a more hypotonic fluid containing less sodium to the distal nephron and result in a more dilute urine. This loss of free water might lead to diuretic potentiation by promoting a rise in serum sodium, which in itself has been reported to effect increased delivery of fluid from the proximal to the distal tubule where most potent diuretics act.

Folic Acid Displacement in Normal Human Subjects. DAVID G. JOHNS AND IAN H. PLENDERLEITH, Montreal, Canada (introduced by Douglas G. Cameron).

When large doses of unlabeled folic acid are administered intravenously to normal human subjects who have previously received tracer amounts of tritium-labeled folic acid, the labeled folic acid is displaced from its intracellular binding sites and can be recovered from the plasma and urine. This observation was the basis of a method employed to study folic acid binding and displacement in man. Seven compounds, either closely related in structure to folic acid, or fragments of the folic acid molecule, were tested for their ability to displace a 5μg per kg dose of tritium-labeled folic acid (20 μc) administered 24 hours previously. The compounds to be tested were administered intravenously at a dosage of 1 μmole per kg, and their ability to displace intracellular folic acid was assessed by measurement of folic acid radioactivity appearing in the urine in the next 6 hours. If the amount of radioactivity flushed by unlabeled folic acid is represented by 100, the amount flushed by the other compounds tested was as follows: pteroyltriglutamate, 91; pteroic acid, 31; 4-amino-10-methylpteroylglutamate (Methotrexate), 16; 5-formyltetrahydropteroylglutamate (folinic acid), 13; *p*-aminobenzoylglutamate, 0; and glutamate, 0. Thus, those compounds in which the structural integrity of the pteridine moiety of folic acid was unimpaired (pteroyltriglutamate and pteroic acid) were effective folic-acid-displacing agents; compounds lacking the pteridine moiety were unable to displace folic acid; compounds having a modified pteridine moiety showed only weak displacing ability, despite the very high *in vitro* affinity of one of them (Methotrexate) for the enzyme dihydrofolic reductase. The results suggest that in man, a structurally intact pteridine moiety is necessary for effective displacement of folic acid from cells.

The Lysis of Artificially Induced Intravascular Clots in Man by Intravenous Infusions of Urokinase. ALAN J. JOHNSON,* W. ROSS MCCARTY, AND J. NEWMAN, New York, N. Y.

Streptokinase (SK) has been found to be a potent thrombolytic agent when infused intravenously in man to produce maximal amounts of plasminogen activator. In the present studies, a plasminogen activator from human urine, urokinase (UK), was used to produce thrombolysis in man. UK was found to be nontoxic and nonantigenic when infused in 26 individuals (1 to 842,000 Ploug units). Since thromboplastic substances are found in urine, Lee-White coagulation time, Quick prothrombin time, and thromboplastin generation test were performed during infusion of UK preparations in man. No change from preinjection assays was shown. Endogenous SK inhibitor and antibody (noncompetitive inhibitors) must be quantitatively neutralized *in vivo* by infused SK. Endogenous UK inhibitor is competitive, not requiring quantitative neutralization. As expected, no antibody to UK was found in man. Thus, the initial, or priming, dose of UK is relatively predictable and constant. SK infusions must be carefully titrated to prevent hyperplasminemia, or excessive plasminogen depletion. Severe bleeding may accompany hyperplasminemia, with hypofibrinogenemia, decreased factor V, and increased coagulation time (antithrombin VI). The UK level required for thrombolysis is much lower than that for hyperplasminemia; a bleeding diathesis is rarely produced with UK. With SK, excessive depletion of endogenous plasminogen causes re-formation of thrombus. If UK infusion is very rapid, plasminogen depletion and clot re-formation also occur. Thrombolysis, however, is easily produced with infusion of small amounts of UK, without excessive depletion of plasminogen. SK must be infused 4 additional hours after thrombolysis to permit intimal repair of traumatized vein and prevent clot re-formation. UK, also, must be infused 4 additional hours to prevent clot re-formation. Thrombolysis occurred in each of 6 patients treated with UK to produce a moderate activator system. Re-formation of clot occurred in 1 (24 hours after clot lysis had occurred).

Low Plasma Lipoprotein Lipase Activity as a Factor in the Pathogenesis of Alcoholic Hyperlipemia. DON P. JONES, MONTY S. LOSOWSKY, CHARLES S. DAVIDSON,* AND CHARLES S. LIEBER, Boston, Mass.

Among several hundred patients hospitalized with acute alcoholism, five had gross serum lactescence with triglyceride, total cholesterol, and phospholipid concentrations ranging from 2,120 to 10,340, 778 to 1,170, and 1,020 to 1,920 mg per 100 ml, respectively, which rapidly decreased after hospitalization. Their triglyceride concentrations fell to 330, 61, 130, 390, and 143 mg per 100 ml within 5, 12, 18, 23, and 35 days, respectively, after hospitalization. Alcohol given to seventeen former alcoholics caused serum lipid elevations in all, with a maximal peak triglyceride concentration of 900 mg per 100 ml, well below the levels observed in the five hyperlipemic subjects. Although one of these latter subjects had evidence of pancreatitis, the other four had no apparent cause for hyperlipemia. To evaluate a possible etiologic factor in addition to alcohol itself, plasma lipoprotein lipase activity (LPL) was evaluated 15 minutes after heparin injection (0.4 mg per kg iv). LPL activity was measured by the microequivalents of fatty acids released per minute per milliliter of postheparin plasma when

incubated with a coconut oil-bovine albumin emulsion. Mean LPL in seven normal subjects was 0.277 μEq $\pm$ 0.062 (SD). Similar values (mean 0.262 μEq $\pm$ 0.057) were observed in six acute alcoholics with normal blood lipids, and also in the one patient with hyperlipemia and pancreatitis (0.242 μEq). In the other four hyperlipemic subjects, however, LPL was abnormally low (mean 0.114 μEq $\pm$ 0.006; $p < 0.001$). LPL measurements were repeated in these subjects when the serum had cleared after periods of 21 to 180 days of abstinence and normal diet. LPL, though somewhat higher than initially (mean 155 μEq $\pm$ 31), was still significantly below normal, without any overlap ($p < 0.01$). Thus, although alcohol administration regularly resulted in hyperlipemia of a mild degree, marked hyperlipemia with gross serum lactescence may require a combination of two factors, the direct effect of alcohol on lipid metabolism potentiated by a reduction of LPL activity.

Role of Streptococcal Infection in Induction of Autoantibodies to Heart in Rheumatic Fever. MELVIN H. KAPLAN,* KATHRYN H. SVEC, AND JOSE ARANA-SIALER, Cleveland, Ohio.

Evidence has been presented that group A streptococcal cell walls contain an antigen which is immunologically related to constituents of human heart tissue, based on observations of a reciprocal cross-reaction between antisera to streptococcal cell walls and heart tissue. This streptococcal cross-reactive antigen has been partially purified by neutral salt fractionation and chromatography. Sera of patients with rheumatic fever were tested by agar diffusion for precipitin reaction with preparations of streptococcal cross-reactive antigen. Sera from 32 of 40 patients exhibited precipitation, usually a single line, with this antigen preparation. The precipitin could be absorbed from the serum with heart tissue, but not kidney. It could be absorbed with streptococcal cell walls, but not protoplasmic membranes or culture filtrate. A more sensitive test procedure based on agglutination of bentonite particles coated with streptococcal cross-reactive antigen gave comparable results. In patients with rheumatic fever, such cross-reactive antibody was found to persist for long periods, in some patients as long as 6 months after onset of the disease. Its presence in the serum could not be directly correlated with clinical activity. It was of interest that patients with rheumatic heart disease, judged clinically inactive, frequently exhibited positive reactions. Autoantibody to heart appearing after cardiac surgery was not related to cross-reactive autoantibody. Cross-reactive antibody was observed also in the sera of several patients with acute glomerulonephritis, and occasionally in patients with uncomplicated streptococcal pharyngitis or scarlet fever. These data support the concept that exposure to a cross-reactive antigen of group A *Streptococci* may result in induction of autoantibodies to heart and may be related to deposition of bound γ-globulin in the heart in rheumatic fever and rheumatic heart disease.

The Effects of Angiotensin II in Patients with Hypertensive Disease. NORMAN M. KAPLAN AND JACK SILAH, Dallas, Tex. (introduced by Jay P. Sanford).

Effects of angiotensin II upon blood pressure and secretory rate of aldosterone (ASR) were observed in patients receiving 102 mEq Na daily. Angiotensin was infused in progressively increasing amounts until either the diastolic pressure rose 20 mm or a level of 20 ng (nanogram) per kg per minute was reached, and was maintained at this rate for 6 hours. ASR were determined on the day of angiotensin infusion and on a control day. The mean pressor doses of angiotensin, in ng per kg per minute, for the various groups were: 12 normotensive patients, 7.9; essential hypertension (in 22), 3.1; malignant hypertension (in 14), 18.9; primary aldosteronism (in 4), 3.3; cirrhosis with ascites (in 5), 16.0; and chronic renal disease with hypertension (in 5), 4.2. The differences between the normotensive and all other groups in the pressor dose are significant ($p = 0.01$). One patient with primary aldosteronism and malignant hypertension responded to 5.3 ng per kg per minute, a dose less than that required for any patient with malignant hypertension and secondary aldosteronism. Two patients with renal hypertension were less responsive than any patient with essential hypertension. After the hypertension of these patients was relieved by nephrectomy, responsiveness to angiotensin increased. Excluding the patients with primary aldosteronism, there was good correlation between the pressor dose of angiotensin and the control ASR, but no correlation with the level of blood pressure, concentration of serum electrolytes, or degree of azotemia. The infusion of angiotensin increased ASR in most patients with normal control rates, but decreased ASR in most patients with elevated control rates. Angiotensin elicited an excessive pressor response in primary aldosteronism and a variable pressor response, inversely proportional to ASR, in other forms of hypertension. This suggests that the pressor response may reflect differences in endogenous angiotensin levels, and may therefore serve as a means for characterizing the role of angiotensin in hypertension and as a method for differential diagnosis.

Effect of Calcium Infusion on Hydroxyproline Metabolism: A New Approach to Parathyroid-Bone Interactions. HARRY R. KEISER, JOHN R. GILL, ALBERT SJOERDSMA,* AND FREDERIC C. BARTTER,* Bethesda, Md.

Bone matrix contains a large percentage of total body collagen. Urinary hydroxyproline peptides (HOPr), an index of collagen metabolism, may be elevated in some patients with bone disease. Parathyroid extract given to normal subjects and to patients with hypoparathyroidism rapidly increases urinary HOPr. This appears to be an effect of the extract on bone rather than on the kidneys. It was of interest to study also the effect of suppression of parathyroid activity on HOPr excretion. Calcium, 15 mg per kg, was given by constant infusion over 4 hours to 6 normal subjects fed constant diets. A decrease of 50% in HOPr excretion occurred during

or within 4 hours after the infusion. The fall in phosphorus excretion lagged by 4 to 8 hours. Excretion of HOPr on the day of infusion (18.8 to 32.1 mg per 24 hours) was 23 to 43% less than on control days (30.9 to 55.3 mg per 24 hours). Blood HOPr concentrations and creatinine clearances showed essentially no changes. There was no decrease in HOPr excretion in 3 patients with hypoparathyroidism given calcium infusions for periods up to 4 days. The results suggest that the effect of calcium infusion on HOPr excretion is mediated by the parathyroid glands, and not an effect of the hormone on the kidney. Unlike the rate of phosphorus excretion, which may reflect direct renal effects and re-entry into bone, the rate of HOPr excretion reflects only its release from bone, since the peptide is not used for new bone formation. Thus HOPr excretion, in providing a sensitive measure of bone destruction, reveals that even physiologic changes of parathyroid activity have immediate effects on bone.

Chronology and Pattern of Human Chromosome Replication. Y. KIKUCHI AND AVERY A. SANDBERG,* Buffalo, N. Y.

The timetable of replication of the 46 chromosomes in normal cells has been delineated in leukocytes after their short-term (72-hour) culture. Over 2,000 metaphases (and their radioautographs) of normal subjects (3 male, 3 female) were examined. The incorporation of tritiated thymidine was used to determine the chronology of chromosome replication. The labeled deoxyriboside was added for 10 minutes to the cultured cells at various intervals (1 to 28 hours) before fixation of the cells. There was a definite sequence to the order in which the chromosomes began and finished DNA replication (S period. (Thus, one of the autosomes of group B is the first *to begin* replication, followed by groups A1–A3, C6–C7, and the X chromosomes; the latest to begin replication are groups F and G. The *completion* of chromosomal replication was characterized by several salient features: *1*) one of the X chromosomes in the female and the Y in the male were the last to replicate, *2*) among the autosomes, members of groups B and D replicated last, and *3*) groups F and G were the earliest to finish replication. The order in which the chromosomal DNA began and finished replication was consistent from subject to subject. As previously reported, one of the X chromosomes in females and one of the Y in the males were more heavily labeled than any other chromosome when the tritiated thymidine was added late in the S period; in contrast, the homologous X in the female and the X in the male were not heavily labeled. Asynchrony and variation in replication were also characteristic of each pair of homologues and for various regions of individual chromatids. The data indicate that the S period begins approximately 18 hours before metaphase in leukocytes. The delineation of the chronology and pattern of human chromosome replication in normal cells can serve as a reference for comparison with similar studies in abnormal cells.

Sites of the Nonmetabolic Component of the Cardiac Output in the Hyperkinetic Circulatory States of Thyrotoxicosis and Cirrhosis of the Liver. HERMES A. KONTOS, WILLIAM SHAPIRO, H. PAGE MAUCK, JR., ALTON R. SHARPE, JR., AND JOHN L. PATTERSON, JR.,* Richmond, Va.

In thyrotoxicosis and in certain patients with cirrhosis of the liver, the cardiac output (CO) is increased in excess of oxygen utilization. The distribution of this nonmetabolic component of the increased CO remains conjectural. This problem was investigated in 8 thyrotoxic patients, 5 cirrhotic patients with hyperkinetic circulation, and in 6 normal subjects. The CO was significantly elevated in both patient groups. Oxygen consumption was significantly increased in the thyrotoxic, but normal in the cirrhotic group. The CO/oxygen consumption ratio was higher in both thyrotoxic (4.44 ± 1.34 L per minute per 100 ml oxygen; $p < 0.01$) and cirrhotic (5.35 ± 1.09; $p < 0.001$) patients than in normal subjects (2.87 ± 0.43). Hand blood flow was higher in the thyrotoxic (18.83 ± 6.35 ml per minute per 100 ml; $p < 0.01$) and cirrhotic (16.05 ± 6.37; $p < 0.05$) group than in normal subjects (7.61 ± 3.74). Forearm blood flow was also higher in thyrotoxic (8.91 ± 2.36 ml per minute per 100 ml; $p < 0.001$) and cirrhotic (6.39 ± 2.36; $p < 0.02$) patients than in control subjects (3.23 ± 0.47). Arterial-deep venous oxygen difference in the forearm was much lower in the thyrotoxic (4.71 ± 1.99 ml per 100 ml; $p < 0.001$) and cirrhotic (4.37 ± 3.67; $p < 0.05$) patients than in controls (8.29 ± 1.05). The arterial molar lactate/pyruvate ratio was not significantly higher in the thyrotoxic patients (5.12 ± 1.41) than in the controls (4.47 ± 0.94), but was significantly increased in the cirrhotic group (7.08 ± 1.92; $p < 0.01$) and in 4 normokinetic cirrhotic subjects (6.56 ± 1.14; $p < 0.02$) studied for comparison. The results, together with reported studies on other organs, indicate that the nonmetabolic component of the increased CO in thyrotoxicosis and in cirrhosis is due to increased blood flow through skeletal muscle and skin, and suggest the possibility of mediation through common mechanisms. Increased anaerobiosis, a potential stimulus to increased CO, was not present in thyrotoxicosis and appeared to have no relationship to the hyperkinetic circulatory state in cirrhosis.

Mechanisms of Uric Acid Production in Chronic Leukemia. IRWIN H. KRAKOFF AND M. EARL BALIS, New York, N. Y. (introduced by Joseph H. Burchenal).

It has been shown by several investigators that uric acid is produced in man by two routes: *1*) *de novo* synthesis of purines from simple precursors with prompt oxidation to uric acid and *2*) degradation of nucleic acids with oxidation of the liberated purines to uric acid. Studies in this laboratory and others have shown overproduction of uric acid in chronic granulocytic (CGL) but not in chronic lymphocytic leukemia (CLL) with similar degrees of leukocytosis. In order to investigate differences in mechanism of uric acid biosynthesis be-

tween the two types of chronic leukemia, sodium formate-C^{14} was given intravenously to patients with each type of leukemia and to nonleukemic "control" patients. Two phases of isotope enrichment of urine uric acid were seen in CGL: *1*) an early peak occurring about 2 days after isotope administration and *2*) a prominent second phase reaching a maximum about 12 to 15 days after isotope administration. The prominent late phase is thought to be derived from the degradation of nucleic acid purines, and dissection of the curve into its two components indicates that the late phase is quantitatively correlated with the overproduction of uric acid. In CLL, in which total uric acid excretion is normal, the later phase was not seen, presumably owing to the longer survival of CLL leukocytes, or the incomplete degradation of their nucleic acids. This demonstration of a more rapid "turnover" of purines in CGL suggests that the clinical responsiveness of that disease to antimetabolites that are inhibitors of purine biosynthesis is due to greater dependence on *de novo* purine synthesis. The absence of increased purine metabolism in CLL, manifested by normal uric acid production, results in less dependence on *de novo* purine synthesis and unresponsiveness to the antimetabolites that inhibit it.

The Secondary Ventilatory Response to Exercise. RICHARD A. KRUMHOLZ AND JOSEPH C. ROSS, Indianapolis, Ind. (introduced by William P. Deiss).

A ventilatory response to work load usually occurs simultaneously with commencement of exercise. This initial increase in ventilation (V) is maintained for several breaths, after which there is a gradual increase in V, followed by a much more pronounced abrupt increase, the secondary ventilatory response. The factors responsible for this secondary ventilatory response are, as yet, undetermined. This investigation was designed to study the effects on this secondary response of procedures that change cerebral blood flow. V was continuously recorded in 12 subjects during exercise on a treadmill (4 mph, 6% grade). Arterial blood samples for determinations of pH and pCO_2 were taken at intervals during exercise. With air breathing, an abrupt ventilatory increase of 14.6 ± 6 L per minute occurred at 67 ± 18 seconds after exercise was begun, while the abrupt increase of 14.3 ± 4 L per minute occurred significantly later (83 ± 10 seconds) ($p < .01$) during 100% O_2 breathing. Six subjects who received $NaHCO_3$ infusion had a secondary ventilatory response at 80 ± 15 seconds before and at 72 ± 20 seconds after the infusion on air, while the response came at 92 ± 19 seconds before and 93 ± 12 seconds after the infusion of 100% O_2 ($p = NS$). In 6 other subjects, the secondary ventilatory response occurred at 53 ± 11 seconds on air and at 71 ± 18 seconds on O_2. After NH_4Cl infusion, the response was significantly later on air (70 ± 10 seconds) and on O_2 (87 ± 15 seconds) ($p < .01$). The initial ventilatory plateau, however, was not affected by either O_2, $NaHCO_3$, or NH_4Cl. In this study, the secondary ventilatory response could not be attributed to changes in pH or pCO_2. The time of onset of the secondary response was altered by substances that change cerebral blood flow. These findings suggest that this abrupt increase in ventilation may be due to a blood-borne stimulus, whose effects are altered by changes in cerebral blood flow.

The Role of Immediate Wheal Reactions (IWR) in Immune Responses. WILLIAM J. KUHNS,* New York, N. Y.

Immunologically competent, Schick-negative persons receiving frequent intracutaneous doses of fluid diphtheria toxoid developed delayed reactions followed by IWR at times of succeeding toxoid injections. Among most severe instances of IWR were persons whose sera contained skin-sensitizing antibodies (SSAb) measured in passive transfer (PK) tests. These findings raised the possibility that activity of cell lines responsible for IWR-SSAb was optimal when antigen persisted in skin in hyperimmune persons. Injections of I^{131}-labeled toxoid into the skin of Schick-negative persons who developed delayed reactions confirmed the impression that in this circumstance intradermal antigen was retained for longer periods (half-life = 5 days) than was observed in unreactive, Schick-negative persons (half-life = about 1 day). IWR and associated skin changes did not occur when frequent doses of toxoid were given to Schick-positive, normal persons and an agammaglobulinemic patient. PK reactions with SSAb derived from other sources, however, could be demonstrated in skin in these persons. The results are consistent with the following interpretation. IWR occur *1*) at passive transfer sites when SSAb attached to cells with ability to mediate IWR combines with antigen and *2*) during active immunity in presence of at least two cell populations, with SSAb-forming cells and "mediator" cells. In experiments to determine the nature of these cells, stained biopsy sections of skin sites prepared with SSAb indicated unusual numbers of perivascular chronic inflammatory cells, including mast cells. Although reasons for specific activity of SSAb are unknown, the presence of a labile carrier globulin may be significant. Thus, in samples of skin sensitizing diphtheria antitoxin, selected methods of serum fractionation and heat at 56° C destroyed wheal reactivity without altering antitoxin content (demonstrated by toxin neutralization).

Intestinal Absorption and Malabsorption of the Amino Acid Analogue Alpha-aminoisobutyric Acid in Man. LEONARD LASTER AND D. M. MATTHEWS, Bethesda, Md. (introduced by Jan Wolff).

Several properties of alpha-aminoisobutyric acid (AIB) indicate its usefulness for assessing human intestinal absorption. First, although mammalian small intestine apparently has an active transport mechanism for AIB, its absorption is not too effective to be influenced by disease. Using everted hamster intestine sacs and Michaelis-Menten analyses of observed kinetics, we found the K_m for AIB approximately 20 times that of L-alanine. Sec-

ond, man appears to be unable to metabolize AIB. Others recovered virtually all of injected AIB-C^{14} unchanged, in urine, after 9 days and detected no conversion to $C^{14}O_2$. Finally, AIB appears to be nontoxic. Rats fed 2 g per kg per day for 14 days showed no disturbances other than reversible amino aciduria. We fed AIB-1-C^{14} to fasted subjects (10 μc, 0.2 g per kg) and determined C^{14} in hourly plasma samples and in urine collected during 5 hours. The results, expressed as highest mg per 100 ml AIB in plasma (1 or 2 hours) and as percentage of dose excreted in urine, were: in seven control subjects, 30 to 42 mg per 100 ml and 27 to 52%; in two patients with fibrocystic pancreatic insufficiency, 29 and 39 mg per 100 ml and 32 and 42%; in one patient with extensive small-intestine resection, 9 mg per 100 ml and 10%; in one patient with sprue-like malabsorption, 13 mg per 100 ml and 3%; in one patient with sprue in partial relapse, 36 mg per 100 ml and 33%, and in remission, 52 mg per 100 ml and 40%; in one patient with sprue in severe relapse, 16 mg per 100 ml and 9%, and again in relapse, 6 months later, 11 mg per 100 ml and 6%; in one patient with Whipple's disease in relapse, 13 mg per 100 ml and 23%, and in remission on antibiotics, 30 mg per 100 ml and 31%; and in one patient with bronchial carcinoma, 17 mg per 100 ml and 22%. Tentatively, AIB absorption seems normal in pancreatic insufficiency and depressed in intestinal malabsorption. Thus, the AIB tolerance test may be a helpful clinical index of amino acid absorption.

Important Determinants in Resistance to Pulmonary Infection. GUSTAVE A. LAURENZI, RAUL B. ENDRIGA, JOSEPH J. GUARNERI, AND JOHN P. CAREY, Jersey City, N. J. (introduced by Timothy J. Regan).

The sterility of the normal bronchial tree is evidence that effective antibacterial factors are active. In this study, the ability of the lower respiratory tract to dispose of bacteria and the effect of various conditions on this function were quantitated in two different strains of mice. Since mice lacking *E. coli* in their intestinal flora are reported to be more susceptible to many infections, *E. coli*-bearing and *E. coli*-free mice were compared. Rapid clearance of *S. aureus* from the lungs of mice in both strains was demonstrated after implantation by a precise aerosol system; 45% disappeared in 1 hour, 70% in 2 hours, and 88% in 4 hours. Variables were introduced and maintained for 4 hours after bacterial implantation. In the *E. coli*-bearing strain, cigarette smoke inhalation markedly reduced 4 hour clearance to 50%, and alcohol had a similar effect. In this group, less interference (65% to 75% clearance) occurred with hypoxia, barbiturates, cortisone, and reticuloendothelial system blockage with colloidal carbon. Cortisone and carbon were administered before bacterial deposition. In the *E. coli*-free mice, bacterial deposition was much lower, and clearance was unaffected by smoke inhalation. Moreover, clearance was slightly less impaired by the other variables. This study demonstrates the remarkable capacity of the lower respiratory tract to clear bacteria, and the results show that cigarette smoke and alcohol have the greatest inhibitory effects. In addition, *E. coli*-free mice are less susceptible to bacterial implantation by the airborne route, and they are resistant to the relatively acute effects of smoke on the bronchopulmonary disposal of bacteria. The importance of indigenous factors such as the intestinal flora and genetic influences in resistance to pulmonary infection is indicated by this study.

A Paradoxical Metabolic Effect Resulting from N-Acetylation of Peptides Related to ACTH and MSH. HAROLD E. LEBOVITZ AND FRANK L. ENGEL,* Durham, N. C.

The validation with synthetic peptides of extra-adrenal actions of corticotropin has stimulated exploration of relationships between molecular structure of synthetic peptides related to ACTH and MSH and their biological actions on adrenal and extra-adrenal tissues. To this end, a series of *in vivo* and *in vitro* bioassay techniques has been developed to evaluate these activities and has been applied to the present study of certain synthetic "blocked" peptides, generously supplied by Dr. K. Hofmann. Included were the N-terminal 13- (trideca-), 20- (eicosa-), and 23- (tricosa-) amino acid peptide analogs of corticotropin in which specific amino acid residues were blocked as follows: *1)* N-acetylation of the N-terminal serine, *2)* amidation of the glutamic acid carboxyl group, and *3)* formylation of epsilon amino groups of lysine residues. Compared to natural corticotropin A_1, these peptides exhibited potencies of 1 to 2% in their ability to elevate plasma corticosterone in dexamethasone-blocked rats, 0.25 to 1.0% in their capacity to stimulate corticosterone production by quartered rat adrenals *in vitro,* and 0.25 to 1.0% in their lipolytic activity on rat adipose tissue *in vitro.* Natural corticotropin A_1 and the synthetic unblocked eicosa- and tricosapeptides induced comparable hypoglycemia on intravenous injection into normal mice, whereas the block peptides consistently elicited hyperglycemia. The latter response was also elicited by natural α-MSH, which differs from the blocked tridecapeptide by having free glutamic and lysine residues. Thus, the hyperglycemic activity appears to be related to the N-acetylation and not to the blocking of glutamic or lysine residues. The mechanism of the hyperglycemia is unexplained. Adrenalectomy does not eliminate it, but 4 mU of insulin blocks it without lowering blood sugar in control mice. Thus, N-acetylation qualitatively alters one extra-adrenal action (hypoglycemia to hyperglycemia), while quantitatively reducing other adrenal and extra-adrenal actions. Some implication of this striking dissociation of biological activities consequent to changes in peptide structure will be discussed.

The Nature of the Proteins Sensitizing the Red Cells in "Autoimmune" Hemolytic Disease. JOHN P. LEDDY, RICHARD W. HILL, SCOTT N. SWISHER,* AND JOHN H. VAUGHAN,* Rochester, N. Y.

Previous studies of the antiglobulin reactions in "autoimmune" hemolytic disease (AHD) have indicated that

the erythrocyte-sensitizing protein is often γ_2-globulin, agglutination being produced by rabbit antisera to human γ_2-globulin, but not by antisera to other serum proteins, In other instances, agglutination was brought about not by antisera to γ_2-globulin, but by antisera to whole human serum completely absorbed with γ_2-globulin (Cohn fraction II), a "non-γ"-globulin reaction. This problem required re-examination because of *1*) recent evidence that antibody activity in man is carried by three distinct but antigenically related immunoglobulins and *2*) recent immunochemical definition of certain human complement (C′) proteins. The present investigation therefore sought to determine whether erythrocytes giving "non-γ"-globulin reactions were sensitized with C′ protein, or with an immunoglobulin other than γ_2-globulin, or with protein entirely unrelated to immunoglobulins or C′. Antisera specific for γ_2, γ_{1A}, and γ_{1M} immunoglobulins and for complement protein (β_{1A}-globulin) were prepared, and their specificities proven by immunodiffusion, passive cutaneous anaphylaxis, and hemagglutination. In addition, an antiserum reactive with numerous other serum proteins, but not with immunoglobulins or C′, was prepared. In 24 patients with AHD, these reagents indicated that the erythrocytes were sensitized with γ_2-globulin, C′ protein, or both. In no instance was sensitization with γ_{1A}- or γ_{1M}-globulin observed; in only 2 instances were weak reactions seen suggesting coating with a small amount of other protein, in addition to γ_2-globulins or C′. Tanned erythrocytes showed no such selectivity of serum protein uptake. Erythrocyte coating with γ_2-globulin was found primarily in idiopathic AHD, whereas C′ sensitization alone was characteristic of AHD secondary to systemic lupus erythromatosis or to lymphatic malignancy. Thus, detectable erythrocyte sensitization in AHD is remarkably limited to γ_2-globulin or complement. Nonspecific adsorption of other serum proteins by "altered" erythrocyte membranes appears to play little or no role in the phenomenon.

Folic Acid and Hepatic DNA Synthesis. CARROLL M. LEEVY, WILLEM TENHOVE, OSCAR FRANK, HERMAN BAKER, AND GILBERT R. CHERRICK, Jersey City, N. J. (introduced by Harold Jeghers).

Our studies show the liver is an important site for conversion of folic acid (FA) to metabolically useful forms, and hepatic disease decreases uptake and storage of this vitamin. Preliminary data suggested FA, essential for DNA synthesis in microorganisms and mammalian hemopoiesis, is also of critical importance for liver regeneration. Investigations were therefore undertaken to elucidate the role of FA in hepatic DNA synthesis and determine if its depletion or excess alters response to liver injury. Observations were made on 150 FA-depleted Sprague-Dawley rats and normal litter-mate controls with CCl_4-induced hepatic necrosis, and on 15 alcoholic patients with FA deficiency and liver disease. Tissue FA and folinic acid were assayed microbiologically with *Lactobacillus casei* and *Pediococcus cerevisiae*, respectively. Hepatic DNA synthesis was evaluated in rats by radioautographic and radiochemical techniques to measure incorporation of tritiated thymidine (H_3T) into DNA. It was assessed in human percutaneous biopsy specimens by *in vitro* uptake of H_3T. Effects of uridylic acid, thymidine, FA, folinic acid, and vitamin B_{12} on conversion of uridylic to thymidilic acid and subsequent formation of DNA was determined by chromatographic and bioautographic analysis. Untreated FA-deficient animals exhibited four- to tenfold reduction in DNA synthesis as compared with those treated with thymidine, FA, and folinic acid. Neither uridylic acid nor vitamin B_{12} influenced DNA synthesis, although uridylic acid accumulated. Differential counts showed FA deficiency delays healing and decreases turnover of liver, mesenchymal, and ductular cells. Specimens from FA-deficient patients exhibited equivalent reductions in DNA synthesis, corrected by FA. Excess FA had no effect on DNA synthesis. These results indicate FA is rate-limiting for hepatic thymidilic acid and DNA synthesis; they emphasize the desirability of identifying and correcting a deficiency of FA and other metabolically related substances that facilitate DNA synthesis after liver injury.

Myocardial Water Shifts Induced by Coronary Arteriography. PATRICK H. LEHAN, MAUREEN A. HARMAN, AND HENRY A. OLDEWURTEL, Jersey City, N. J. (introduced by Harper K. Hellems).

The effects of vasoactive drugs have been studied by coronary arteriography without adequate information on changes induced by the hypertonic contrast agents *per se*. In 15 dogs, coronary blood flow, electrocardiograms, and systemic and coronary perfusion pressures were continuously monitored after injection of 4 ml Hypaque (90% Na methylglucamine diatrizoate) or isotonic NaCl as control. Serial 10-second samples of blood from the coronary sinus and aorta were analyzed for changes in hematocrit, hemoglobin, plasma protein, and osmolality. In the period of radiographic visualization of the coronary tree (10 to 15 seconds), there was a massive transfer of tissue water to the capillaries, as indicated by a 35 to 50% fall in coronary sinus hematocrit, hemoglobin, and protein. Despite the injected material's osmolality of 2,500 mOsm per kg, osmolality in blood after injection rose only 40%. During this period of myocardial water loss, coronary blood flow fell and coronary vascular resistance increased. As the radiopaque dye left the vasculature, coronary vasodilatation occurred. During the next 2 minutes, all the above coronary sinus blood determinations exceeded control values, suggesting a return of water to the myocardium. These compartmental water shifts and resultant changes in cellular milieu may account for the reduced myocardial contractility and reduction in cardiac output and systemic pressure. The validity of using hypertonic contrast agents, especially with repeated injections, to assess vasoactive drugs is challenged by these findings.

Production, Excretion, and Net Balance of Fixed Acid in Patients with Renal Acidosis. JACOB LEMANN, JR., A. DAVID GOODMAN, EDWARD J. LENNON, AND ARNOLD S. RELMAN,* Boston, Mass., and Milwaukee, Wis.

A previously described technique for the measurement of net external acid balance has been applied to patients with stable renal acidosis, with and without azotemia. The results: *1)* In eight acidotic patients, the net balance of acid was positive (mean: 16.9 ± 6.3 [SD] mEq per day) despite a constant level of extracellular bicarbonate. This retention of acid constituted approximately 40% of the daily endogenous load. (In sixteen studies on non-acidotic normal subjects taking the same diet, the mean balance was -0.5 ± 6.0 mEq per day.) *2)* Reduced production of acid was not a factor in maintaining the steady extracellular bicarbonate because acidotic patients produced as much acid as did normal subjects on the same liquid formula diet. Nitrogen balance and weight were maintained. *3)* When acidosis was corrected in six of these patients by the continuous administration of alkali, the positive balance was abolished in five, and the mean daily balance was -4.5 ± 7.5 mEq. These data indicate that in patients with renal disease acidosis calls into play an *extrarenal* mechanism that disposes of a large fraction of the endogenous acid load and thus is critical in the stabilization of acid-base equilibrium. The nature of this mechanism is not demonstrated by these studies, but for many reasons, neutralization of the retained acid by continuous slow dissolution of alkaline bone salts seems the most probable explanation.

The Mechanism of Sodium Diuresis after Saline Loading: Evidence for a Factor Other than Increased Filtered Sodium and Decreased Aldosterone. NORMAN G. LEVINSKY AND RICHARD C. LALONE, Boston, Mass. (introduced by Chester S. Keefer).

It has occasionally been suggested that increased sodium excretion after saline loading is due to decreased tubular reabsorption of sodium. Glomerular filtration rate (GFR), however, commonly increases during saline loading; the increment in filtered sodium is usually sufficient to account for increased excretion. Even during saline diuresis, sodium excretion is a small fraction of filtered sodium, and errors in measurement of GFR are relatively large. Therefore, in experiments in which sodium excretion increases when filtered sodium is apparently stable or decreased, it is difficult to rule out the possibility that increases in GFR too small to measure are nevertheless sufficient to account for increased excretion. To circumvent this difficulty, the mechanism of sodium diuresis was studied in dogs in which filtered sodium could be reduced during saline loading by means of an aortic clamp. Mineralocorticoids were administered to eliminate changes in endogenous aldosterone activity as a factor. In 22 acute experiments, control periods were obtained, after which dogs were infused with 1,400 to 5,400 ml of isotonic saline or modified Ringer's solution; plasma sodium was stable. When sodium excretion after saline loading was reduced by aortic clamping to a rate 20 to 280 μEq per minute above control, filtered sodium was regularly significantly (300 to 4,700 μEq per minute) below control values. When filtered sodium was comparably reduced in 7 control studies without saline infusion, excretion always fell, showing that no artifact was introduced by clamping. Neither increased filtered sodium nor decreased aldosterone can account for increased sodium excretion after saline loading. Part of the increase is due to an additional factor that decreases tubular reabsorption of sodium.

The Effect of Anesthesia and Adrenalectomy on the Renal Response to Angiotensin. HOWARD LEVITIN, WILLIAM B. LEHMANN, GILLES PIGEON, YVES WARREN, AND IRA S. GOLDENBERG, New Haven, Conn. (introduced by Franklin H. Epstein).

Valine-5 angiotension II has been infused into anesthetized and unanesthetized dogs, during a diuresis induced by water or 0.5 N saline. In addition, adrenalectomized dogs have been studied under the same experimental conditions. The intravenous infusion of 2 μg per minute of angiotensin for 30 minutes into trained, unanesthetized dogs resulted in marked antidiuresis and, to a variable degree, antinatriuresis. Potassium excretion is variable, but tends to be decreased. Modest reductions in C_{pah} and C_{cr} are observed. The osmolar clearance and free water clearance are significantly reduced. Adrenalectomized dogs maintained on cortisone and desoxycorticosterone acetate responded to the same dose of angiotensin in an identical manner, with antidiuresis and antinatriuresis. C_{pah} and C_{cr} also fall. In addition, C_{osm} and C_{H_2O} are reduced. Under Pentothal anesthesia, however, normal dogs respond differently to angiotensin. In these animals, angiotensin induces a marked diuresis and natriuresis. Potassium excretion also increases, but less prominently. No significant changes in C_{cr} and C_{pah} are noted. The C_{osm} and C_{H_2O} increase in proportion to the diuresis. Adrenalectomized dogs under Pentothal anesthesia do not alter their response to angiotensin, which is similar to that obtained in such dogs without anesthesia. The magnitude of the change in blood pressure induced by angiotensin was similar in all 4 groups of animals. There was no difference between animals in which diuresis had been induced with water or 0.5 N saline. These results demonstrate a marked influence of Pentothal anesthesia on the renal response to angiotensin in the dog. It is suggested that Pentothal anesthesia minimized the renal hemodynamic effect of angiotensin, thus making apparent a potent depressing effect of angiotensin on the renal tubular reabsorption of sodium.

The Upper Limb-Cardiovascular Syndrome: Effect of an Autosomal Dominant Gene on Embryogenesis. K. B. LEWIS, R. A. BRUCE,* D. BAUM, AND A. G. MOTULSKY, Seattle, Wash.

Clearcut genetic patterns are rarely seen in congenital heart disease. A large family studied clinically and by

autopsy demonstrated the operation of a specific genetic mechanism affecting the heart, great vessels, and upper extremities. There were 15 affected persons out of 19 at risk in 3 generations. Affected patients had both cardiovascular and skeletal malformations to a variable degree. The cardiac anomalies included a single right coronary artery, atrial septal defect, anomalous right subclavian artery, and probable transposition of the great vessels. The cardiac lesions led to death in infancy in 5 cases. Skeletal malformations were limited to upper extremities, and included shortened clavicles, absent or rudimentary thumbs, syndactyly, hypoplastic arms, forearms, wrists, and hands. The clinical pattern varied from patient to patient. The association of upper limb skeletal defects and atrial septal defect has previously been documented in 5 patients from 3 families. The syndrome under study appears to be identical with a much broader clinical spectrum than previously described. Since the limb buds and primitive heart tube differentiate at the fourth week of fetal life, a specific gene or environmental effect at that time would disrupt these structures selectively. Thalidomide usually affects limb buds, but in 25% of cases, also produces cardiovascular malformations. The upper limb-cardiovascular gene appears to act at a similar stage and causes both upper limb and cardiovascular malformations. Transmission is that of an autosomal dominant trait. Chromosome studies in one affected member were normal. Since embryological development is a dynamic and individual process, the observed variation in clinical expression in both heart and upper limbs would be compatible with a time-specific genetic defect affecting organ differentiation. The upper limb-cardiovascular syndrome may be considered the genetic counterpart of the thalidomide syndrome.

Cell Proliferation Kinetics in the Gastrointestinal Tract of Man. MARTIN LIPKIN, BERTRAND BELL, AND PAUL SHERLOCK, New York, N. Y. (introduced by Thomas P. Almy).

The kinetics of cellular proliferation in the human gastrointestinal tract have been studied after intravenous injection of tritiated thymidine into 10 selected subjects and microradioautographic analysis of specimens of biopsied mucosa of stomach, ileum, colon, and rectum. Biopsy specimens were obtained from these areas at ½- to 1-hour intervals for the first 12 hours after injection of thymidine, every 2 hours for the next 12 hours, at daily intervals for 1 to 2 weeks, and 1 to 2 times weekly thereafter to a maximum of 6 months. The proliferative cycle of the gastrointestinal epithelial cells is divided into several phases: a DNA synthesis (S) phase lasting 10 to 15 hours; a premitotic (G_2) phase lasting 1 to 2 hours; a mitosis (M) phase lasting 1 hour or less; and a postmitotic (G_1) phase lasting the remainder of the proliferative cycle. The mean generation time of epithelial cells in all areas examined is approximately 1 day. Zymogen and parietal cells of the stomach renew very slowly. At a given time, 10 to 16% of the epithelial cells in each area are in S phase. Epithelial cell renewal proceeds at a mean rate of 1% of cells per hour, and the mucosa is replaced in about 4 days. Cells migrate toward the lumen of the gastrointestinal tract at a mean rate of 0.9 cell positions per hour. The data indicate that occasional cells normally die immediately after cell division, and do not proceed with the migrating column. After administration of the antimetabolites 5-fluorouracil and 5-fluorodeoxyuridine, the incorporation of thymidine into DNA is rapid, but the G_2 and mitosis phases are delayed. Rectal carcinoma cells proliferate at a slower rate than normal rectal epithelial cells. Although most epithelial cells are rapidly extruded into the lumen within several days, a few epithelial cells remain in the mucosa for several months. Prolonged interphase and slow migration contribute to retention of these cells, which constitute a population thus far indistinguishable from other epithelial cells, except by identification of their unusual kinetics.

A Substance in Plasma Lethal for Candida Albicans. DONALD B. LOURIA AND ROBERT G. BRAYTON, New York, N. Y. (introduced by Claude E. Forkner).

A substance has been found in serum and plasma of normal persons that kills *Candida albicans,* but not other *Candida* species or other yeasts. One million *Candida* cells are added to 1 ml of serum, the tube is rotated for 24 hours, and the number of viable *Candida* cells determined at 6 to 24 hours by pour-plate enumeration techniques. A positive result is tenfold reduction in *Candida* census at either period. The substance has been found in the serum of 38 to 40 normal persons (95%) and in each of 8 cord bloods. None was detected in urine or cerebrospinal fluid. Rabbit and guinea pig plasma have the factor, but mouse and rat plasma do not. The factor was present in 11 patients with leukemia, lymphoma, or myeloma, in 10 of 12 with carcinoma (83%), and in 10 of 12 nonazotemic diabetic subjects (83%). Only 10 of 16 patients (62.5%) with azotemia and 1 of 8 azotemic diabetic subjects (12.5%) possessed the substance. It was absent in 3 siblings with congenital hypoparathyroidism-hypoadrenalism and in 13 of 17 persons with mucocutaneous or systemic candidiasis. The substance is dialyzable through cellophane, partially through collodion, but does not appear in serum ultrafiltrates. It is not modified by heating plasma to 70° C or refrigeration and is active over a wide pH range. It is inactivated by alcohol and trypsin. Its activity is not modified by adding antibiotics, glucocorticoids, or high concentrations of glucose. It can be adsorbed on *Candida* cells, but rabbits infected intravenously with 10^7 cells do not lose it. Patients without the factor who have moniliasis develop high titers of agglutinating antibody, and their plasma remains effective in opsonizing *Candida.* The data currently available suggest this substance may be important in protecting against severe *Candida albicans* infections.

Comparative Studies of Ventricular Vulnerability to Fibrillation. BERNARD LOWN, SAMI KAID BEY, MARK PERLROTH, AND TADAAKI ABE, Boston, Mass. (introduced by Frederick J. Stare).

Within each cardiac cycle, there is a period especially susceptible to ventricular fibrillation (VF). The present investigation demonstrates the occurrence and documents some properties of the vulnerable period in 5 mammalian species: rabbits, cats, dogs, sheep, and subhuman primates. The cardiac cycle was systematically explored at 10-msecond intervals with a capacitor discharge of 2.5 mseconds across the intact chest of anesthetized animals. In approximately 100 animals, more than 10,000 synchronized shocks failed to produce VF unless they fell just before the apex of the T wave of the surface electrocardiogram. VF was consistently produced in these 5 species when the ratio of Q shock/QT interval ranged from 0.65 to 0.80. The duration of the vulnerable period varied from 20 to 40 mseconds. In any one animal, the vulnerable period was the same whether this was determined transthoracically or from the surface of the heart, or measured from within the myocardium. The energy necessary to produce VF varied from 0.5 to 10 watt-seconds. For each species, there is an optimal energy level that consistently produces VF. The persistence of VF is related to heart weight. In dogs and sheep with hearts weighing over 50 g, the arrhythmia was permanent. In rabbits, cats, and Cebus monkeys with hearts weighing from 10 to 15 g, VF tended to be transient. These findings suggest that ventricular vulnerability is a physiologic property of the mammalian heart. The significance of these results to the occurrence of sudden death in man will be discussed.

Uric Acid Metabolism in Acute Intermittent Porphyria. GEORGE D. LUDWIG,* Philadelphia, Pa.

Although the precise biochemical lesion of acute intermittent porphyria remains obscure, impaired purine synthesis has been implicated. Aldrich and co-workers found decreased uric acid and increased porphyrin concentrations in allantoic fluids from chick embryos rendered porphyric by Sedormid injection, and postulated decreased utilization of delta-aminolevulinic acid (ALA) as a single carbon donor for purine synthesis, with resultant diversion of accumulated ALA to porphobilinogen (PBG) and porphyrins. Were reduced purine synthesis involved in human acute porphyria, low serum uric acid concentrations might be anticipated. In four patients in whom serial measurements were made during acute attacks, the serum urate concentration, which was normal at onset, fell significantly to as low as 1.0 mg per 100 ml. Greatly increased urinary urate excretion, however, with parallel increases in urate clearance and urate/creatinine clearance ratios invariably accompanied decreases in serum concentrations, which, with a slight lag, followed the pattern of the tubular urate reabsorption. Aminoaciduria coincided with peaks of urate excretion, and the excretory pattern of both correlated better with ALA excretion than with PBG or porphyrins, suggesting that this organic acid may alter renal handling of both amino acids and urate. Maximal urate and α-NH_2-nitrogen excretion coincided with maximal severity of the hyponatremic syndrome due to probable inappropriate secretion of antidiuretic hormone, which we have found almost invariably to accompany acute attacks of porphyria. The observation that ALA, or its methyl analogues, induces oliguria when injected into animals or man suggests that it may also be responsible for "triggering" this syndrome. Since dilutional effects of net water retention and renal loss fully account for the decreases in serum urate, the hypothesis that a block in uric acid production exists in the natural disease gains no support, and the rationale for the therapeutic use of adenine compounds is rendered questionable.

Staphylococcal Infections in Chick Embryos. WILLIAM R. MCCABE, Chicago, Ill. (introduced by Mark H. Lepper).

A previous report demonstrated the sensitivity of embryonated eggs to allantoic infection with pathogenic *Stapyhlococcus aureus* and described some aspects of this infection. The potential value of this model for the study of staphylococcal infections prompted extension of these studies and further investigation of its characteristics to define bacterial virulence factors and defense mechanisms. Coagulase-positive and -negative *Staphylococci* attained similar maximal population densities in allantoic fluid *in vivo,* and both invaded the amnion and embryo. Coagulase-positive strains produced striking inflammatory reaction and early abscess formation throughout the embryo, while coagulase-negative strains produced only minimal inflammatory reaction histologically. Virulence for chick embryos tended to reflect the pathogenicity in humans of the 87 strains studied. Coagulase-negative *Staphylococci* (30) were no more lethal than either sterile saline or broth with an inoculum of 10^3 to 10^4 bacteria. Coagulase-positive strains (27) isolated from the nares of healthy outpatients were more lethal ($p < .0.001$) than coagulase-negative strains and less lethal ($p < 0.01$) than coagulase-positive isolates (25) from definite human staphylococcal infections. Pathogenic *Staphylococci* produced fatality rates in excess of 50% after the injection of less than 100 bacteria. The fatality rates produced by individual strains were reproducible when repeated over a period of several months. The age of the embryo appreciably influenced the outcome of infections with both coagulase-negative and -positive strains. Specific factors responsible for lethality to embryonated eggs have not been identified. Gelatinase production, pigment, egg yolk factor, and the quantity and type of coagulase produced did not correlate with lethality. Although α-hemolysin production *in vivo* greatly exceeded that observed in broth culture, hemolysin production was not a prerequisite for lethality. Gamma globulin containing agglutinating antibody against the challenge organism afforded significant protection ($p < 0.001$), while gamma globulin without

appreciable agglutinating antibody (< 1:20) was not protective.

The Apparent Eradication of Human Tubercle Bacilli in Mouse Tissues. ROBERT M. MCCUNE AND FLOYD M. FELDMANN, New York, N. Y. (introduced by Walsh McDermott).

As reported previously, large populations of tubercle bacilli in mouse tissues were rendered nonculturable by 3 months' administration of pyrazinamide-isoniazid, but in the months after treatment, they reappeared naturally in most of the animals. The sterile state was shown to endure for 1 month to more than a year thereafter. Subsequent experiments revealed that when a 4-week course of 1.0 mg of cortisone was administered 2 months after cessation of therapy, microbial revival occurred in all the animals. In the undetectable phase, the bacilli could be present in some altered form, or they could be unaltered, but so few as to escape detection. In view of the increased sensitivity of the culture techniques employed, only rarely could an *unaltered* bacillus escape detection. Sterilization without death, however, could lead to the "vanishing" of large populations of tubercle bacilli. When intensive microscopy, however, aided by fluorescent strains was conducted (7,000 fields per slide examined for 4 to 6 hours), acid-fast structures with the appearance of tubercle bacilli were observed in tissues that were negative on culture. Whether these represent dead organisms or nonculturable bacilli that will revive later is not known. In 4 experiments in which treatment was extended to 6 months, no culturable bacilli appeared naturally 3 or 6 months after cessation of treatment. In one of these experiments, therapy was started 8 weeks after infection, when the lesions were grossly necrotic. Moreover, no culturable bacilli were evoked in these experiments by a 4-week course of 1.0 mg cortisone administered throughout the third post-treatment month. Intensive microscopy, however, revealed a few bacilli, some of which stained irregularly. Whether extension of therapy has effected total eradication, or merely prolongation of the sterile state must await further studies.

Epithelial Renewal in the Stomach, Duodenum, and Rectum of Man: Radioautographic Studies. WALTER C. MACDONALD, JERRY S. TRIER, AND NEWTON B. EVERETT, Seattle, Wash. (introduced by Cyrus E. Rubin).

This study was undertaken in man because extensive data on gastrointestinal epithelial proliferation and migration was available only in animals. Two ambulatory patients with incurable nongastrointestinal cancer were selected because of their excellent general health. Each was given 10 mc of tritiated thymidine intravenously. One hour later and at frequent intervals up to 14 days, suction biopsies were taken from the fundal gland area of the stomach, the distal duodenum, and the rectum. Within one hour, many labeled cells were evident in the gastric pits and gland isthmuses, and in duodenal and rectal crypts. Parietal cells were not labeled, but occasionally chief or mucous neck cells were. Rarely, duodenal goblet cells appeared to be labeled within the first hour; labeling of rectal goblet cells was not certain until later. Migration from gastric pit to surface usually took 4 to 6 days, although cells from a few pits reached the surface in 36 hours. Duodenal cells migrated from the crypt mitotic zones up the villi to be extruded from the tips in 5 to 6 days. Rectal epithelial cells reached the surface in 6 days, but discrete extrusion zones were not apparent. A few well-labeled cells persisted in the mitotic regions of all three organs at 14 days. The human duodenal and rectal epithelial migration time is 5 to 6 days, which is two to three times that reported in rodents. That of the human gastric fundal epithelium varies greatly between pits, but is also probably longer than in rodents. The labeling of goblet cells within 1 hour suggests that all goblet cells do not differentiate from columnar cells, but that they may reproduce themselves. From this study, it is apparent that animal data on gastrointestinal epithelial proliferation cannot be applied unreservedly to man.

Reversal of Angiotensin Vasoconstrictor Activity during Ischemia. JOHN C. MCGIFF AND HAROLD D. ITSKOVITZ, Philadelphia, Pa. (introduced by Calvin F. Kay).

Present hypotheses relating the role of angiotensin during renal ischemia to restoration of renal perfusion pressure do not account for the observed changes in renal blood flow. A striking reduction in renal blood flow measured by either direct or indirect means has been a universal finding. The activity of angiotensin II was studied on the renal bed before and during reduction in renal blood flow in chloralose-anesthetized dogs, with a rotameter to measure renal venous outflow continuously. During graded reduction of renal blood flow by a clamp placed on the renal artery, angiotensin (0.025 to 2.5 μg per kg) increased renal blood flow. Before and after renal artery constriction, angiotensin decreased renal blood flow from 50% to 90%. Constriction of the aorta between the origin of the renal arteries during measurement of both renal flows demonstrated a differential effect elicited by angiotensin. The renal flow below the constriction increased while that above the constriction decreased after administration of angiotensin. Levarterenol in equipressor amounts elicited only reduced renal blood flow in the presence or absence of constriction. Analyses of pre- and postglomerular resistance changes induced by angiotensin suggest that the postglomerular vessels are primarily involved when angiotensin elicits a reduction in renal blood flow. On the other hand, preglomerular vessels are the primary site of the resistance changes when angiotensin elicits an increased renal blood flow during renal artery constriction. Simultaneously measured limb and renal blood flows demonstrated that the limb as well as the kidney will increase its flow during angiotensin administration

in the presence of ischemia. These findings permit a more rational assignment of angiotensin as a compensatory humoral agent elicited in response to renal ischemia. It appears that the effect of angiotensin on the renal vasculature is determined by the presence or absence of renal ischemia.

Enzymic Hydrolysis of Thyrotropin and the Long-acting Thyroid Stimulator. J. M. McKENZIE, Montreal, Canada (introduced by J. C. Beck).

The long-acting thyroid stimulator found in the blood in Graves's disease was recovered with the 7 S proteins when appropriate sera were filtered in the gel Sephadex G-200. On the other hand, thyrotropin, whether endogenous or added *in vitro* (Condliffe, human pituitary thyrotropin), was found with the 4 S fraction when serum from normal or hypothyroid persons was filtered. These fractions were prepared, and the effect of various proteolytic enzymes on the two thyroid stimulating substances was studied; the extent of hydrolysis was observed by use of a "pH-stat" and subsequent agar gel immuno- and starch gel electrophoresis. Papain and subtilopeptidase destroyed thyrotropin in these preparations, and trypsin had a partially destructive effect. The long-acting thyroid stimulator was not, however, adversely affected by the proteolytic enzymes trypsin, subtilopeptidase, Panprotease (Worthington), or papain. The hydrolysate resulting from limited digestion by papain was filtered on Sephadex G-25; a macromolecular component, representing approximately 13% by weight of the initial serum proteins and giving only 4 precipitation bands on immunoelectrophoresis, was obtained. This fraction had all of the long acting thyroid stimulator, and the biological activity remained despite further hydrolysis with leucine aminopeptidase, when the immunoelectrophoretic pattern was reduced to faint albumin and γ-globulin bands and one other component. More extensive digestion with papain gave a hydrolysate in which approximately half of the thyroid-stimulating activity was of sufficiently small molecular size to be retarded in a column of Sephadex G-25. Conclusions were that *1*) thyrotropin and the long-acting thyroid stimulator, in serum, differ markedly in resistance to proteolytic enzymes; *2*) proteolysis and gel filtration allowed concentration of the latter principle; and *3*) by these means, a relatively small molecule ($<$ 5,000 mol wt) capable of prolonged thyroid stimulation was found.

Evidence for a Stimulatory Feedback of Ketone Acids on Pancreatic Beta Cells. LEONARD L. MADISON,* DAVID MEBANE, AND AMANDA LOCHNER, Dallas, Tex.

The marked hypoglycemic effect of ketone acids and their sodium salts recently reported from this laboratory was shown to be the result of a decrease in the hepatic output of glucose and to be accompanied by a simultaneous fall in FFA. Since these effects of ketones qualitatively resembled those of insulin, the present study was designed to determine whether ketones (β-hydroxybutyric and acetoacetic acids) stimulate endogenous insulin secretion directly, or have an insulin-like action per se. In one group of studies, the effect of intravenous administration of ketones on blood glucose concentration was followed for 120 minutes in completely depancreatized and in alloxan-diabetic dogs. In depancreatized dogs, ketone administration had no hypoglycemic effect; mean blood glucose rose from 185 to 239 mg per 100 ml. In contrast, in alloxan-treated dogs still capable of secreting insulin, ketone infusion resulted in a fall in blood glucose from 217 to 169 mg per 100 ml. In another group of studies, pancreatic venous insulin concentrations were measured by the immunoassay of Berson and Yalow in 10 nondiabetic dogs before and during both femoral venous and pancreatic arterial infusion of ketones. Ketone administration into a peripheral vein produced an increase in mean pancreatic insulin concentration from 275 to 650 μU per ml. Finally, pancreatic arterial infusion of minute amounts of ketones resulted in an eightfold increase in pancreatic venous insulin from 105 to 840 μU per ml. These data show that ketone acids have a direct stimulatory effect on endogenous insulin secretion and suggest that this feedback of ketones on beta cells is operative in preventing progressive ketoacidosis during starvation by modulating hepatic ketogenesis.

The Oxidation of Thiocyanate by an Enzyme System of Thyroid Tissue. F. MALOOF* AND M. SOODAK, Waltham and Boston, Mass.

Thiocyanate ion, SCN^-, is oxidized by the thyroid *in vivo*. This report describes a cytoplasmic particulate system of thyroid tissue which oxidizes SCN^- (180 mμmoles per g per hour) to sulfate, *in vitro*. The system is heat-labile, nondialyzable, and pH-dependent. Liver and kidney tissues are inactive. A salivary enzyme system also oxidizes SCN^-, but it differs from the thyroid system in that it is nonparticulate, is relatively resistant to heating, and is not inhibited by thiourea (10^{-3} M). L-Ascorbic acid (4×10^{-4} M) or D-araboascorbic acid is the cofactor in this reaction. 2-O-Methyl-L-ascorbic acid (10^{-3} M) is inactive in replacing or blocking the effect of ascorbic acid. The oxidation of SCN^- is inhibited by anaerobiosis, fresh thyroid supernatant fluid (1.0 mg protein), iodide (10^{-4} M), azide (10^{-4} M), cyanide (10^{-4} M), or aromatic antithyroid compounds (4×10^{-3} M). It is also inhibited (80%) by preincubation with sulfite (10^{-2} M) or borohydride ($NaBH_4$, 10^{-2} M), compounds that cleave disulfide (-S-S-) bonds. The inhibition by $NaBH_4$ is partially (40%) reversible by aeration. Thiourea is a competitive inhibitor. The values for K_i20 and K_i40 are 3.5×10^{-6} M and 4.8×10^{-6} M. The enzyme system has been solubilized by sonication of an acetone powder of the cytoplasmic particles. The presence of thiocyanate (10^{-3} M) or iodide (10^{-4} M) during the solubilization is essential to prevent inactivation. Spectroscopic studies reveal a Soret band at 412 mμ, with peaks at 530 and 560 mμ upon reduction with dithionite. The addition of alkaline-pyridine yields a hemochromagen with

peaks at 418, 523, and 555 mμ. The enzyme system is inhibited (90%) by native globin (1.0 mg) and can be reactivated by the addition of hematin (10^{-4} M). This soluble enzyme system not only oxidizes thiocyanate, but is effective in the desulfuration of thiourea and the iodination of organic molecules. These activities are inhibited by similar compounds and are contingent upon the presence of an intact disulfide bond and a heme protein in thyroid tissue. Hence it is proposed that a common enzyme system is involved in all three reactions.

Enhanced Mutagenicity of Virus-bound Carcinogens. CHRISTOPHER M. MARTIN AND DEAN F. GRAY, Jersey City, N. J. (introduced by Harry J. Robinson).

Studies from this laboratory have demonstrated that carcinogenic polynuclear hydrocarbons bind to Poliovirus 2, and that concurrent administration of common human viruses enhances tumor formation by such carcinogens in mice. This phenomenon has been analyzed in simpler biological systems. *In vivo* studies of the mutagenicity of 9,10-dimethylbenzanthracene-1,2 (DMBA) were performed with a strain of *Escherichia coli* B and a virulent coliphage T_5. Bacteria were grown and bacteriophage propagated in an aqueous medium of inorganic salts and 1% glycerol. Although water-insoluble, DMBA existed in glycerol as a finely dispersed, metabolically available colloid. When propagated in bacteria grown in the presence of DMBA-C^{14}, 5 to 500 $\times 10^{-8}$ M, T_5 bound carcinogen in amounts proportional to DMBA concentration—from 12,000 to 970,000 molecules per plaque-forming unit. Roughly 90% was weakly bound to bacteriophage protein, and 10% firmly bound to bacteriophage nucleic acid. Over a range of multiple conditions, T_5 containing firmly bound DMBA significantly increased the rate of formation of T_5-resistant bacterial mutants. DMBA firmly bound to T_5 was 100 times more mutagenic than free or weakly bound carcinogen. *In vitro,* DMBA-C^{14} bound firmly to mammalian and bacterial DNA and RNA and to bacteriophage DNA in concentrations consistent with the intercalating theory of Lerman. DMBA-C^{14} did not bind firmly to nucleotides, oligonucleotides, or denatured nucleic acids. Studies of the effects of bound DMBA on the transforming activity of pneumococcal DNA are in progress. The data suggest that *1*) an intact nucleic acid helix is necessary for firm binding, and *2*) virus nucleic acid-binding significantly enhances the mutagenicity of organic carcinogens. The data are consistent with a hypothesis that viruses may serve as carcinogen vectors.

The Typhoid Carrier State: Quantitative Bacteriology and Preliminary Results of Therapy. JOHN G. MERSELIS, JR., DONALD KAYE, C. STEPHEN CONNOLLY, AND EDWARD W. HOOK,* New York, N. Y.

Ten typhoid carriers (*Salmonella typhosa* present in stool more than 4 years) were studied by quantitative bacteriologic techniques to determine sites of multiplication of *S. typhosa* and the effect of therapy with ampicillin. *S. typhosa* was present in gastric juice in only 3 patients. Duodenal contents were obtained before and after pancreozymin was administered intravenously to produce gallbladder contraction. *S. typhosa* was isolated from duodenal aspirates from 5 of the 10 patients before pancreozymin; in contrast, duodenal contents obtained from every patient after pancreozymin contained *S. typhosa* (10^4 to 10^7 per ml). The number of *S. typhosa* in the postpancreozymin specimen exceeded by 10 times the number in the prepancreozymin specimen in 9 of 10 patients; the patient who showed no increase had had cholecystectomy. Excretion of *S. typhosa* in feces was not intermittent; 31 of 32 stool specimens were positive and 28 contained 10^6 to 10^9 *S. typhosa* per gram. Each patient received 4 to 6 g ampicillin daily for 6 weeks; the titer of *S. typhosa* in daily stool specimens rapidly decreased, and stools were negative for *S. typhosa* by 5 days and remained so for the duration of therapy. *S. typhosa* reappeared in stools of 5 patients by 1, 4, 5, 7, or 9 weeks after ampicillin was discontinued; 1 patient had cholelithiasis and 1 had had cholecystectomy. At present, 5 patients have stools negative for *Salmonella* 1, 1, 7, 17, or 26 weeks after completion of therapy; none have cholelithiasis or have had cholecystectomy. These studies indicate that in chronic typhoid carriers, *S. typhosa* multiplies in the biliary tract and is consistently excreted in large numbers in the stool. Ampicillin rapidly eliminates culturable *S. typhosa* from feces and may occasionally be effective in cure of the typhoid carrier state; additional follow-up studies are in progress.

Release of Histamine Activity by Lysolecithin. ELLIOTT MIDDLETON, JR., AND GERALD B. PHILLIPS,* New York, N. Y.

Previous investigators have noted that lysolecithin caused release of histamine when perfused through dog liver and an anaphylactoid reaction in guinea pigs after intravenous administration. These observations suggest that lysolecithin may be operative in the mechanism of histamine release in other systems, and prompted the following experiments. Lysolecithin was prepared by the action of snake venom (*Naja Naja*) on purified beef brain lecithin. After isolation, it was found to be pure by chromatography on silicic acid-impregnated paper. Saline solutions of this preparation, after Seitz filtration, were analyzed for phosphorus to determine concentration and were shown to be bacteriologically sterile. Intradermal injection in two normal subjects of 0.025 to 0.05 μmole of this lysolecithin preparation in a volume of 0.1 ml resulted in the formation of typical wheal and erythema reactions. When Benadryl (0.5 mg) was injected simultaneously, the reaction was abolished. A comparable effect of the Benadryl was noted on the formation of a wheal and erythema by histamine in the same subjects. These experiments indicate that lysolecithin can act as a histamine liberator in human skin. In other experiments, lysolecithin (0.1 to 0.4 micromole per ml) was incubated with washed human or rabbit

buffy coat (with some erythocytes) in Tyrode's solution for 20 minutes at 37° C. After centrifugation, the lysolecithin-treated preparations showed a red supernatant fluid, indicating hemolysis, and at the bottom of the tube, a gelatinous, stringy, pale material. The control preparation showed a clear supernatant fluid and a button of packed cells. Wright's stain of smears of both preparations showed that the cells exposed to lysolecithin had undergone marked lytic changes within 2 minutes at the higher lysolecithin concentrations. By the bioassay technique, there was evidence that lysolecithin had liberated histamine from the damaged cells.

Effect of Acetylphenylhydrazine on In Vitro Incorporation of Amino Acid Precursors into Erythrocyte Glutathione. AARON MILLER AND MARTHA HORIUCHI, Boston, Mass. (introduced by Belton A. Burrows).

Acetylphenylhydrazine and other Heinz-body hemolytic anemia-producing drugs cause an *in vivo* and *in vitro* fall in erythrocyte glutathione, the fall ascribed to increased glutathione degradation. The effect of acetylphenylhydrazine on *in vitro* biosynthesis of glutathione from amino acid precursors was therefore investigated. Normal erythrocytes were incubated (3 hours) with acetylphenylhydrazine (8×10^{-4} to 1.28×10^{-2} M) and C^{14}-glycine or S^{35}-cystine in phosphate buffer at pH 7.4, and the following were then studied: *a*) glutathione and methemoglobin concentrations, *b*) erythrocyte entry of radioactive amino acids, *c*) specific activity of erythrocyte gluathione, *d*) lactate, sodium, and potassium concentrations of extracellular fluid, and *e*) presence of Heinz bodies. Incorporation of C^{14}-glycine and S^{35}-cystine into glutathione was decreased by acetylphenylhydrazine concentrations as low as 1×10^{-3} M (15 mg per 100 ml) to 2×10^{-3} M (30 mg per 100 ml). Decreasing incorporation was noted with increasing concentrations of acetylphenylhydrazine, i.e., 22 to 33% of controls at 6.4×10^{-3} M, and 32 to 54% of controls at 1.28×10^{-2} M. Glutathione concentrations were unaffected by concentrations up to 6.4×10^{-3} M. A decreased entry of C^{14}-glycine and S^{35}-cystine into erythrocytes was found, the decrease paralleling their decreased incorporation into glutathione. When erythrocytes were preincubated (2 hours) with C^{14}-glycine or S^{35}-cystine and then washed and reincubated (3 hours) with acetylphenylhydrazine (8×10^{-3} to 1.0×10^{-2} M), the content of labeled amino acids and their incorporation into glutathione were similar to their control. Acetylphenylhydrazine also caused decreased erythrocyte entry of α-aminoisobutyric acid, a nonmetabolizable amino acid. Methemoglobin appeared at concentrations of 3.2×10^{-3} M and above, Heinz bodies at 8×10^{-3} and above. A leak of erythrocyte potassium and an effect on lactate production was not observed at any drug concentration. It is concluded that: *1*) acetylphenylhydrazine damages the erythrocyte membrane, reducing its permeability to amino acids; *2*) the decrease in radioactive amino acid incorporation into glutathione in acetylphenylhydrazine-exposed erythrocytes is due to the decreased cellular entry of amino acids rather than to impaired biosynthesis of glutathione; and *3*) the membrane change is the first abnormality detected by low levels of acetylphenylhydrazine and may play a role in the *in vivo* destruction of drug-exposed erythrocytes.

Abnormal Antibody Production, Autoimmune Disease, and Allergic Disease in Patients with Lymphosarcoma and Chronic Lympocytic Leukemia. DANIEL G. MILLER, New York, N. Y. (introduced by Martin Sonenberg).

In studying the course of patients with lymphosarcoma and chronic lymphocytic leukemia for infectious complications, it became apparent that autoimmune and allergic diseases were also prominent. The autoimmune diseases included rheumatoid arthritis and other connective tissue diseases, hemolytic anemia, and thrombocytopenic purpura. The allergic processes included dermatitis, allergic purpura, and anaphylactic reactions. In contrast to patients with frequent infection, those with autoimmune and allergic disease had a tendency to high serum γ-globulin levels, and in some, progression of the lymphoproliferative disease led to further elevation of γ-globulin. Nine of the thirty-five patients studied had γ-globulin levels over 1 g per 100 ml; all had autoimmune or allergic processes, and eight produced abnormal antibodies. Thirteen patients had γ-globulin levels below 0.6 g per 100 ml; five had autoimmune or allergic complications, and three produced abnormal antibodies. One additional patient with disseminated lupus erythematosus and low serum γ-globulin had marked proteinuria. Of the entire group, twenty-two manifested autoimmune or allergic diseases. The leukocytes of four patients were coated with globulin, as demonstrated by fluorescein-labeled antihuman globulin. Seven patients had positive Coombs tests, and four had elevated titers of cold agglutinin. Platelet agglutinin was found in four patients, leukocyte agglutinin in five. Leukocyte preparations for the lupus phenomenon were positive in two patients and suspicious in four. Serological confirmation for lupus erythematosus was obtained for two patients, and autopsy confirmation for one. Six patients had positive latex fixation tests in low titers. The presence of abnormal antibodies did not always correlate with the clinical findings.

Binding of Triiodothyronine-I^{131} by Inter-Alpha-Globulins in Thyroid Disease and in Pregnancy. MARVIN L. MITCHELL, ANNE H. BRADFORD, AND SONIA COLLINS, Boston, Mass. (introduced by Joseph M. Hayman, Jr.).

Quantitative differences in the binding of I^{131}-labeled thyroid hormones by sera from patients with thyroid disease have been demonstrated previously, using erythrocytes or resin to compete with the plasma proteins for the radiohormone, *in vitro*. Earlier studies had shown that triiodothyronine-I^{131} (T-$3I^{131}$) was associated in part with the inter-α-globulins from which the T-$3I^{131}$ could be displaced by the addition of stable thyroxine. Therefore, it seemed that a procedure might be developed based upon the change in the distribution of T-$3I^{131}$ between the inter-α-globulins and the remainder of the

serum proteins that would reflect qualitative as well as quantitative differences in thyroid function. Sera from 67 normal controls, 32 hyperthyroid patients, 15 hypothyroid patients, and 11 pregnant women, containing T-3I^{131}, were subjected to zone electrophoresis on Whatman 3 filter paper in barbital buffer at pH 8.6. The partition of T-3I^{131} among the protein fractions was determined by monitoring the paper strip with a scanner connected to a continuous recorder, and then the areas under the recorded curves were integrated, and the fraction of radioactivity bound to the inter-α-globulins was expressed as percentage of the total radioactivity on the strip. The inter-α-T-3I^{131} binding (T-3IAB) was significantly reduced or absent in hyperthyroidism (mean T-3IAB value, $19.4 \pm 14.4\%$ [1 SD]) and increased both in myxedema and in pregnancy (mean T-3IAB values, respectively, $70.4 + 8.3\%$ and $65.6 + 9.0\%$) when compared to those of the normal controls (mean T-3IAB value, $42.6 + 8.7\%$; $p < 0.01$ in the comparisons). Enrichment of serum from normal subjects with stable thyroxine to levels exceeding 30 μg per 100 ml was necessary in order to obtain the decreased T-3IAB values seen with serum from most of the untreated hyperthyroid patients. Successful treatment of thyrotoxic patients with antithyroid materials, or with radioiodine increased the previously low T-3IAB values to those of the normal.

Phosphate Transport by Suspensions of Tubules from Rat Kidney Cortex. LEONARD A. MOROZ AND STEPHEN M. KRANE, Boston, Mass. (introduced by Marian W. Ropes).

Interest in the renal handling of phosphate has prompted a search for *in vitro* preparations providing intact tubular cells free of stroma. From collagenase-treated rabbit kidney cortex, Burg and Orloff prepared suspensions of convoluted tubules and tubular fragments with greater oxygen consumption and para-aminohippurate accumulation than tissue slices. In the present study, this technique was used to examine orthophosphate (P_i) transport by rat kidney cortex. Tissue suspensions were incubated aerobically at 25° C in Krebs-Ringer bicarbonate buffer at pH 7.4 containing P_i^{32}. Radioactivity in the trichloroacetic acid-soluble tissue fraction was determined. Uptake of P^{32} proceeded rapidly, approaching equilibrium after 30 minutes, when wet tissue concentrations averaged 2.5 times the concentration in the medium (1.0 mM P_i). The P^{32} in the acid-soluble fraction reacted almost entirely as P_i by precipitation as magnesium ammonium phosphate at alkaline pH. Accumulation of P_i was abolished by incubation at 2° C and by preheating the tissue to 60° C and was inhibited by anoxia and by metabolic inhibitors (dinitrophenol, iodoacetate, fluoride, azide, $HgCl_2$, phlorizin, and *p*-chloromercuribenzoate). Glucose in the medium was without effect. Accumulation against higher gradients (6:1) occurred at lower phosphate concentrations (0.25 mM P_i); at higher medium levels, a saturation effect was observed. Tissue accumulation of P_i was abolished by substituting lithium, choline, or potassium for sodium in the medium. Uptake was restored at sodium levels above 40 mM. P_i accumulation in this system has many features of active transport: dependence upon metabolic integrity of the tissue, temperature effect, saturation kinetics, and movement against apparent concentration gradients. In addition, there appears to be an absolute requirement for sodium in the medium.

Effects of Variation in Sodium Chloride Intake on the Acid-Base Balance of Patients with Renal Tubular Acidosis (RTA). R. CURTIS MORRIS, JR., AND E. AUDIOUN, San Francisco, Calif. (introduced by Malcolm McIlroy).

In renal tubular acidosis, diminished hydrogen ion excretion leads to acidosis, reduction of serum HCO^-_3, and hyperchloremia. To test whether NaCl balance per se might affect the hyperchloremic acidosis, the NaCl intake of a 55-year-old white woman with the characteristic biochemical features of renal tubular acidosis was varied over a wide range under balance conditions. On a daily NaCl intake of 55 mEq, NaCl balance and weight were maintained; the hyperchloremic acidosis was corrected, as measured by arterial pH, CO_2 content, and Cl; the urinary calcium excretion diminished from > 200 to < 75 mg per day; and the bone pain associated with roentgenographically demonstrated osteomalacia disappeared. In 3 separate balance studies on a daily NaCl intake of 166 mEq per L, a stable, partially compensated hyperchloremic acidosis occurred, as indicated by an arterial pH of 7.35, HCO^-_3 of 17.3 mEq per L, pCO_2 of 32.5 mm Hg, and Cl of 114 mEq per L. On a daily intake of 66 mEq of NaCl, a lesser degree of hyperchloremic acidosis occurred, as indicated by measured arterial pH of 7.37, pCO_2 of 36.3 mm Hg, HCO^-_3 of 20.7 mEq per L, and Cl of 109 mEq per L. On a daily NaCl intake of 50 mEq, weight and normal blood pH were maintained, but hyponatremia resulted, as indicated by serum Na ranging from 131 to 134 mEq per L. An infusion of normal saline resulted in a fall in arterial blood pH from 7.44 to 7.38 and a fall in pCO_2 from 38.4 to 34.5 mm Hg. In neither the chronic nor acute studies did changes in the measured rate of urinary excretion of titratable acidity and ammonia account for the changes in blood acidity. We interpret these findings to mean that the NaCl balance per se is a determinant of acid-base balance in patients with renal tubular acidosis.

Identification of Complement Antigens Coating Red Cells in Acquired Hemolytic Anemia. H. J. MÜLLER-EBERHARD,* H. FUDENBERG, M. HARBOE, AND P. L. MOLLISON, New York, N. Y.

Red cells of certain patients with acquired hemolytic anemia are coated *in vivo* with serum components other than γ-globulin. Such cells can be agglutinated with an antiserum to whole human serum, but often fail to react with anti-γ-globulin serum. Recently, it has been possible to obtain specific antisera to two individual human complement components with the aid of which the nature

of the non-γ-globulin material could be elucidated. One of the antisera is directed against β_{1C}-globulin, a serum protein that has previously been obtained in highly purified form and that is a moiety of the third component of complement. The other antiserum is directed against β_{1E}-globulin, a heretofore unrecognized serum constituent. β_{1E}-Globulin was isolated by a combination of chromatography and preparative electrophoresis and was found to represent the fourth component of hemolytic complement. Both β_{1C}- and β_{1E}-globulin proved to be highly antigenic proteins. *In vitro* studies utilizing human red cells and human antibody showed that both proteins become firmly attached to cells in the course of a typical complement reaction, with β_{1E} at the reaction step involving the fourth component of complement and β_{1C} during the step of the third. β_{1C}- and β_{1E}-globulin could be identified as the non-γ-globulin material coating cells of patients with acquired hemolytic anemia and as the two main antigens responsible for the non-γ-globulin Coombs reaction. Addition of small amounts of both proteins virtually abolished agglutination by an antiserum to whole serum.

Angiotensin Concentrations in Plasma of Sodium-depleted Humans. PATRICK J. MULROW,* NANCY A. POWELL, AND RICHARD L. KAHLER, New Haven, Conn.

Indirect evidence in the dog and rat suggests that sodium depletion stimulates aldosterone secretion through the renin-angiotensin system. The present study investigated whether sodium depletion *in man* stimulated aldosterone secretion through this system by measuring plasma concentrations of angiotensin during sodium deprivation. The angiotensin method involved rapid cooling of 65 to 280 ml of shed blood, separation of plasma at −2° C, and precipitation of proteins with trichloroacetic acid. After extraction with ether, the supernatant fluid was chromatographed on two successive resin columns. The angiotensin activity in this purified extract was estimated by a rat pressor bioassay that was sensitive to 0.2 mμg angiotensin. This method gave a mean recovery of 43 ± 6% SE when 10 to 500 mμg aspartyl angiotensin II was added to 9 whole blood specimens. Infusions of small doses of aspartyl angiotensin II (0.23 and 0.36 μg per minute) into 2 normal subjects, sufficient to raise systolic blood pressure 9 and 12 mm Hg, resulted in angiotensin plasma concentrations (95% confidence limits) of 5 to 7 and 7 to 8 mμg per 100 ml, respectively. Arterial or venous plasma concentrations of angiotensin in 15 control subjects ranged from 1 to 6 mμg per 100 ml (mean 2.6 ± 0.38 SE). Arterial plasma concentrations in 9 subjects after 4 to 8 days on a 10 mEq sodium diet ranged from 1 to 5 mμg per 100 ml (mean 2.7 ± 0.32 SE). These concentrations in the sodium-depleted subjects were not significantly different from the control concentrations, yet the subjects excreted increased amounts of aldosterone, 18 to 49 μg per 24 hours. These data suggest that salt depletion *in man* stimulates aldosterone secretion by a mechanism different from the renin-angiotensin system.

Movement of Inulin and Bicarbonate Ion across the Bladder of an Aglomerular Teleost, Lophius americanus. H. VICTOR MURDAUGH, JR., PETER SOTERES, WILLIAM PYRON, AND EDWARD WEISS, Birmingham, Ala., Pittsburgh, Pa., and Salisbury Cove, Me. (introduced by Jessica H. Lewis).

Studies of renal function in aglomerular fish have contributed greatly to modern concepts of renal physiology. Such studies have assumed that substances appearing in urine result solely from renal activity. Recent studies reporting excretion of carbohydrates like inulin and pentoses in urine of aglomerular fish have been disturbing. In a study of the renal response to HCO_3^- infusion in the aglomerular teleost *Lophius americanus,* it was noted that both HCO_3^- and inulin appeared in bladder urine after intravascular administration. Solutions of inulin and HCO_3^- were instilled into the bladder in order to determine whether the urinary bladder in this species was permeable to these substances. Under these conditions, inulin and increased HCO_3^- concentrations were found in plasma. This suggested that the inulin and HCO_3^- found in urine after intravascular injection may have resulted from direct penetration of the bladder without renal mediation. This hypothesis was tested by cannulating one ureter to obtain renal urine and ligating both ureters above the bladder. Previously collected *Lophius* urine was instilled into the bladder. Inulin and HCO_3^- were given intravascularly; under these conditions, they appeared in the bladder urine, whereas the composition of renal urine was unchanged. These data establish that the urinary bladder of *Lophius* is capable of altering urinary composition and suggest that previous studies assuming that urinary composition results solely from the function of *Lophius* kidney require re-evaluation. Bladder contributions to urine in mammals should likewise be investigated.

Evidence of a Biochemical Basis for the Involuting Effect of Iodine in Graves's Disease. SHIGENOBU NAGATAKI AND SIDNEY H. INGBAR,* Boston, Mass.

In hypophysectomized animals, the hormonogenetic response to exogenous thyrotrophin (TSH) varies inversely with the thyroidal content of organic iodine. Experiments were undertaken to determine whether this alteration in sensitivity could be ascribed to changes in glandular intermediary metabolism. Diverse aspects of thyroidal intermediary metabolism were studied *in vitro* in thyroids of hypophysectomized rats. Animals had previously been given either no TSH or standard doses of this hormone *in vivo.* In addition, glandular organic iodine content had been increased by addition of iodide, or decreased by addition of propylthiouracil or perchlorate to the diet. Variations in glandular organic iodine greatly altered the thyroidal metabolic response to TSH. Thus, dietary iodine supplements decreased, while propylthiouracil, or perchlorate, or both, increased: *1)* oxygen consumption, *2)* total glucose assimilation, *3)* conversion of uniformly labeled glucose to CO_2, lactic acid, lipid, and nucleic acid, *4)* formation of pyrimidine

nucleotides from labeled orotic acid, and *5*) incorporation of labeled amino acids into thyroprotein. In thyroids of hypophysectomized animals given no TSH, effects of changes in glandular iodine content on glucose metabolism were similar, though less striking. Thus, the thyroidal content of organic iodine broadly influences the basal intermediary metabolism of the thyroid and alters its responsiveness to TSH stimulation. Glandular growth, thyroglobulin synthesis, and iodine metabolism are dependent upon thyroidal oxidative metabolism. Therefore, the findings may explain the inverse relationship between thyroidal organic iodine content and the hormonogenetic response to TSH. The data further suggest that when hormone synthesis is impaired and glandular organic iodine declines, the resulting increase in oxidative metabolism facilitates adaptive goitrogenesis as well as hormone formation. Finally, inhibition of anabolic and energy-generating processes and decreased production of acid metabolites after diets high in iodine may respectively explain the involution of hyperplasia and reduction of vascularity that iodine induces in the toxic goiter of Graves's disease.

Clotting Properties of Water-insoluble Thrombin. T. F. NEWCOMB AND Q. Z. HUSSAIN, Gainesville, Fla. (introduced by S. P. Martin).

Water-insoluble thrombin is a valuable tool because it can be added to or removed from clotting mixtures rapidly and quantitatively by physical means. It is prepared by coupling thrombin to a copolymer of phenylalanine and leucine, or by coupling prothrombin to the copolymer followed by thromboplastic activation. The insoluble thrombin has esterase properties similar to soluble thrombin; it fails, however, to clot fibrinogen solutions. That this is partly because of the physical properties of the copolymer particle is demonstrated as follows. *1*) Addition of insoluble thrombin to platelet-rich plasma accelerates O_2 consumption, induces viscous metamorphosis, and results in fibrin formation when observed microscopically. These changes occur first around the polymer particle. *2*) Fibrinogen exposed to insoluble thrombin will gel in the cold (4° C) in a fashion similar to the formation of cryoprofibrin by fibrinogen cleavage with limited amounts of thrombin. *3*) Insoluble thrombin is mixed with fibrinogen under conditions that allow splitting of the fibrinogen, but prevent polymerization of the fibrin monomer (1 M NaBr, pH 5.3). After 30 minutes at 37° C, the insoluble thrombin is removed. Fibrin appears after the conditions are changed to allow polymerization (restoring the ionic strength to 0.154 by dialysis and the pH to 7.6). We conclude that insoluble thrombin is able to induce clotting. The weak activity is in part related to the physical state of the thrombin and might be due to occlusion of the particle surface by fibrin as it is first formed. Since coupling to the copolymer is thought to occur through the aromatic amino acids of the thrombin, it may be that these are not essential for thrombin's enzymatic activity. Since this macroscopic particle has thrombic activity, it can be used to study intermediate reactions in clotting, heretofore inaccessible to direct analysis.

Chylous Joint Effusion: A Study of Intra-articular Lipid Synthesis. DAVID S. NEWCOMBE AND ALAN S. COHEN, Boston, Mass. (introduced by Robert W. Wilkins).

Remarkably few analyses have been carried out on synovial fluid lipids. Only recently have increased total cholesterol (124 mg per 100 ml) and phospholipids (101 mg per 100 ml) in joint effusions of patients with rheumatoid arthritis as compared to normal fluids (cholesterol = 7 mg and phospholipid = 14 mg per 100 ml) been reported. The startling demonstration of a massive chylous knee effusion in a patient with chronic rheumatoid arthritis led to detailed chemical and electron-microscopic studies of the phenomenon of lipid segregation in the synovial space. Light and polarization microscopy of the chylous fluid disclosed typical birefringent cholesterol crystals and Sudan III-positive droplets. Initial total synovial fluid cholesterol and phospholipid values were 1,236 and 228 mg per 100 ml, respectively, while serum lipids were normal. A nonchylous effusion in the opposite knee of the same subject had levels of 67 and 78 mg per 100 ml, respectively. After intra-articular sodium acetate-1-C^{14} injection into the chylous joint, synovial fluid and parallel serum samples were removed at specific intervals for 4 weeks for analysis of the various lipid moieties. The early appearance and greater magnitude of radioactivity in the total lipid fraction and in the digitonin-precipitated fraction of the chylous fluid (as compared to the parallel serum samples) indicated local fatty acid and sterol synthesis. Electron-microscopic studies of a synovial membrane biopsy specimen obtained from the chylous joint showed large dense osmiophilic intracellular inclusions. These chemical and electron-microscopic data suggest that intra-articular accumulation of lipids may be explained by local synthesis as well as by diffusion and segregation of serum lipids as previously proposed.

Evolution of Delayed Hypersensitivity Observed in the Electron Microscope with Ferritin-conjugated Tuberculin Antigen. WALTER L. NORTON AND MORRIS ZIFF,* Dallas, Tex.

The mechanism of delayed hypersensitivity is unknown; high affinity and "cell-bound" antibody have been suggested, and evidence for the selection of specifically sensitized, circulating lymphocytes at the skin test site is conflicting. To establish the subcellular localization of antigen in the delayed reaction, a conjugate of ferritin and purified protein derivative of *M. tuberculosis* (PPD) was prepared. Tuberculin-sensitive guinea pigs were simultaneously skin-tested with PPD-ferritin conjugate and unconjugated PPD mixed with ferritin. Test sites were excised at intervals and sections viewed in the electron microscope. Uptake of conjugated ferritin into macrophages and pericapillary cells occurred within 15 minutes and was complete within 8 hours. Uptake of unconjugated ferritin in the control sites tested with free

PPD occurred in a similar manner, as recently noted in another test system. At 8 hours, significant degeneration of macrophages was observed, and at 24 hours, extracellular ferritin reappeared in the immediate area of fragmented macrophages in both the conjugated and unconjugated PPD test sites. Cell fragments containing ferritin were associated with intact granulocytes. Lymphocytic infiltration began at about 24 hours. Endothelial cells were free of ferritin, and at no time was there endothelial separation, as has been described in the immediate type of skin reaction. These experiments demonstrate that: *1*) there is an initial, nonspecific cellular uptake of the antigen in the delayed hypersensitivity reaction, *2*) extracellular antigen disappears almost completely during the initial stages of the reaction, and *3*) with macrophage degeneration, there is reappearance of antigen extracellularly coincident with onset of the inflammatory reaction. These findings suggest that the initial step in "recognition" is carried out by the tissue macrophage. The subsequent appearance of lymphocytes at the site may well be mediated by some product originating in the macrophage, rather than by unaltered antigen.

A Study of Staphylococcal Carriers in Normal Families. ALVIN NOVACK AND HARRY A. FELDMAN,* Syracuse, N. Y.

A population of "normal" families has been under continuous surveillance for 45 months to determine the relation of age, sex, and season to the nasal carriage of coagulase-positive *Staphylococci* (CPS). Nasal cultures have been obtained seasonally each year from all subjects. The antibiotic sensitivities and bacteriophage types of the 1,430 (35%) CPS isolated from the total of 4,143 cultures have been determined. The highest carrier rates (45%) were noted in school-age children, and the lowest (19%) in those over 30 years. Highest carrier rates were noted during the summer (44%), and the lowest (29%) during the winter. The CPS were generally sensitive to commonly used antibiotics, especially penicillin G (93%). By bacteriophage typing, 278 (21%) were NT, 298 (23%) were group III, 109 (8%) were "80/81," and the remainder were scattered through a variety of groups. The antibiotic sensitivities of the various phage groups were similar except that group III and "80/81" strains were less sensitive to penicillin. Among the 192 persons who provided cultures for from 21 to 39 months, 25 (13%) never were positive for CPS, and 68 (35%) were positive less than 25% of the time. Of the remainder, 78 (41%) were positive 25 to 74% of the time, and 21 (11%), 75 to 100% of the time. The latter, continuous carriers, tended to carry one phage type. Intermittent (25 to 74%) carriers often harbored different types at different times. Family groups behaved like individuals in these respects and appeared to be bacteriologically homogeneous. Carrier rates were not related to sex. Thus, age, season, and family staphylococcal prevalence appear to be important determinants of the staphylococcal carrier state. Nonhospital-acquired *Staphylococci* in this community are almost uniformly sensitive to pencillin G.

Plasmas Haptoglobin Kinetics, WARD D. NOYES AND LARS GARBY, Gainesville, Fla. (introduced by George T. HARRELL).

Data on haptoglobin turnover has been obtained from six normal adults. After an intravenous infusion of 1 to 4 g of hemoglobin, the major portion of plasma haptoglobin was complexed and removed from circulation. The subsequent slow rise in plasma haptoglobin concentration was observed as an index of its rate of synthesis and of catabolism. Similar experiments were conducted on dogs with cannulation of the thoracic duct and in humans with repeated samples of ascitic fluid as an estimate of extravascular haptoglobin concentration. Haptoglobin was determined by the method of Connell and Smithies, and haptoglobin phenotype by disc electrophoresis. These studies indicate that lymph or that portion of extravascular space represented by ascites does not act as a precursor for plasma haptoglobin, but maintains a slow exchange with the circulating pool. After depletion of plasma haptoglobin, there is a return to baseline levels in 5 to 8 days, with a value of 50% reached within 36 to 40 hours. Previous data on plasma hemoglobin kinetics indicate that normally somewhat less than 0.5 g of haptoglobin is destroyed daily by this pathway. This would now appear to represent some 25 to 50% of the total daily haptoglobin catabolism. A two- to threefold increase in hemolysis therefore might be anticipated to lower haptoglobin levels, consistent with similar figures obtained from clinical studies in other laboratories. The detection of brief episodes of hemolysis by lowered haptoglobin levels would be possible only for 2 to 3 days, since the return towards normal is so prompt. These kinetic studies emphasize the value of understanding haptoglobin physiology in relation to clinical problems.

Increased Thyroid Function and Choriocarcinoma. WILLIAM D. ODELL, ROBERT W. BATES, RICHARD S. RIVLIN, MORTIMER B. LIPSETT,* AND ROY HERTZ, Bethesda, Md.

Thyroid function was assessed in 94 patients with metastatic trophoblastic disease. Seven had laboratory evidence of increased thyroid function. There was uniform elevation of 24-hour radioiodine uptake (range +46 to 82%), serum protein-bound iodine (9.2 to 17.0 μg per 100 ml), and BMR (+7 to +75%), and the serum cholesterol (109 to 150 mg per 100 ml) was depressed. Clinical evidence of hyperthyroidism was either not present or minimal, and thyroid gland size was normal. On admission, these 7 patients had 24-hour urinary gonadotropin excretions ranging from 2 to 10×10^6 mouse uterine U. Approximately 50% of the patients without abnormalities in thyroid function, however, had gonadotropin excretions greater than 1×10^6 mouse uterine U. Plasma thyrotropin (TSH) levels were determined in 2 patients and found to be above the average of 3 mμ per

100 ml (10 and 13 mμ); assay of the tumor from 2 patients revealed TSH activity (40 and 300 mμ per 100 g) greater than equivalent amounts of blood. Chorionic gonadotropin was shown by bioassay to be devoid of TSH activity. In 4 patients whose tumors responded to treatment with chemotherapy, the thyroid function tests returned to normal. It is postulated that these alterations in thyroid function stem from production of a substance with TSH activity by the tumor tissue and that this represents another example of the varied hormonal syndromes associated with a variety of neoplasms.

The Extracellular Space in Brain. W. WALTER OPPELT, CLIFFORD S. PATLAK, AND DAVID P. RALL, Bethesda, Md. (introduced by C. Gordon Zubrod).

The extent of extracellular space (ECS) in brain is vital to the problem of transfer of metabolites and drugs from blood to nerve cells. The 150-A intercellular spaces seen in electronmicrographs represent an ECS of 2 to 4 %. This is too small to permit free diffusion and would therefore require specific transcellular transport of most substances. To investigate this problem, ECS in brain was determined in anesthetized Rhesus (*M. mulatta*) and Green (*C. aethiops sabaeus*) monkeys and cats (*F. domesticus*) by analysis of the entry of inulin from the ventricular system into the brain. Synthetic CSF with inulin-C^{14} was perfused from a lateral ventricle to the cisterna magna for 2 to 4 hours. After sacrifice, the brain was removed, and 3-mm coronal sections were made through caudate nucleus, thalamus, and pons. Serial blocks of tissue about $3 \times 3 \times 1$ mm were taken on a perpendicular axis from the ventricular surface, and radioactivity was determined in each. Inulin decreased as a function of distance from the ventricle in a manner consistent with free diffusion of inulin through a liquid. Negligible inulin entered blood. ECS by this method was estimated to be 9 to 13% for the three areas analyzed. The discrepancies between these data and the 2 to 4% ECS seen in electronmicrographs might be due to a rapid shift of water into the cells after death. Similar experiments therefore were performed in freshly killed animals. These showed an ECS of 5 to 6%, suggesting that the 150-A space in electronmicrographs may be artifactually small. These various observations confirm our preliminary experiments with similar inulin perfusions in the dog and suggest that the ECS in the live brain is around 10%, an amount sufficient for diffusion of molecules from blood to nerve cell.

Role of an Abnormal, Myeloma-Type, Serum Gamma Globulin in the Pathogenesis of the Skin Lesions of Papular Mucinosis (Lichen Myxedematosus). ELLIOTT F. OSSERMAN * AND KIYOSHI TAKATSUKI, New York, N. Y.

Papular mucinosis is a rare, chronic, progressive skin disorder of obscure etiology. The lesions initially are pruritic, erythematous papules superimposed upon a brawny induration (myxedema) of the underlying and surrounding subcutaneous tissues. With relentless progression over the course of months or years, the skin of the head, trunk, and extremities becomes markedly thickened, furrowed, and thrown into heavy, disabling, and disfiguring folds. Histologically, the lesions are characterized by infiltration of the upper corium with mucicarmine-staining material associated with fragmentation and disorganization of collagen bundles. Systemic manifestations are usually absent. In one case reported by Perry, Montgomery, and Stickney, the coexistence of multiple myeloma was considered coincidental. Studies of a 46-year-old Caucasian woman with papular mucinosis of 13 months' duration revealed the presence in the serum of an exceptionally basic (pH 8.1), myeloma-type, electrophoretically homogeneous protein (350 mg per 100 ml) that migrated in paper and agar electrophoresis (pH 8.6) in the post-gamma region. The abnormal protein was further characterized as a euglobulin (Sia water dilution test, positive); sedimentation constant, 7 S; immunological type, gamma-2 (Korngold; Mannik and Kunkel, group 2). Normal serum gamma globulin concentration was at the lower limit of normal (900 mg per 100 ml). There was no Bence Jones proteinuria. Marrow aspiration demonstrated an increase in plasma cells with some abnormal forms. Skeletal X rays were negative. Immunohistochemical studies of the patient's skin demonstrated that the infiltrated superficial portions of the corium stained intensely and specifically with fluorescein-labeled rabbit antihuman gamma-2 globulin. It is postulated that the fundamental disorder in papular mucinosis is a plasmocytic dyscrasia and that the dermal infiltrates represent insoluble conjugates of an abnormal gamma globulin (exceptionally basic euglobulin in the present case) and constituents (possibly, acidic mucopolysaccharides) of the connective tissue ground substance.

Lung Volume and Pulmonary Vascular Resistance in Man. H. W. PALEY AND JOHN BUTLER, San Francisco, Calif. (introduced by Meyer Friedman).

In previous work, we demonstrated a marked rise of pulmonary vascular resistance (PVR) in response to an increased functional residual capacity (FRC) in man. To evaluate the mechanism of this increase in PVR, ten healthy male subjects in the sitting position were studied in *1*) the control state, *2*) with a reduced FRC (−0.9 L, −15% of vital capacity determined by body plethysmography) induced by negative-pressure breathing at −6.6 mm Hg, and *3*) with an increased FRC (+1.3 L, +26% of vital capacity, determined from spirometry) induced by a chest cuirass. Pressures from the pulmonary artery (Pa), Pa "wedge" (Paw), and an esophageal balloon (Pes) were recorded using strain gauges at the level of the sternal angle. Cardiac output (CO) was determined by indicator dilution (indocyanine green) using central injection and arterial sampling through a cuvette densitometer. PVR was calculated as (Pa − Paw) mm Hg/CO (L per minute) and we assumed an average pulmonary vascular pressure (APVP)

as (Pa + Paw) mm Hg/2. There was no significant change of CO (6.0 ± 1.0 L per minute) with the small FRC or large FRC (5.4 ± 0.96 L per minute) compared with control (5.9 ± 1.1 L per minute). Control Pes was −5.4 ± 0.7 mm Hg and Pa was 4.9 ± 1.8 mm Hg. Pes decreased with small FRC (−10.2 ± 1.6 mm Hg) and Pa fell (−0.5 ± 2.5 mm Hg). With large FRC, however, Pes fell (−12.0 ± 1.9 mm Hg), but Pa rose (5.4 ± 3.0 mm Hg). The following analysis supports the concept that there are two vascular "compartments" in the pulmonary vascular system, one exposed to intrapleural pressure and the other, which is the site of increased PVR with the large FRC, to alveolar pressure. When APVP was plotted with airway (alveolar) pressure, a distending transmural gradient (TMG) was observed during control (0.6 ± 1.3 mm Hg) and small FRC (2.1 ± 1.7 mm Hg), but a collapsing TMG (−0.7 ± 2 mm Hg) with large FRC. These changes could account for the marked rise of PVR (2.5 ± 0.63 U, $p < 0.005$) with large FRC compared with small FRC (1.4 ± 0.44 U) and control (1.3 ± 0.34 U).

Serological Studies in Penicillin Allergy. C. W. PARKER AND J. A. THIEL, St. Louis, Mo. (introduced by H. N. Eisen).

Sera from more than 100 patients with penicillin allergy have been evaluated by the hemagglutination assay. Red cells were prepared by incubation with penicillin in barbital buffer followed by thorough washing. The majority of sera from allergic subjects had positive reactions (a dilution of 1:16 or higher). Positive reactions with sera from "normal" subjects were unusual. Detailed hapten inhibition studies indicated that the red cell agglutination was inhibited most effectively by penicilloyl-α-amides. The presence of antibodies specific for penicilloyl in sera of subjects allergic to penicillin was confirmed by an independent assay utilizing a radioactive antigen. I^{131}-labeled penicilloyl-bovine serum albumin was incubated with various human sera. The amount of radioactivity precipitated after the subsequent addition of an excess of rabbit antihuman gamma globulin afforded an estimate of the amount of penicilloyl-protein specifically bound by human gamma globulin. The precipitation of labeled antigen with sera from patients with penicillin allergy varied from 8 to 75% of total radioactivity (normal control values = 5 to 10%). The coprecipitation of radioactivity was inhibited by penicilloyl-α-amides in low concentration. The relative effectiveness of various derivatives (penicilloyl, penicillin, and penicillinate) in inhibiting precipitation of radioactivity or hemagglutination correlated well with the results of hapten inhibition of the precipitin reaction between rabbit and antipenicilloyl antibody and penicilloyl proteins. These findings, taken in combination with cutaneous studies with penicilloyl-polylysine, provide strong evidence that antibodies specific for the penicilloyl group are very commonly present in humans with penicillin allergy. Although there is a significant relationship between the results of the cutaneous and the serological assays, in some subjects the serological assays do not correlate with one another, or with the degree of cutaneous reactivity to penicilloyl-polylysine.

Platelet Preservation Studies Using Platelets Labeled with C^{14}-5-Hydroxytryptamine. E. JOHN PARKER-WILLIAMS, PHIN COHEN, PATRICIA WATROUSE, AND FRANK H. GARDNER,* Boston, Mass.

Platelet life-span and preservation studies have been undertaken with C^{14}-serotonin (C^{14}-5HTA). Normal human volunteers were bled into disodium ethylenediaminetetracetic dihydrate (Na_2EDTA), citrate phosphate dextrose (CPD), and acid citrate dextrose (ACD) anticoagulants. Whole blood was labeled and stored at 4° C for varying periods before autologous transfusion. Platelets collected in Na_2EDTA had lower specific activity for Cr^{51} or C^{14} than those collected in ACD or CPD as anticoagulant. After transfusion, platelet sequestration was more marked with Na_2EDTA than with ACD or CPD. Fresh whole blood labeled with C^{14}-5HTA had a plateau of radioactivity after day 5, with greater than 20% radioactivity after 10 days. The life-span curves of platelets stored in whole blood for all intervals up to 48 hours (26 experiments) were identical in form and duration with those of fresh blood. Direct *in vivo* labeling by intravenous injection of C^{14}-5HTA gave life-span curves similar to those obtained with the infusion of labeled whole blood stored for 48 hours. Platelet concentrates prepared from whole blood collected in Na_2EDTA showed a prolonged plateau of radioactivity after day 5. Platelet concentrates prepared in ACD or CPD invariably showed gross clumping of the platelets, unless Triton or EDTA were added to the platelet-rich plasma before centrifugation to prepare the platelet concentrate. This study provides substantial evidence for *in vivo* reutilization of C^{14}-5HTA. Despite the physiologic advantage possessed by C^{14}-5HTA, the problem of reutilization invalidates its use for determining platelet life-span. The reproducibility of the Cr^{51} technique makes it a better technique for studies of thrombocytopenic states and platelet preservation.

The Differential Effects of Na^+ and K^+ on Active and Nonactive Membrane Transport in Striated Muscle. JAMES E. PARRISH AND DAVID M. KIPNIS,* St. Louis, Mo.

The active transport (i.e., accumulation against a chemical gradient) of sugars by intestinal and renal tubular epithelium is Na^+-dependent. In the present study, the effects of both Na^+ and K^+ on an *active* carrier-mediated transport system (i.e., amino acid transport) and a *nonactive* carrier-mediated process (i.e., sugar transport) have been examined using the isolated, intact, rat diaphragm preparation. Amino acid transport was studied with α-aminoisobutyric acid and glycine; sugar transport, with 2-deoxyglucose and galactose. Concomitant measurements were made of insulin responsiveness and the intracellular/extracellular distribution of

tissue water. The iso-osmotic replacement of Na^+ with either Tris-chloride or choline-chloride, or inhibition of the Na^+ pump with strophanthin K (1×10^{-3} M) did not affect the sugar transport system or its insulin responsiveness. The active transport of amino acids, however, and its acceleration by insulin progressively diminished with decreasing [Na^+] and was totally abolished in the absence of Na^+. The diffusion component of amino acid transport was unaffected. Similar results were obtained with strophanthin. Iso-osmotic replacement of Na^+ did not significantly alter either tissue water content or distribution. Increasing [K^+], 10 to 150 mM, progressively inhibited and eventually abolished both sugar and amino acid transport and insulin responsiveness. In high K^+ isotonic medium (150 mM), the cell became impermeable to 2-deoxyglucose and α-aminoisobutyric acid, remained impermeable to sucrose and inulin, but developed a marked increase in intracellular water (30%). These data indicate that *1*) *nonactive* carrier-mediate sugar transport does not require Na^+, *2*) *active* carrier-mediate amino acid transport is Na^+-dependent, *3*) increasing K^+ inhibits both *active* and *nonactive* transport systems, *4*) the insulin effect on sugar and amino acid transport can be dissociated, and *5*) marked increases in intracellular water can occur in the face of diminished permeability to other solutes.

Interrelationship of Renal Tubular Sodium Transport and 1,2-Dehydrogenation of Steroids. MAURICE M. PECHET * AND EVELYN L. CARROLL, Boston, Mass.

The effects of *d*-aldosterone on renal transport of sodium are markedly altered by 1,2-dehydrogenation. Thus in metabolic balance studies carried out with a variety of subjects, *d*-aldosterone, the naturally occurring epimer, induced sodium retention when administered im in doses of 0.15 to 4 mg. In a normal subject given 2 mg daily, the average daily balance deviations from control values were: sodium, +17.0 mEq; chloride, +26.12 mEq; and potassium, −21.21 mEq. Doses of 4 mg daily caused marked sodium retention (+84.32 mEq), marked chloride retention (+51.22 mEq), and potassium loss (−13.99 mEq). In a panhypopituitary subject, 1 mg *d*-aldosterone daily caused marked sodium retention (+91.0 mEq), chloride retention (+59.5 mEq), and potassium loss (−8.97 mEq), with corresponding changes in serum sodium, chloride, and potassium, but without significant changes in endogenous creatinine clearances. The administration of 1 mg daily of 1,2-dehydro-*d*-aldosterone, prepared in our laboratories, to the same panhypopituitary subject induced insignificant sodium retention (+0.46 mEq), slight chloride retention (+1.57 mEq), insignificant potassium loss (−0.98 mEq), and no significant changes in serum sodium, chloride, potassium, or endogenous creatinine clearance. Two mg of 1,2-dehydro-*d*-aldosterone daily caused a much smaller sodium retention (+23.86 mEq) and chloride retention (+12.31 mEq) than did 1 mg of *d*-aldosterone, although the potassium loss was greater (−15.57 mEq). The sodium-retaining properties of *d*-aldosterone are thus markedly impaired by 1,2-dehydrogenation. Since this change occurs without significant changes in glomerular filtration rate, it is apparent that 1,2-dehydrogenation decreases the sodium-retaining properties of *d*-aldosterone by diminishing its capacity to promote renal tubular reabsorption of sodium.

Demonstration of Impaired Collagen Aggregation in Isolated Rat Cartilage Induced by Beta Amino Proprionitrile. WILLIAM A. PECK AND WILLIAM H. DAUGHADAY,* St. Louis, Mo.

The inhibition of collagen aggregation by lathyrogenic agents demonstrated in experimental animals and chick embryo has provided a useful investigative model that may have relevance to the underlying disorder in collagen metabolism in Marfan's syndrome. We have been able to demonstrate impaired collagen aggregation in rat cartilage, incubated for 24 hours in an enriched medium containing proline-U-C^{14} and beta amino proprionitrile (BAPN). This experimental system permitted the chromatographic isolation and measurement of the specific activity of medium free hydroxyproline, and of the hydroxyproline bound in 0.45 M saline-soluble collagen and in the insoluble collagen residues. With BAPN concentrations of 1 and 10μg per ml incubation medium, the incorporation of radioactivity into soluble collagen hydroxyproline increased from 14 to 167% of the control values, with a concomitant decreased incorporation into insoluble collagen. The amount of labeled hydroxyproline appearing in soluble collagen was not increased further with 50 of 100 μg per ml BAPN. With 100 μg per ml BAPN, incorporation of proline into hydroxyproline of soluble cartilage was slightly decreased, and into hydroxyproline of insoluble cartilage, greatly decreased. The incorporation into medium free hydroxyproline was uninfluenced by the increased radioactivity of soluble collagen induced by BAPN, supporting the previous contention that medium free hydroxyproline is derived from an active small molecular hydroxyproline intermediate rather than the breakdown of soluble collagen. In the doses of BAPN employed in these experiments, no consistent major changes of sulfate S^{35} incorporation into cartilage were observed. These results support the view that the primary effect of lathyrogenic agents is confined to inhibition of collagen aggregations without influencing the formation of free hydroxyproline or the processes of collagen synthesis.

Control of Cellular Metabolism by Hormonally Sensitive Pyridine Nucleotide Transhydrogenase of Mitochondria. LEROY PESCH AND JOSEPH MCGUIRE, New Haven, Conn. (introduced by S. R. Lipsky).

The aerobic oxidation of pyruvate via the mitochondrial tricarboxylic acid cycle supplies a large proportion of the energy for cellular activity. Humoral regulation of this process would be expected to exert a profound regulatory influence on many vital cellular processes within the normal or diseased cell. Enzymic pyridine

nucleotide transhydrogenase activity was isolated by 2-methyl-2-butanol extraction of mitochondrial fractions from whole homogenates of liver and pituitary. This enzyme catalyzes the following reaction: $NADPH + NAD^+ \rightarrow NADP^+ + NADH$. It is known that this transhydrogenation of pyridine nucleotides is the rate-limiting reaction in the oxidation of isocitric acid by isocritic dehydrogenase of liver mitochondria. Since the tricarboxylic acid cycle is a unidirectional pathway, the rate of this transhydrogenase would thus control the over-all oxidative activity of mitochondria. The *in vitro* addition of 10^{-7} M estradiol or diethylstilbestrol, or 10^{-4} M serotonin, epinephrine, or norepinephrine caused a two-fold stimulation in the rate of this transhydrogenase. Furthermore, this stimulation of pyridine nucleotide transhydrogenation was found to be coupled to NADP-malic dehydrogenase activity of pituitary mitochondria and to NADP-isocitric dehydrogenase activity of liver mitochondria. These results indicate that this hormonally sensitive pyridine nucleotide transhydrogenase of mitochondria plays a fundamental role in the physiologic regulation of cellular metabolism.

Increased Lactic Dehydrogenase (LDH) Activity and Mitochondrial Abnormalities in the Nephron of Hypokalemic Man. VICTOR E. POLLAK, HERMANN MATTENHEIMER, AND ROBERT C. MUEHRCKE, Chicago, Ill. (introduced by Robert M. Kark).

LDH activity was assayed quantitatively by ultramicrobiochemical techniques in individually dissected and weighed glomeruli, proximal and distal convolutions, medullary rays, and medulla of eleven healthy human kidneys and of kidneys from four patients with prolonged severe hypokalemia. Excessive potassium losses were gastrointestinal in two patients (self-inducing diarrhea and vomiting), and urinary in two (potassium-losing nephritis, primary hyperaldosteronism). LDH activity in healthy glomeruli was 38.1 ± 12.2 (SD) moles per kg dry weight per hour (MKH), and in hypokalemia was 50.5 ± 16.8 MKH. In hypokalemia, LDH activity doubled in proximal convolutions (in normal subjects, 84.1 ± 21.2 MKH; hypokalemia, 154.0 ± 12.2 MKH; $X^2 = 10.2$; $p = < 0.002$), distal convolutions (in normal subjects, 72.0 ± 17.4 MKH; hypokalemia, 149.0 ± 29.6 MKH; $X^2 = 10.2$; $p = < 0.002$), but not in medullary rays, medulla, and papilla. LDH activity was intermediate between normal and severe hypokalemia in convoluted tubules of one patient with secondary hyperaldosteronism and moderate hypokalemia. Preliminary studies indicate no change in carbonic anhydrase, glutamate oxalacetate, and pyruvate transaminase activity in convoluted tubules. LDH activity was not increased in convoluted tubules in lupus nephritis or various renal tubular disorders. It was increased in convoluted tubules in prolonged severe metabolic acidosis (one patient) and NH_4Cl-induced acidosis (two of three patients). In hypokalemic rats, in contrast, LDH activity was increased only in medullary collecting ducts. Electron-microscopic studies indicate gross morphologic abnormalities of convoluted tubule mitochondria in hypokalemic man and of collecting duct mitochondria in hypokalemic rats. Thus, LDH activity was increased in both species only in tubules whose mitochondria were abnormal. If, as is probable, mitochondrial damage in these tubules results in decreased oxidative phosphorylation, an alternate source for ATP synthesis is necessary. In the glycolytic pathway, DPN, essential for generation of high-energy phosphate bonds, could be regenerated by reduction of pyruvate to lactate, thus possibly explaining increased LDH activity.

Treatment of Acute Lymphoblastic Leukemia in Relapse Using Internally Administered Y^{90}-DPTA Irradiation. MYRON POLLYCOVE, H. SAUL WINCHELL, WILLIAM D. LOUGHMAN, AND JOHN H. LAWRENCE,* San Francisco, Calif.

Using intravenously administered Y^{90}-DPTA, a procedure has been developed that gives relatively selective irradiation of lymphatic structures. A constant rate of tissue irradiation is maintained for 6 hours by continuously recycling the patient's urine intravenously, and irradiation is terminated quickly by cessation of urine recycling; urine excretion $t_{\frac{1}{2}} = 1$ hour. This procedure, initially investigated in a large series of dogs, has been utilized in the therapy of acute lymphoblastic leukemia. Clinical and hematological remissions were obtained in each of four patients in relapse treated with Y^{90}-DPTA recycling 50 to 200 r to the lymph nodes; side effects were minimal or absent. The remissions lasted 2 to 4 months after a single treatment with Y^{90}-DPTA without subsequent antileukemic therapy. After relapse, three of the four patients were sensitive to standard chemotherapeutic agents, and further remissions were obtained with these drugs. The first patient received only 50 r to lymph nodes, yet his circulating lymphoblasts decreased at a constant exponential rate, $t_{\frac{1}{2}} = 7$ hours, from 55,000 to 200 per mm^3 in 50 hours. One patient received an excessive radiation dose to the bone marrow and developed complete marrow aplasia with absence of granulocyte, platelet, and red cell production persisting for 3 months. During this period, the patient was treated with fresh whole blood or plasma and kept in strict reverse isolation without prophylactic antibiotic therapy. Autogenous return of normal marrow function returned during the fourth month after irradiation. One patient with D+ erythrocytes who, after irradiation, was given an homologous bone marrow transplant from her father, who had D− erythrocytes, developed a positive Ashby differential agglutination with 4 to 10% unagglutinated cells above control levels (1 to 1.5% unagglutinated) after mixing with D+ antisera. This finding persisted until the time of her death 6 months after treatment with Y^{90}-DPTA and homologous bone marrow.

Relationships between Steroid Structure and Action on Active Sodium Transport, In Vitro. G. A. PORTER AND I. S. EDELMAN,* San Francisco, Calif.

Crabbe reported that aldosterone increased the average rate of Na transport across the urinary bladder of the

toad. The present study was designed to *1*) define the conditions needed for reproducibly significant response to aldosterone *in vitro* and *2*) explore the relationship between steroid structure and action on Na transport. Active Na transport across paired urinary hemibladders of *Bufo marinus* was continuously measured by the short-circuit current technic. In preliminary studies, it was found that preincubation of the bladders in glucose-free, steroid-free Ringer's solution for 12 to 15 hours, followed by incubation in glucose-fortified (5.5×10^{-3} M) Ringer's for 2 hours, yields a system in which aldosterone regularly produces significant and sustained increases in Na transport. This sequence, in paired studies, was then used to measure the action of aldosterone analogues, all steroids being present at 7×10^{-7} M. The order of effectiveness in accelerating Na transport was found to be: desoxycorticosterone actetate $\geq$ *d*-aldosterone > cortisol > prednisolone > 2-methyl,9α-fluorocortisol > 2-methyl cortisol > progesterone $\geq$ no steroid. From these results, it was concluded that the mineralocorticoid action depends on the hydroxyl function at the 21 carbon and is suppressed by hydrophobic functions (methyl groups) at the 2 carbon, as in mammals.

Bile Formation during Hepatic Ischemia. R. PREISIG, J. G. RANKIN, J. G. SWEETING, R. WILLIAMS, AND S. E. BRADLEY,* New York, N. Y.

Bile formation appears to vary in the dog chiefly with bile acid excretion and endogenous release of secretin. In order to evaluate local circulatory determinants, the effect of hepatic ischemia upon bile flow and composition was studied in anesthetized (Nembutal) animals, previously cholecystectomized and fitted with Thomas cannulae, in which bile acid supply was kept constant and secretin release suppressed. The total biliary output was collected continuously by catheter, and hepatic blood flow (EHBF) was measured by the BSP or colloid clearance methods. Constant intravenous infusion of sodium taurocholate (1.3%, at 15 μmoles per minute) and an anticholinergic agent (Piptal, Lakeside) was maintained throughout. Hepatic venous obstruction by inflation of a balloon in the inferior vena cava (5 experiments) was always followed by a significant (average, 32.5%) reduction in bile flow, with little change in BSP, taurocholate, or electrolyte content, a fall in EHBF (62%) and mean arterial pressure (MAP, 54%), and a rise in hepatic venous pressure (13 mm Hg). BSP Tm decreased in one study, and in another, increase in taurocholate infusion rate affected neither bile flow nor taurocholate output. In contrast, a similar change in EHBF (53%) with hemorrhage (4 to 5% body weight) that lowered MAP 48% (15 experiments) did not affect bile formation. Secretin and taurocholate still appeared to be major determinants, since both elicited (4 studies each) typical choleretic responses. Since BSP Tm and S remained unchanged (2 studies), it may be inferred that hemorrhage resulted in relatively uniform ischemia without elimination of parenchymal tissue from function. With venous obstruction, the data suggest that a reduction in parenchymal functioning mass did occur.

The Antibody Response to Various Forms of Pork and Beef Insulin. THADDEUS E. PROUT, DEAN H. LOCKWOOD, AND BENJAMIN ROTHFELD, Baltimore, Md. (introduced by Samuel P. Asper, Jr.).

Pork insulin differs from human insulin in only one amino acid, whereas bovine insulin has three such differences. This suggests that pork insulin might be less antigenic than bovine insulin when administered to man under identical conditions. Moreover, since the antigenicity of pork insulin in man is likely to be related to the single amino acid not shared by these two species, it was further postulated that modification of pork insulin in such a way as to eliminate this difference might also eliminate the antigenicity. Thus, the C-terminal alanine from the B-chain of pork insulin was removed using carboxypeptidase at pH 8.6. A biologically active insulin residue (modified pork insulin) remained that had a structure common to both species. The 11 insulins compared in this study were: lente, crystalline zinc, and globin beef insulins; lente, crystalline zinc, and NPH pork insulins; semilente, lente, and ultralente insulins in mixtures of beef and pork insulin; and amorphous and NPH modified pork insulin. Antibodies to insulin were determined by two independent methods. Thirty-eight human subjects not previously treated with insulin were given one of the various forms of beef, pork, or modified pork insulin by daily injection under hospital supervision for 3 to 8 months. Studies of insulin antigenicity have also been carried out in the rabbit and pig. Results from these studies are consistent with the conclusions that: *1*) elimination of the differences between an injected insulin and the homologous insulin of the injected animal did not eliminate the antigenicity; *2*) antibody production to insulins with identical primary structures is influenced by the forms in which the insulins are administered; and *3*) the tertiary structure, in addition to the primary amino acid sequence, of insulin appears to be an important determinant of insulin antigenicity.

Studies on Resistance to Alkylating Agents: The Role of Immunological Tolerance. REGINALD P. PUGH, WILLIAM J. HARRINGTON,* AND VICTORIA K. MAYER, St. Louis, Mo.

It has been suggested that immunological tolerance may modify leukemia and its response to treatment. Since alkylating agents apparently induce leukocyte mutations with new antigenic specificities, tolerance could be a factor in development of resistance to these agents. To test this hypothesis, the following studies were undertaken. Bone marrow from C57-B1 mice was harvested in Hank's solution. The donors were either normal or had been given busulfan for 7 days (2.5 mg per kg per day). Nucleated cells, 8×10^6, were injected subcutaneously into newborn isologous animals. When 6 weeks of age, each recipient was again injected with the cor-

responding marrow cells now incorporated in Freund's adjuvant. A control group of the same age was similarly immunized but without prior neonatal preparation. After two booster injections of cells in adjuvant, busulfan (2 mg per kg per day) was given to all of the mice. Only the control recipients of alkylated marrow had an exaggerated granulocytopenic effect ($p < 0.01$). It is probable that this effect represents immunologic rejection of granulocytes containing new antigens induced by the latter course of busulfan. Tolerance to these cells with altered antigenicity was presumably induced by the neonatal preparation. The control animals (i.e., those not prepared in the neonatal period) were allowed to recover and were then rechallenged with busulfan. Again, those immunized with alkylated marrow developed an exaggerated granulocytopenia, but to a less marked degree. After their recovery, the mice were given a third course of bulsulfan with significant, but still less evidence of difference between the two groups. A fourth challenge brought about no difference in degree or duration of granulocytopenia, or completeness of recovery. This loss of selective susceptibility may have more than one basis. Current evidence does not suggest immunoparalysis from repeated courses of an alkylating agent. The present studies and related observations favor the development of tolerance to antigenic mutations randomly induced by busulfan. In addition, there may be emergence of a population of granulocytes possessing new antigenic specificities that were not represented adequately, if at all, in the original marrow material used for immunization. If these interpretations are correct, they indicate need for agents that induce more potent antigens and do so less randomly.

An Interaction between Chylomicrons and a Gamma Globulin on Dilution of Serum in Sucrose Solutions. PAUL G. QUIE AND JAMES G. HIRSCH,* New York, N. Y.

In the course of experiments involving dilution of fresh human serum with isotonic sucrose solutions, it was noted that chylomicrons in such preparations were deposited as a sediment on centrifugation, rather than rising to the top of the tube. This report deals with studies attempting to explain this unexpected phenomenon. Chylomicrons sedimented under these conditions appeared morphologically unchanged on dark-field microscopy, and contained lipids essentially identical to those in floated chylomicrons from the same undiluted serum. The marked increase in density of chylomicrons on dilution of serum in sucrose was found to be due, at least in part, to deposition on their surface of a gamma globulin fraction. This adsorbed gamma globulin was apparently aggregated; it displayed anticomplementary activity when incubated with human or guinea pig serum, and caused an immediate reaction in guinea pig skin. The phenomenon occurred on dilution of serum with 2 to 4 volumes of isotonic sucrose, but not on dilution with various salt solutions. No euglobulins were precipitated in the absence of chylomicrons under the conditions employed. The reaction between chylomicrons and gamma globulin was blocked above pH 8, and was also prevented by heating the serum at 56° C, or by adding agents that chelated divalent cations. Washed, floated chylomicrons suspended in certain preparations of human gamma globulin also became sedimentable on dilution in sucrose solutions. Chylomicrons placed in albumin from human serum did not exhibit similar behavior. Lipemic serum from rats and rabbits showed a similar alteration in chilomicron density on dilution in ion-free media. These observations give rise to speculation on a possible role of serum proteins in clearing chylomicrons *in vivo,* and also point to a mechanism other than heating or antigen-antibody reaction for formation of aggregated gamma globulin.

A Metabolic Regulating Device Based on Actions of Human Growth Hormone and of Insulin, Singly and Together, on the Forearm. DAVID RABINOWITZ AND KENNETH L. ZIERLER,* Baltimore, Md.

Concentration of circulating insulin varies during the day, largely increasing during absorption of food and having its nadir in the remote postabsorptive state. Whether or not there are variations in circulating growth hormone is undetermined, but it is generally assumed that its concentration is relatively constant during a day. Thus, the body is exposed to the combined influence of insulin and growth hormone at mealtime, but mainly to growth hormone alone during food-free intervals. We have, therefore, examined the effects of physiologic amounts of human growth hormone (HGH) and of insulin, singly and together, on forearm metabolism in man, by intra-arterial infusion in normal subjects, in acromegalic subjects, and in normal subjects after 3-day fast. Results are: HGH alone increased release of FFA from forearm adipose tissue, increased FFA uptake, and decreased glucose uptake by muscle; insulin plus either exogenous or endogenous HGH stimulated less glucose uptake than occurred with insulin alone, but FFA release from adipose tissue was obliterated exactly as with insulin alone. In other words, the arm is insulin-resistant with respect to glucose uptake, but insulin-sensitive with respect to FFA movement when both insulin and HGH are presented to it, and insulin inhibits the lipid-catabolic effects of HGH. The consequence is that carbohydrate and fat storage is promoted during the immediate postprandial period, and lipid catabolism is made easy during the postabsorptive period, so that the supply of substrates is smoothed out over the day, independent of ingestion.

The Relationship between Vitamin D and Parathyroid Hormone. HOWARD RASMUSSEN,* HECTOR F. DELUCA, JOHN D. SALLIS, AND GEORGE W. ENGSTROM, Madison, Wis.

A comprehension of the relationship between the activities of vitamin D and parathyroid hormone is of central importance to an understanding of many clinical

disorders of calcium metabolism. Recent evidence has stressed that the hormone does not exert its characteristic calcium-mobilizing action in D-deficient rats, but has left unexplained the pathogenesis of hypophosphatemia in osteomalacia that has been considered to result from parathyroid hyperactivity. We have reported previously that calcium and stoichiometric amounts of phosphate are accumulated by isolated mitochondria, and are released under the influence of vitamin D and parathyroid hormone. The presence of vitamin D is necessary for the demonstration of this hormonal effect. In addition, the hormone, but not the vitamin, stimulates a noncalcium-dependent uptake of phosphate. From these findings, it was predicted that the hormone would exert its characteristic effects upon phosphate, but not upon calcium metabolism in D deficiency. This has been confirmed in D-deficient rats. Within three hours after parathyroidectomy, there is a slight fall in plasma calcium (4.9 to 4.1 mg per 100 ml), but a dramatic rise in plasma phosphate (12.0 to 19.2 mg per 100 ml). The administration of 50 to 100 U of hormone prevents the hyperphosphatemia without preventing the slight fall in plasma calcium. The administration, however, of a massive dose (2,000 U) does induce a rise in plasma calcium (5.4 to 10.1 mg per 100 ml) without causing the nephrocalcinosis or hypercalcemia that it would produce in a normal rat. Thus the studies upon the mitochondrial exchanges of calcium and phosphate have led to predictions concerning exchanges of these ions in the intact organism and seem to offer the first substantial basis for defining vitamin D-parathyroid relationships. Furthermore, the results suggest that the primary effect of the hormone is upon phosphate translocations and that vitamin D functions to couple the hormonal effect upon phosphate translocations to those upon calcium translocations.

Effect of L-*Leucine on Gluconeogenesis.* GERALD REAVEN AND ROBERT E. GREENBERG, Palo Alto, Calif. (introduced by John A. Luetscher).

Previous results from this laboratory have indicated that experimentally induced "leucine hypoglycemia" is accompanied by a marked fall in hepatic glucose output. The ketogenicity of L-leucine, and the situations in which it has produced hypoglycemia, suggested decreased gluconeogenesis as the most likely possibility by which L-leucine reduces the hepatic glucose output. Consequently, the current experiments were undertaken in order to evaluate the effect of L-leucine on gluconeogenesis, and were performed in mice that respond to L-leucine like other species studied. Assays of hepatic glucose 6-phosphatase and fructose 1,6-diphosphatase activity in a number of experimental conditions indicated that leucine hypoglycemia is not associated with a decrease in activity of either of these enzymes that could be responsible for a reduction in the conversion of amino acids to glucose. On the other hand, administration of L-leucine to normal mice did reduce the production of glucose-C^{14} from glycine-C^{14} *in vivo*. These observations were confirmed *in vitro* with mouse liver slices; L-leucine also decreased production of glucose-C^{14} from both alanine-C^{14} and pyruvate-C^{14}. Since L-leucine also reduced the production of both C^{14}-labeled glycogen and CO_2 from pyruvate-C^{14}, it is concluded that the effect of leucine is to divert pyruvate from the gluconeogenetic pathway, and not to decrease glycogenolysis, or increase glucose catabolism. Demonstration that L-leucine reduces the production of glucose-C^{14} from amino acids and pyruvate does not necessarily mean that leucine hypoglycemia results from decreased gluconeogenesis. The observation, however, that L-leucine appears to divert 3-carbon fragments from conversion to glucose offers a reasonable explanation for the fact that L-leucine barely lowers blood glucose concentration in normal subjects, yet will produce profound hypoglycemia in a variety of situations characterized by the need for increased hepatic glucose output for maintenance of euglycemia.

Plasma Insulin-like Activity (ILA): Insulin Plus Adipose Tissue Inhibitors? LILLIAN RECANT,* HALUK ALP, MARY KOCH, AND JOANNE EGGEMANN, St. Louis, Mo.

Measurements of plasma ILA have been made, using the *in vitro* effect of plasma on the conversion of 1-C^{14}-glucose to $C^{14}O_2$ and 6-C^{14}-glucose to lipid by rat epididymal fat. These parameters were chosen because diabetogenic substances (growth hormone, ACTH, glucagon) enhance 1-C^{14}-glucose to CO_2 and depress 6-C^{14}-glucose to fat. In contrast, crystalline insulin stimulates both parameters in a straight-line relationship to the insulin concentration. Fasting plasma levels of 1-C^{14}-CO_2 ILA in μU per ml were as follows: in 45 normal subjects, 442 with a range of 69 to 1,400; in 47 untreated diabetic subjects, 734 (110 to 2,400); in 10 acromegalic subjects, 1,159 (272 to 3,200); in 7 newborn infants, 737 (408 to 1,100); in 4 pituitary dwarfs, 300 (164 to 520); and in 6 obese subjects, 1,037 (138 to 1,540). 6-C^{14}-Lipid ILA levels in μU per ml were 453 (50 to 1,600) in normal subjects; 519 (61 to 2,000) in diabetic subjects; 517 (132 to 1,500) in acromegalic subjects; 304 (144 to 400) in newborn infants; 347 (160 to 672) in dwarfs; and 693 (324 to 1,148) in obese patients. Oral glucose tolerance tests with 1-C^{14}-CO_2 ILA measurements were performed in 33 normal, 35 diabetic, and 3 obese subjects. Maximal ILA increments were: 334 μU per ml in normal, 756 in adult diabetic, 340 in 2 juvenile diabetic, and 1,553 in obese subjects. These data suggest that fasting ILA levels represent insulin plus additional substances, since with insulin alone the ratio of C_6-lipid ILA : C_1-CO_2 ILA would be 1.0. In normal subjects and hypopituitary dwarfs, this ratio approached 1.0. Diabetic, acromegalic, newborn, and obese subjects, however, showed deviations from the theoretical ratio with relatively decreased C_6-lipid ILA and increased C_1-CO_2 ILA. In addition, the C_1-CO_2 response to glucose was exaggerated in adult diabetic and in obese subjects. Similar responses have been reported after growth hormone administration to man. It seems likely that in diabetic and acromegalic subjects as contrasted with normal ones,

plasma factors exist that metabolically resemble a combination of insulin and diabetogenic substances in their action on fat tissue. An intensive investigation of isolated plasma protein fractions for identification of inhibitors is under way.

The Effect of Amelioration of Anemia on the Proportion of Fetal Hemoglobin in the Erythrocytes of Patients with Sickle-Cell Anemia. L. JUDEN REED, THOMAS B. BRADLEY, JR., AND HELEN M. RANNEY,* New York, N. Y., and Jersey City, N. J.

The presence of increased amounts of fetal hemoglobin in the erythrocytes of patients with certain congenital hemolytic and acquired anemias has not been satisfactorily explained. The present study was designed to ascertain the effect of amelioration of the anemia on the amount of fetal hemoglobin in the erythrocytes of patients with sickle-cell anemia. Two such patients (blood groups A and AB) received transfusions of normal blood group O erythrocytes sufficient to maintain hemoglobin concentrations between 11 and 15 g per 100 ml for 4 months. Suppression of erythropoiesis with marked decline in reticulocyte counts and disappearance of erythroid hyperplasia from the bone marrow occurred, and hemoglobin S constituted less than 10% of circulating hemoglobin. At weekly intervals, the recipient's own (group A or AB) erythrocytes were agglutinated with commercial antisera and separated by differential sedimentation. Efficacy of the separation was confirmed by absence of hemoglobin A on electrophoresis of hemolysates prepared from agglutinated erythrocytes. In one patient, the concentration of hemoglobin F in the patient's erythrocytes rose from 12 to 30% during the first month, then declined abruptly. After 4 months, hemoglobin F comprised 6% of her total hemoglobin. In the second patient, hemoglobin F rose from 11 to 18% and declined slowly to 11%. After 4 months, continuing hemoglobin F synthesis was demonstrated by Fe^{59} incorporation studies. The initial rises in hemoglobin F in both patients were attributed to preferential destruction of the erythrocytes containing larger proportions of hemoglobin S; consequently, the red cells surviving after 2 to 3 weeks were those containing relatively larger amounts of hemoglobin F. While fetal hemoglobin synthesis was probably reduced by transfusion therapy in the first patient, synthesis of fetal hemoglobin persisted in both patients despite prolonged correction of anemia.

Diffusion of Gases Out of Distal Nephron during Antidiuresis in Man: CO_2. EDWARD L. REID AND A. GORMAN HILLS,* Miami, Fla.

Urine concentration through abstraction of "solute-free" water from distal tubule and collecting duct must result in rise of diffusion pressure of substances dissolved in luminal fluid. If, in consequence, the highly diffusible gases NH_3 and CO_2 diffuse out of the distal nephron, the rate of excretion of total ammonia and total CO_2 will decrease as urine flow rate declines, and because each gas forms one element in a buffer system, acid-base composition of urine will also change predictably. The following indicates indirectly that CO_2 diffuses out of the distal nephron of man during antidiuresis: *1)* data presented elsewhere indicate that NH_3 diffuses out of the distal nephron during antidiuresis, with H^+ generation; *2)* rates of urinary excretion of H_2CO_3 and HCO_3^- both regularly decline as flow declines (13 experiments, $p < 0.01$); and *3)* in alkaline urine, reduction of urine flow rate regularly causes the ratio $HCO_3^-:H_2CO_3$ in urine to rise, with urine pH necessarily also rising (10 experiments, $p < 0.01$), whereas in acid urine containing little HCO_3^-, urine pH parallels flow. It is concluded that gas diffusion out of the nephron is the major cause of changes of urine pH with changing flow. Since water reabsorption continues to the papilla during antidiuresis, two consequences of water reabsorption, concentration of the CO_2 system and H^+ generation, both tending to raise urine $[H_2CO_3]$, also continue to the papilla. A major part of the pCO_2 elevation of low-flow alkaline urine above arterial pCO_2 is abolished by maximal water diuresis (10 experiments, $p < 0.01$), notwithstanding the more rapid transit of luminal fluid. Much of the elevation of pCO_2 of low-flow alkaline urine therefore appears to be due to delayed dehydration of the elevated $[H_2CO_3]$ generated by the concentrating process.

Immunologic Response of Heifers to the Administration of Porcine and Bovine (Homologous) Insulin. ALBERT E. RENOLD,* JÜRGEN STEINKE, J. STUART SOELDNER, RUSSELL E. SMITH, AND HARRY N. ANTONIADES, Boston and Amherst, Mass.

Of 15 heifers, 5 had 7 biweekly (every 2 weeks) injections of crystalline pork insulin in adjuvant, 5 had 10 or more biweekly injections of beef insulin in adjuvant; and 5 had biweekly injections of adjuvant alone (insulin dose, 150 U per 100 pounds animal). Sera were obtained biweekly, and distribution of added insulin-I^{131} (beef and pork) was examined after electrophoresis on cellulose acetate strips. Sera from animals injected with adjuvant *alone* never exhibited beta-gamma globulin distribution of added insulin-I^{131}. In all animals injected with *pork* insulin, beta-gamma distribution occurred after 4 weeks, and was most marked with pork insulin-I^{131}, but also was definite with beef insulin-I^{131}. This was confirmed by microimmunoelectrophoresis using either whole serum or its purified gamma globulin. In the 5 heifers injected with *beef* insulin, beta-gamma distribution of added insulin-I^{131} was not observed until 8 weeks, but was definite after 12 to 32 weeks. In their sera, the beta-gamma distribution did not differ when measured with either beef or pork insulin-I^{131}. An *in vivo* immunologic response to both pork and beef insulin could be demonstrated by the disappearance rate of intravenous insulin-I^{131}. One of the beef-injected animals died, and examination of its pancreas revealed lymphocytic infiltration into islets without such infiltration into exocrine pancreas. These observations suggest that heifers exhibit an immunologic

response not only to pork, but also to *prolonged* administration of beef (homologous) insulin; they also suggest that insulin in the circulation is not present in a form identical with that of extracted crystalline pancreatic insulin, injected in adjuvant.

Observations on the Role of Ethanol in the Pathogenesis of Alcoholic Cirrhosis. TELFER B. REYNOLDS,* ALLAN G. REDEKER, AND OLIVER T. KUZMA, Los Angeles, Calif.

Others have shown that moderate amounts of ethanol can be ingested by patients with alcoholic cirrhosis, in a hospital environment, without harmful effects. We pursued this further by giving larger doses of ethanol (3 ml per kg per day) to patients critically ill with "decompensated" cirrhosis and jaundice. Ethanol was given four times daily for several weeks in a flavored mixture containing casein hydrolysate. Fifteen patients treated in this fashion improved satisfactorily and showed no deleterious effects from the ethanol. Serum bilirubin fell to normal, serum protein and prothrombin levels improved, body weight increased, and general condition improved markedly. Daily caloric intake from food was 1,200 to 2,500 with an additional 800 to 1,600 kcal from ethanol. Protein intake usually exceeded 70 g per day. In 12 additional patients, ethanol intake was adjusted to equal the calculated daily capacity for ethanol metabolism. Ethanol metabolic rate (EMR) was determined by measuring the rate of decline of blood ethanol after ingestion of a known quantity. It ranged from 4.0 to 7.6 (mean 5.3) g per hour. EMR increased as liver function improved, averaging 6.8 g per hour after several weeks of hospitalization, and the dose of ethanol was increased appropriately. Improvement in these patients was consistent and satisfactory. In 3 patients, ethanol was given in an amount 25% greater than calculated EMR, still with no apparent injurious effect. Morning blood ethanol levels showed no accumulation until intake exceeded EMR by more than 25%. This was explained by finding an increase in EMR at high blood ethanol levels, which is not consistent with the usually accepted theory of ethanol metabolism. We conclude that the liver can metabolize ethanol at a maximal rate over a prolonged period without harmful effect, provided food intake is adequate.

The Effect of Infused Norepinephrine on the Coronary Circulation and Myocardial Metabolism. JOSE RIBEILIMA, VERNON E. WENDT, HERMINIO R. RAMOS, PETER SCHOLLMEYER, AND RICHARD J. BING,* Detroit, Mich.

It has been observed that epinephrine and norepinephrine cause a rise in coronary flow secondary to increased metabolic demands of the heart. The effect of these catecholamines on cardiac metabolism, however, is less known. This work is designed to study coronary circulation, myocardial efficiency, and myocardial substrate utilization induced by infusion of epinephrine in 13 normal subjects. Patients without cardiovascular disease were studied in fasting nonpremedicated state. Cardiac output was calculated by dye dilution, and coronary blood flow by the nitrous oxide method. Simultaneous arterial and coronary sinus samples were obtained for determination of oxygen, carbon dioxide, pyruvate, lactate, glucose, and free fatty acids. Infusion of norepinephrine commenced until an elevation of the mean arterial blood pressure of 30 mm Hg could be maintained. The determinations were then repeated. Coronary vascular resistance, tension time index, and myocardial efficiency were calculated. The myocardial oxidation reduction potential (ΔE_h) was obtained from the difference in the ratios of lactate/pyruvate in coronary vein and arterial blood. During infusion, the heart rate slowed without electrocardiographic evidence of myocardial ischemia; cardiac output and stroke volume increased, while coronary blood flow did not change. The coronary vascular resistance and myocardial efficiency rose. The redox potential of the heart became more positive, suggesting absence of glycolysis and improved oxygenation of the heart muscle. Arterial level and myocardial extraction of free fatty acids increased.

Sonic Measurement of Bone Mass. CLAYTON RICH, ELI J. KLINK, GAY L. MULLINS, AND C. BENJAMIN GRAHAM, Seattle, Wash. (introduced by Belding H. Scribner).

By use of equipment we have constructed that measures the transit time of a sonic pulse between piezoelectric transducers fixed to the ends of a U-shaped frame, we have determined the velocity of sound in compact bovine bone, muscle, and fat to be 0.288, 0.163, and 0.157 cm per μsec, respectively. When samples of bone are placed between the transducers so that the sonic pulse passes through different thicknesses of bone, the change in transit time is proportional to the mass of bone or calcium per unit area traversed. In numerous measurements, it was determined that the change in transit time in bone was 0.811 ± 0.057 μsec per mg calcium per mm^2 of cross-section of the sonic path. When the thigh of a rat is passed at a constant rate between the transducers, a recording potentiometer produces a curve that reflects the transmission time in soft tissues and a decrease in transit time as the transducers pass bone. If the contribution from soft tissue is subtracted, the resulting curve, representing bone, is identical with that obtained when the transducers scan the isolated femur of the other leg. When the instrument is calibrated by reference to curves from bones of known calcium content, the mass of calcium in a bone *in situ* can be measured directly. In several studies of a rat thigh, we found 4.32 mg calcium per mm^2 of mid-shaft of the femur. Subsequent chemical analysis of the same area showed 98% of this amount.

The Hypercalcemia of Magnesium Depletion. J. A. RICHARDSON AND L. G. WELT,* Chapel Hill, N. C.

This investigation was designed to examine the mechanism responsible for the hypercalcemia of Mg^{++} depletion.

Four groups of rats were pair-fed an identical diet except that one had all the essential minerals (C), one had all but Ca^{++} (NoCa), another had all but Mg^{++} (NoMg), and the last had neither Ca^{++} nor Mg^{++} (DD). It was observed that there was striking hypocalcemia in NoCa, that there was the usual hypercalcemia in NoMg, and that there was no statistically significant difference in the level of serum Ca^{++} between NoMg and DD, nor between DD and the controls. When the NoCa and DD groups were compared, there was no difference in the fecal or urinary excretion of Ca^{++}. Furthermore, the total carcass Ca^{++} of these two groups was no different. When the C and NoMg groups were compared, there was a difference of borderline significance ($p < .05$) between fecal excretion of Ca^{++} and total carcass Ca^{++}, but there was no difference in urinary excretion of Ca^{++}. Thus, it is clear that the hypercalcemia of Mg^{++} depletion is *not* dependent on either an increase in gastrointestinal absorption or diminished urinary excretion of Ca^{++}. Alternatively, it appears that the hypercalcemia is a result of a redistribution of Ca^{++} within the animal. The presumption is that this is a mobilization of bone Ca^{++} consequent to Mg^{++} depletion.

Evaluation of the Rate of Interaction between Thyroxine and Serum Proteins. JACOB ROBBINS,* J. E. RALL,* MONES BERMAN, AND MARIO ANDREOLI, Bethesda, Md.

Circulating thyroxine in man is almost entirely bound to three proteins: an α-globulin, a "pre-albumin," and albumin. Although the equilibrium constants for these interactions are known approximately, the rates of association and dissociation are unknown, and might affect availability of free hormones to tissue. Measurement of these rates by two methods was attempted. The rate of dialysis from a thin layer of liquid covering a large surface of stretched cellophane sac was measured. Free thyroxine-I^{131} diffused across at 16% per minute (pH 7.4, 25° C); thyroxine added to undiluted serum (.045 to .18 μg per ml) crossed at $< .05\%$ per minute. Dilution of the thyroxine-serum mixture increased the rate by favoring dissociation. With dilutions of 1:20 and 1:200, no difference was observed between *1*) diluted thyroxine-serum mixtures preincubated for 90 minutes, *2*) mixtures incubated in normal serum and diluted just before dialysis, and *3*) dilute mixtures prepared as quickly as possible. The results indicated that thyroxine-protein equilibration was complete in less than 2 minutes, the time required for measurement. A fluorescence method allowed faster monitoring of the reaction. Thyroxine bound to serum albumin quenches tryptophane fluorescence (excited at 290 mμ) owing to the proximity of the bound group (absorbing at 360 mμ) to the chromaphore. A flow-through cell was filled with bovine albumin (5.5×10^{-7} M, ph 7.4, 23° C), and fluorescence before and during injection of thyroxine (final concentration, 0.5 to 2×10^{-6} M) was monitored automatically. Injection required 40 msec, and concentration-dependent quenching of fluorescence (identified with the rate of association) was complete within 150 msec. Since this rate is directly proportional to protein concentration, binding must be extremely rapid in undiluted serum. A minimal dissociation rate of 6.7 $second^{-1}$ ($t_{\frac{1}{2}} = 97$ msec) can be calculated. It is evident that association and dissociation of the thyroxine-protein complexes occur with great rapidity.

A New Technique for Differential Renal Function. KATHLEEN E. ROBERTS * AND C. ALLEN WALL, San Francisco, Calif.

Current techniques for the study of differential renal function are subject to many artifacts, including *1*) undetermined leakage from ureteral catheters that negates accurate collection, *2*) spasm of ureters that causes mechanical impediment to flow, and *3*) bleeding, which presents chemical errors. A recently devised technique eliminating these artifacts is presented here. Small, non-obstructing catheters (no. 4) are placed in each ureter. Urine is collected in a given period without regard to the individual flow from either kidney. With the plasma concentration of inulin and para-aminohippurate (PAH) sufficiently elevated and adequate urine flow established, a timed collection of urine is made from each ureter and the bladder. A mid-point blood is obtained. These are analyzed for inulin (glomerular filtration rate) and PAH (renal plasma flow). All urine from the bladder and both ureters is consigned to one sample except for the small ureteral samples, which are chemically analyzed and then added mathematically to the entire sample. The total sample of urine is designated C, the concentration of inulin or PAH = C_c, and the total flow C_f. The flow in the right ureter is termed A_f, and the concentration A_c. Similarly, in the left ureter flow is labeled B_f and concentration B_c. Since the only unknown factors are the flow through each ureter, it is possible to calculate the individual kidney flow mathematically. By using established formulas, glomerular filtration rate and renal plasma flow can then be calculated: $A_cA_f + B_cB_f = C_cC_f$ and $B_f = C_f - A_f$. By substituting B_f above, $A_f = (C_cC_f - B_cC_f)/(A_c - B_c)$. The practical significance of this work is the simplification of evaluating data obtained with differential renal function studies in patients with renal disease.

A Model for the Study of Anaerobic Metabolism in the Intact Animal: Facultative Aerobiosis in the Freshwater Turtle. EUGENE D. ROBIN,* JOHN W. VESTER, H. VICTOR MURDAUGH, JR., AND J. EUGENE MILLEN, Pittsburgh, Pa., Birmingham, Ala., and Salisbury Cove, Me.

Comparative physiology and biochemistry provide rich areas for exploring phenomena that permit deeper understanding of analogous processes in man. There has been recent emphasis on the importance of anaerobic metabolism. The availability of an animal in which intimate details of anaerobic metabolism could be studied *in vivo* for extended periods would be of interest. The turtle's ability to survive prolonged diving is well-known.

Experiments indicate that survival is possible because sufficient energy can be obtained anaerobically to meet metabolic requirements. Changes in blood gases, acid-base parameters, and lactate-pyruvate concentrations during diving, during 100% N_2 inhalation, and after massive doses of NaCN were studied. P_{O_2} falls during diving and N_2 inhalation, and rises after CN^-, reflecting decreased intracellular O_2 supply in the former cases and decreased intracellular O_2 utilization with CN^-. P_{CO_2} falls with N_2 and CN^-, and increases during diving, reflecting net balance between pulmonary CO_2 excretion and buffer-generated CO_2. $[H^+]$ increases during diving, and tends to fall with N_2 and CN^-, reflecting net balance between anaerobically generated H^+ and H^+ equivalents lost by hyperventilation. Plasma lactate concentration rises and $[HCO_3^-]$ falls during the three experimental circumstances, reflecting the utilization of anaerobic pathways. The hyperlacticacidemia is essentially an increase in "excess lactate." Parallel studies indicate that energy for maintenance of ion gradients by isolated turtle bladder (Na^+ pump) can be obtained anaerobically. Since a Na^+ pump can function anaerobically in turtle bladder, it seems reasonable that prolonged anaerobic survival is possible because ion pumps in turtle brain are likewise capable of using glycolysis for energy. These observations suggest that the turtle is a suitable model for investigating such problems as relationships between external ventilation and tissue metabolism, whole-body Pasteur effect, relations between energy supply and ion transport, and kinetics of buffer-produced CO_2.

Studies on Release of Iodide from the Thyroid. I. N. Rosenberg,* J. C. Athans, M. J. R. Dawes, and G. H. Isaacs, Boston, Mass.

Transfers of iodide between the thyroid gland and circulation were studied in the dog before and after administration of perchlorate. Animals that had been given I^{131} 2 to 7 days earlier to label glandular iodine were given I^{125} intravenously to label circulating iodide, and 2 hours later a cannula was placed in the inferior thyroid vein, and serial sampling of arterial and thyroid venous blood was begun. Plasma I^{125} and trichloroacetic-acid-soluble I^{131} were considered to be iodide. In 5 dogs given thyrotropin (10 U) 24 hours before thyroid venous cannulation, the observed thyroidal extraction ratio for I^{125} greatly exceeded that for iodide-I^{131}. In the pre-perchlorate period, glandular release of iodide-I^{131} (calculated from I^{125} clearance, arterial iodide-I^{131} concentration, and observed arteriothyroid venous plasma iodide-I^{131} concentration difference) was usually comparable in magnitude to protein-bound I^{131} release. From these data and chemically determined concentrations of stable protein-bound iodine (PBI) and iodide in arterial and thyroid venous plasma, glandular release of stable iodide was estimated to be approximately half that of stable PBI and twice the uptake of stable iodide. Intravenous administration of perchlorate approximately doubled the release of iodide. In 2 of 4 dogs not pretreated with thyrotropin, the thyroidal extraction ratio for I^{125} was appreciably greater than that for iodide-I^{131} in the control period; in 3 of these 4 dogs, release of iodide-I^{131} in excess of I^{125} was observed after perchlorate injection. The results support the idea that appreciable quantities of iodide not newly accumulated from the circulation may be released from the thyroid gland, especially when the latter has been stimulated, and that release may be enhanced by inactivation of the trapping mechanism for iodide.

Myocardial Blood Flow Measurement by the Injection of Radioactive Gases Directly into Coronary Arteries. Richard S. Ross,* Keiji Ueda, Paul R. Lichtlen, and J. Russell Rees, Baltimore, Md.

Myocardial blood flow has been measured in man by precordial counting after the injection of solutions of radioactive inert gas (Xe^{133} and Kr^{85}) directly into the coronary arteries. The radioactive solution is injected into the coronary ostia through a Sones catheter introduced via the right brachial artery for selective coronary arteriography. Essentially all of the radioactive material is delivered into the coronary artery, and therefore, the exponential rate of disappearance of precordial radioactivity is predominantly a function of myocardial blood flow. Right and left coronary flow can be measured independently by this method. Five patients have been studied, and the $t_{\frac{1}{2}}$ for precordial disappearance range from 0.66 to 1.0 minutes, which can be converted to values for flow ranging from 48 to 99 ml per minute per 100 g. The method is based on animal experiments designed to evaluate the factors influencing the rate constant (k) for the disappearance of precordial activity. This constant (k) is related to flow (F), distribution volume (V), and the partition coefficient (λ) as follows: $k = F/\lambda V$. This relationship has been investigated in dogs in which total flow in a coronary artery or its branch was measured by a rotameter, and a close correlation with k demonstrated over a fourfold range of flow. The inverse relation between k and volume was demonstrated by comparing segments of the coronary arterial tree with different volumes of distribution. The effect of λ was assessed by the use of two inert gases (Xe^{133} and Kr^{85}) with differing partition coefficients. The effect of various injection sites on the disappearance curve has also been evaluated, and the importance of direct coronary injection confirmed. The clearance of 95% of injected Xe^{133} during the first passage through the lung has been demonstrated.

Molecular Similarities in Erythropoiesis-stimulating Factors (Erythropoietins) from Various Sources. Wendell F. Rosse and Thomas A. Waldmann, Bethesda, Md. (introduced by N. I. Berlin).

Erythrocytosis has been associated with severe cyanotic congenital heart disease, renal cysts, and cerebellar hemangioblastomas. This erythrocytosis is probably secondary to erythropoiesis stimulating factor(s) demonstrated in the serum or cyst fluid of these patients.

This study was designed to compare the molecular characteristics of erythropoiesis-stimulating factors found in serum from patients with *a*) aplastic anemia and *b*) erythrocytosis secondary to arterial hypoxia (transposition of the great vessels), and in cyst fluid from patients with erythrocytosis due to *c*) a renal cyst and *d*) a cystic cerebellar hemangioblastoma. Since techniques for purifying erythropoietin are not available, the molecular characteristics of these biologically active materials were studied by analyzing the effect of physical and chemical procedures upon that activity. The polycythemic mouse assay measuring the incorporation of Fe^{59} into the peripheral blood was used to determine the erythropoietin-like activity of the preparations. We have previously shown by radiation inactivation that the molecular weight of these erythropoietive factors was in the range 25,000 to 35,000. The erythropoiesis-stimulating activity in all four materials migrated as an α_2-globulin on Geon-Pevikon block zonal electrophoresis. The biologic activity of each of the four materials was destroyed by reaction with sialidase or with trypsin. An antibody was made in rabbits with a urine extract from the anemic patient as antigen. When each of the four materials reacted with this antiserum, the erythropoiesis-stimulating activity was completely neutralized. Therefore, the erythropoiesis-stimulating factor in each of these materials must be an α_2-globulin of molecular weight 25,000 to 35,000 requiring protein structure and sialic acid for biologic activity and having common antigenic determinants. The erythropoiesis-stimulating factors in anemic serum, anoxic serum, cerebellar hemangioblastoma cyst fluid, and renal cyst fluid appear to be similar, if not identical.

The Paradoxical Effect of Plasmin on Staphylococci: Simultaneous Lysis and Growth Stimulation. EDWARD B. ROTHERAM, JR., AND ABRAHAM I. BRAUDE,* Pittsburgh, Pa.

Plasmin, a proteolytic enzyme of blood, was examined for antibacterial action in order to assess its role in resistance to infection. Pathogenic *Staphylococci* grown 18 hours in broth were resuspended in a non-nutrient buffer (pH 7.4). During 48 hours at 37° C, the optical densities of these suspensions fell approximately 64% before stabilizing, and virtually all cocci became gram-negative and smaller. Residual turbidity almost disappeared on subsequent addition of 2 mg per ml of thrombolysin (lysed suspensions), but was unaffected by heat-inactivated thrombolysin (turbid controls) or by Varidase. Paradoxically, viability assays showed as many surviving bacteria in lysed suspensions as in turbid controls, and 24 hours later viable organisms in lysed suspensions increased 2 to 5 times, while little if any growth occurred in turbid controls. As thrombolysin removed turbidity, the gram-negative cocci vanished and large, dividing, gram-positive cocci became prominent. These effects of thrombolysin were reproduced by as little as 5 μg per ml of highly purified trypsin. Nutrients for the renewed growth seen in suspensions lysed by trypsin were derived, therefore, solely from lysed and digested bacteria. Addition of amino acids stimulated equal growth in lysed suspensions and turbid controls, obscuring the stimulatory effect of trypsin. Added protein (casein), on the other hand, greatly augmented growth only in suspensions containing trypsin. Thus, it appears that plasmin and trypsin stimulate growth by providing peptides from lysed bacteria. *Staphyloccocci* killed by 32% acetone (to preserve intracellular enzymes) became gram-negative and were rapidly lysed by plasmin, whereas heat-killed *Staphylocci* remained gram-positive and were much more resistant to lysis. These results indicate that plasmin can hydrolyze bacterial proteins altered by autolytic enzymes that become activated in dead or injured *Staphylococci,* and that nutrients are thereby provided that stimulate growth. Host resistance might benefit from such enzymatic removal of dead bacteria that could otherwise immobilize phagocytes.

Liver Interstitial Albumin: A Possible Regulator for Albumin Synthesis. M. A. ROTHSCHILD, M. ORATZ, S. S. SCHREIBER, AND C. D. EVANS, New York, N. Y. (introduced by E. C. Franklin).

Previous studies showed that dextran infusions were associated with a lowered albumin synthesis and an increase in the fraction of the albumin pool located extravascularly, and suggested the existence of an extravascular colloid regulatory mechanism effecting control by altering albumin synthesis. The present study describes measurements of extravascular albumin concentration in control, dextran-, and dextran and cortisone-treated rabbits. The intravascular and extravascular distribution of albumin-I^{131} was determined for the whole body and the liver. Extracellular volume was measured with C^{14}-sucrose. During dextran infusions, the body plasma volume rose from 34 to 41 ml per kg, while plasma albumin levels fell 27% from 3.7 to 2.8 g per 100 ml. Extracellular volume was unaltered. Liver plasma volume increased from 9.3 to 12.0 ml per 100 g. Liver equilibrium albumin space increased from 11.7 to 16.6 ml per 100 g. Liver extracellular volume increased from 21.8 to 24.8 ml per 100 g. The calculated liver interstitial albumin concentration rose 37% from 0.71 to 0.97 g per 100 ml. Upon the addition of cortisone (shown to result in an increase in albumin synthesis), the body plasma volume rose to 62 ml per kg with an increase in extracellular volume to 26.9% from control and dextran levels of 19%. Plasma albumin levels rose to 3.0 g per 100 ml. The dextran-induced changes in the liver were reversed. The plasma volume decreased to 9.1, equilibrium albumin space to 11.1, and liver C^{14}-sucrose space to 19.8 ml per 100 g. The interstitial albumin concentration fell to 0.51 g per 100 ml. This study demonstrates that the concentration of albumin in liver interstitial fluid varies inversely with albumin synthesis and supports the concept that changes in albumin concentration in this area could be the effective stimulus regulating albumin synthesis.

An Elevated Microglobulin in Uremia. ALBERT L. RUBIN, KURT H. STENZEL, GLENN D. LUBASH, DORTHE PFAHL, AND PETER F. DAVISON, New York, N. Y., and Boston, Mass. (introduced by David D. Thompson).

Many studies have attempted to correlate chemical abnormalities with clinical status in uremia; compounds of low molecular weight often give poor correlation, and there has been no demonstration of a significant macromolecular abnormality. In this study, a nondialyzable protein has been found in markedly higher concentrations in uremic than in normal subjects, and has been isolated and characterized by physical and chemical technics. Blood is collected with disodium EDTA as the anticoagulant and is centrifuged at 4° C. Plasma proteins are precipitated in 5% trichloroacetic acid. Measurable protein material remains in the supernatant fluid, and is present in markedly higher concentrations in plasma from uremic patients than from normal subjects. The supernatant fluid is dialyzed against a triethylamine-acetic acid-water buffer to remove compounds of low molecular weight and to prepare the material for additional studies. The protein material is not dialyzable, confirming the clinical observation that little reduction in concentration was achieved in patients treated by peritoneal or hemodialysis. Electrophoresis on paper at pH 8.6 shows migration in the α-globulin fraction of serum. On this medium and on cellulose acetate and starch gel, there is migration as a single band. Affinity for amido black and periodic-acid-Schiff stains, and a protein-bound hexose content of 16 to 28% indicate that the material is a protein with a high carbohydrate content. Its electrophoretic migration in different buffers and its amino acid composition characterize it as a strongly acidic glycoprotein. Ultracentrifugal patterns show a near-symmetrical peak, and a molecular weight of approximately 40,000 was estimated by the method of Ehrenberg and by assuming a partial specific volume of 0.74. The protein is of relatively low molecular weight, but may also be considered macromolecular because of its nondialyzable properties and ultracentrifugal behavior. In all 27 patients with uremia studied thus far, this protein has been found in markedly increased amounts.

A Role for Alkaline Phosphatase in Controlling DNA Synthesis. J. R. RUBINI, Dallas, Tex. (introduced by Ben Friedman).

While the widespread occurrence of tissue alkaline phosphatase (AP) has long been appreciated, the physiological role of this enzyme has remained unknown. The present experiments examined the hypothesis that AP may impair DNA synthesis by dephosphorylating thymidylate (TDRP) to thymidine (TDR). Since endogenous TDR is a minor source of DNA-thymine, such AP effects could serve to "divert" significant amounts of TDRP essential for DNA synthesis. To test this hypothesis, partially purified and dialyzed AP from normal human granulocytes, normal human placenta, and calf intestinal mucosa were shown readily to dephosphorylate TDRP. The varied AP were then preincubated with cell suspensions of normal dog marrow, lymph, and human leukemic blood leukocytes. After subsequent incubation with H^3-TDR for 1 hour, slide radioautographs of the cells were prepared. Tritiated DNA synthesis by AP-treated cells was seriously impaired ($> 50\%$) as compared to control incubations without AP or containing boiled AP. Further inhibition of H^3-DNA formation was achieved by adding metal ions known to activate selectively AP of these various tissues. The present results demonstrate impaired H^3-DNA synthesis, presumably resulting from pinocytosis of AP by proliferative cells. If H^3-DNA is a tracer for DNA synthesis, then AP may play an important role in controlling DNA synthesis and growth.

Inactivation of Adipokinetic Peptides by Adipose Tissue. DANIEL RUDMAN,* STANLEY J. BROWN, MARTIN F. MALKIN, AND LUIS A. GARCIA, New York, N. Y.

Several hypophyseal peptides stimulate adipose tissue to convert stored triglyceride into free fatty acids. Adipose tissue from different species varies in its responsiveness to these adipokinetic peptides. Thus, rabbit and guinea pig adipose tissue responds to ACTH, β-MSH, vasopressin, and fraction H; rat adipose tissue responds to ACTH, but not to the latter 3 peptides. Rat adipose tissue homogenate contains a peptidase, or group of peptidases that abolish the adipokinetic activity of ACTH, β-MSH, vasopressin, and fraction H. The peptidase system, which is attached to a particulate component of the homogenate, cleaves at least 3 of the 38 peptide bonds in ACTH. Homogenates of adipose tissue from the rabbit or guinea pig do not reduce the adipokinetic activity of any of the peptides above. Operation of the peptidase system in surviving slices of rat adipose tissue is suggested by these characteristics of this tissue: *a*) rapid disappearance of added adipokinetic peptides from the incubation medium; *b*) absence of response to β-MSH, vasopressin, or fraction H; and *c*) dependence of the response to ACTH upon continuous supply of the peptide to the tissue. In contrast, slices of adipose tissue from the rabbit or guinea pig do not remove a detectable amount of hormone from the medium; these slices are responsive to β-MSH, vasopressin, and fraction H; their response to ACTH continues for at least 2 hours after exposure to the hormone is terminated. Differences between species in responsiveness of their adipose tissue to hypophyseal peptides appear to be related to differences in peptidase content of the fat cell.

Pathways of Transport and Metabolism of C^{14}-Vitamin D_2 in the Rat. DAVID SCHACHTER,* JAMES D. FINKELSTEIN, AND SZLOMA KOWARSKI, New York, N. Y.

Randomly labeled C^{14}-vitamin D_2 was prepared by aerobic incubation of yeast with C^{14}-acetate, isolation of the C^{14}-ergosterol as the digitonide, ultraviolet irradia-

tion of the ergosterol, and crystallization of the radioactive vitamin D_2 as the 3,5-dinitrobenzoyl ester. Three pathways have been identified and studied with the radioactive vitamin. After oral administration to rats with a lymph fistula, C^{14} appeared in the intestinal lymph within 20 to 60 minutes, and absorption continued for 18 to 20 hours. The radioactivity was largely in the chylomicron fraction of the lymph, and it was identified as mainly vitamin D_2 by thin-layer chromatography in two solvent systems. No esterified vitamin was detected. The intestinal absorption, studied further with intestinal loops *in vivo,* was more rapid from duodenal and jejunal segments than from ileal segments. Vitamin D_2 is also transported into the small intestinal mucosa from the bloodstream, as demonstrated by intravenous injection of the radioactive sterol. Moreover, the physiological action of the vitamin in the mucosa, i.e., restoration of the active transport mechanism for calcium adsorption in vitamin-deficient rats, is demonstrable 6 hours after a large dose intravenously. The effect is more rapid than that observed after oral administration. A third pathway of transport of C^{14}-vitamin D_2 is from the bloodstream to the bile, as demonstrated in rats with a bile duct fistula. This route involves significant metabolism of vitamin D_2 to water-soluble products in the bile. Ligation of the common bile duct does not interfere with restoration of the calcium transport mechanism when the vitamin is given intravenously to depleted rats.

Effects of Ethanol on the Liver: Evidence for the Preferential Synthesis of Triglycerides. ROBERT L. SCHEIG AND KURT J. ISSELBACHER,* Boston, Mass.

In the fatty liver produced by ethanol, there is a predominant accumulation of triglycerides as compared with phospholipids. Considerable debate has arisen concerning the source of the increased amounts of fatty acids in the liver, but little attention has been directed to the mechanism whereby the fatty acids, irrespective of their source, are esterified primarily to triglycerides. Studies were carried out with liver slices from female rats given either ethanol (7.5 g per kg), or isocaloric amounts of glucose. The *in vivo* and *in vitro* effects of ethanol on the incorporation of acetate-1-C^{14} and palmitate-1-C^{14} into lipids were compared. Thin-layer and gas-liquid chromatographies were employed to separate lipid components, and radioactivity was measured by liquid scintillation spectrometry. Addition of ethanol to the medium resulted in increased fatty acid synthesis and labeling of glycerides as described previously by others. These *in vitro* effects were enhanced when liver slices from ethanol-treated animals were used. *In vivo* effects of ethanol were studied by comparing slices from ethanol against glucose-treated animals. Here, unlike the *in vitro* effects, ethanol did not cause increased fatty acid synthesis. However, a striking shunt of fatty acids into the tryglyceride moiety was noted. In this fraction, up to 100% more label from either acetate or palmitate occurred. Accompanying this increased synthesis of triglyceride, there was a reciprocal decrease in phospholipid formation. Changes in fatty acid composition observed by gas chromatography coupled with the failure to find increased fatty acid synthesis under the conditions of the *in vivo* experiments suggest that the fatty acids esterified were primarily derived from peripheral fat depots. These studies indicate that ethanol *in vivo* promotes in the liver a preferential esterification of fatty acids to triglycerides. This finding appears relevant to the pathogenesis of the fatty liver produced by ethanol.

The Coefficient of Retraction—A Useful Method for Assessing Pulmonary Elasticity. DONALD P. SCHLUETER AND WILLIAM W. STEAD,* Milwaukee, Wis.

Identification of the patient with incipient pulmonary emphysema continues to be unsatisfactory. In an attempt to improve the laboratory diagnosis of this disease, we have devised an expression that gives weight to the two most common abnormalities noted in emphysema and in pulmonary fibrosis: change in pulmonary volume and elasticity. The volume is taken at 0.2 L less than the total lung capacity (TLC, measured by plethysmography and corrected for height), and the transpulmonary pressure is measured at that volume (by the esophageal balloon method during interruption of air flow). We have termed this relationship the "coefficient of retraction," since it is a measure of the static elastic forces of the fully expanded lungs. It may be expressed: CR = transpulmonary pressure at TLC − 0.2 L/(TLC − 0.2 L). Three groups of subjects have been studied, and the results compared: 10 patients aged 40 to 55 years with pulmonary emphysema identified by usual clinical and laboratory criteria (group I), 11 normal subjects of the same age range (group II), and 26 normal young subjects aged 18 to 22 years (group III). The results are: compliance—group I, 0.34 ± 0.10; group II, 0.22 ± 0.054; and group III, 0.22 ± 0.058. Coefficient of retraction—group I, 2.28 ± 0.51; group II, 6.89 ± 1.78; and group III, 6.85 ± 1.40. In contrast, two patients with pulmonary fibrosis showed CR of 11 and 17.5. There was considerable overlap of the compliance between the normal and emphysematous subjects, despite a significant difference between the means. But with the CR there was no overlap between the groups, even ± 2 SD. Pulmonary emphysema and pulmonary fibrosis are many-factored disorders. The method described constitutes an improved means of detection of alterations in one of the important factors involved: change in pulmonary retractility.

Organization of Enzymes in Erythrocyte Membranes. STANLEY L. SCHRIER, LYDIE S. DOAK, AND BRITA ROHDIN, Palo Alto, Calif. (introduced by David A. Rytand).

Transport of inorganic phosphate (P_i) into mature human erythrocytes is an active process. When whole erythrocytes are incubated with P_i^{32}, the first labeled

compound that appears within them is ATP^{32}. This observation led Bartlett and others to an hypothesis for P_i transport in which control was mediated by glyceraldehyde phosphate dehydrogenase (GAPD) located in the erythrocyte membrane: glyceraldehyde-3-phosphatase + DPN + $P_i \overset{GAPD}{\rightleftharpoons}$ 1,3-diphosphoglycerate + DPNH. Subsequently, phosphoglycerate kinase (PGK) completed conversion of incorporated P_i into ATP: 1,3-diphosphoglycerate + ADP $\overset{PGK}{\rightleftharpoons}$ 3-phosphoglycerate + ATP. In order to test this hypothesis, human erythrocyte membranes were prepared by stepwise osmotic lysis. These membranes are whole, biconcave, and disk-shaped, have 0.36% of their original hemoglobin, and, as previously reported, contain both GAPD and PGK. To determine where in the membrane GAPD and PGK were located, whole membranes were sonicated so that their structure was completely destroyed as seen by phase microscopy. Whole and sonicated membranes were then assayed for GAPD and PGK along with similarly treated, commercial, soluble GAPD and PGK. Sonication induced a 50% increase in membrane PGK that was detectable in two assay systems. In contrast, sonication resulted in a 30% decrease in both membrane GAPD and commercial GAPD, while commercial PGK was unchanged. Sonication produced no change in other membrane enzymes tested: aldolase, transketolase, and phosphoribiosomerase. These studies demonstrating a distinct increase in PGK after sonication can be interpreted as showing that a significant portion of PGK is located within erythrocyte membranes, whereas all the GAPD is on the surface. The hypothesis regarding P_i transport in erythrocytes can be restated as follows: extracellular P_i first comes into contact with GAPD on the membrane outer surface, but final conversion to ATP is catalyzed by PGK located within the membrane. This scheme also implies formation in the membrane of ATP, which is required for Na^+, K^+ transport in erythrocytes.

Abnormal Anal Sphincteric Reflex in Patients with Scleroderma. MARVIN M. SCHUSTER, PERRY HOOKMAN, DONALD F. TOW, AND ALBERT I. MENDELOFF,* Baltimore, Md.

We have demonstrated abnormality of the internal anal sphincteric reflex in patients with scleroderma by use of a new manometric technique. This abnormality is correlated with clinical symptoms. Patients with scleroderma may have severe upper or lower gastrointestinal symptoms. Although motor abnormalities of the upper gastrointestinal tract are readily demonstrated by radiologic techniques and esophageal pressure recordings, motor studies of the lower bowel, particularly the rectum and anus, have been more difficult to perform and even more difficult to assess. To meet these objections, a technique was devised to record simultaneously pressure from *1*) the rectum, *2*) the internal anal sphincter, and *3*) the external anal sphincter. In fifteen normal subjects, balloon distension of the rectum produced reflex relaxation of the internal anal sphincter and reflex contraction of the external anal sphincter. On the other hand, in three patients with scleroderma, the internal sphincter reflex was completely absent. All three patients had symptoms of constipation or incontinence. In four patients with scleroderma who had no lower gastrointestinal symptoms, a normal reflex was present. The external sphincter response was present in all seven patients. This study of the anal sphincter reflexes suggests selective impairment of smooth muscle function (internal sphincter) with sparing of striated muscle function (external sphincter) in patients with scleroderma.

A Functional Locus in the Insulin Molecule. I. L. SCHWARTZ, N. DI FERRANTE, N. D. LEE, P. M. EDELMAN, AND H. BURLINGTON, Cincinnati, Ohio, Memphis, Tenn., and Upton, N. Y.

In studies of the binding of I^{131}-labeled iodoinsulin to rat epidymal fat pads, skeletal muscle, and partially purified skeletal muscle cell membranes, we have obtained evidence that either the cyclic disulfide, or at least one interchain sulfur atom of the A chain is involved in the hormone-tissue interaction, the latter alternative implying cleavage of the hormone before fixation of the A and B chains to separate sites on the receptor. While attempting to determine whether the A-chain cysteine residues are involved as functional groups in the hormone-receptor reaction, or whether they serve only as sites of attachment, we had an opportunity to appraise the tyrosyl residues as functional groups in the physiological action of insulin. Insulin was iodinated under several different sets of conditions, one of which resulted in selective labeling of the A chain. The iodoinsulin preparations obtained were tested for binding to tissues and physiologic activity (hypoglycemic potency, potentiation of sugar transport into skeletal muscle, and CO_2 evolution from adipose tissue). Samples of these preparations were subjected to performic acid oxidation, tryptic hydrolysis, or to both procedures successively; the cleavage products were then separated and assayed for radioactivity. It was found that physiologic activity was not correlated per se with the degree of iodination, at least over the range of 1 to 4 atoms of iodine per molecule of insulin. Also, the amount of labeled hormone bound per unit weight of tissue proved to be similar for active, partially active, and inactive preparations of iodoinsulin. Furthermore, the hormonal activity was depressed specifically in proportion to the degree of iodination of the tyrosyl residue in position 26 on the B chain, suggesting this group as a functional locus on the insulin molecule.

Systemic Lupus Erythematosus in the Dog. ROBERT S. SCHWARTZ, ROBERT M. LEWIS, WILLIAM B. HENRY, AND CHARLES E. GILMORE, Boston, Mass. (introduced by William Dameshek).

Seven dogs that spontaneously developed typical features of systemic lupus erythematosus have been

studied. They had the following features: severe autoimmune hemolytic anemia in each case, idiopathic thrombocytopenic purpura in four cases, and severe glomerulonephritis in four cases. The disease was confined to young adults, there was no relation to breed, and there were five females and two males in the series. One dog had the sequential development of salicylate-responsive lameness, an eruption on the butterfly area of the face, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, and glomerulonephritis. Biopsy of this dog's skin rash showed lesions identical to diskoid lupus. The LE clot test was positive in six dogs. Two of four animals tested had antinuclear antibody (fluorescent method), as did the asymptomatic daughter of one of the propositi. The latex fixation test was positive in two, and three animals had positive thyroid antibody tests. The antiglobulin test was positive during the phase of the disease in which hemolytic anemia was present. Eluates prepared for antiglobulin-positive erythrocytes sensitized randomly selected, normal, dog red cells. The Hinton test was negative in all. Pathological findings included wire loop lesions of glomeruli, myocarditis, and hepatitis. It is thus evident that systemic lupus erythematosus may occur in at least three vertebrate species: mice, dogs, and man. The sequential development of multiple immunologic abnormalities in these dogs bears a strong resemblance to systemic lupus erythematosus in man.

Characterization of the Mechanism of Hemolysis of Human Erythrocytes by Antibody and Complement. DAVID A. SEARS, ROBERT I. WEED, AND S. N. SWISHER,* Rochester, N. Y.

This study was undertaken to determine whether complement-dependent antibody injury to human erythrocytes results in hemolysis by a colloid-osmotic mechanism as shown by Green and associates in ascites tumor cells and mouse erythrocytes. The size of the antibody-complement-induced membrane defect also has been studied. Human erythrocytes were incubated with rabbit antihuman red cell serum, or complement-fixing human anti-A and fresh autologous human serum. The amount of hemolysis produced was compared to the percentage of erythrocyte potassium lost into the medium. In the studies with rabbit antibody, addition of osmotically active macromolecules, including albumin and a series of dextran fractions, in extracellular concentrations sufficient to balance the osmotic pressure of intracellular hemoglobin prevented hemoglobin escape from the cells, while potassium loss was practically complete. This type of "protection" against hemolysis was provided by bovine serum albumin (molecular weight 69,000) and by Dextran-40 (number average mol wt 26,200) but not by Dextran-10 (number average mol wt 6,200). In the studies with human anti-A, neither albumin nor hemoglobin added to the medium provided significant protection. Thus, hemolysis of human erythrocytes by *rabbit* antibody and complement in this system occurs by a colloid-osmotic mechanism. Loss of cation is related to creation in the membrane of functional "holes" through which hemoglobin does not pass. Induction of cation permeability is followed by osmotic swelling and rupture. From the size of the macromolecules required to protect against hemolysis in this system, it can be estimated that the membrane "holes" are between 17 and 35 A in radius. Failure of macromolecules to protect against hemolysis by *anti-A* indicates that hemoglobin is lost directly through membrane defects that are larger than 35 A in radius.

Human Adaptation to Chronic Anemia. DEAN J. SEIBERT AND FRANKLIN B. EBAUGH, JR.,* Hanover, N. H.

This study is concerned with the mechanisms of human adaptation to chronic anemia. The level of hemoglobin at which tissue hypoxia develops at rest was determined by measure of the arterial lactate-pyruvate ratio (L/P). The mean L/P ratio of 15 chronically anemic patients (hemoglobin 2.6 to 7 g per 100 ml) was 4.46 ± 1.26 and $3.84 \pm .793$ in the same series of patients after correction (hemoglobin 9.4 to 15 g per 100 ml for at least 2 weeks). The fall in L/P ratio after correction of the anemia was not significant ($p > 0.1$). There was a significant inverse correlation between the L/P ratio and the level of circulating hemoglobin from 7 to 2.6 g per 100 ml ($Y = 8.32 - 0.73x$); $r = -0.635$; $p < .01$). L/P ratios of 7.20, 7.32, 6.34, 4.82, and 4.29 for patients with circulating hemoglobin of 4.3, 4.4, 2.6, 3.9, and 4.0 g per 100 ml, respectively, were obtained. The first three were more than 2 SD away from the mean for all patients (3.98 ± 1.17). The ratio capillary/deltoid muscle fiber was 0.86 ± 0.24 for 8 anemic patients and was 1.07 ± 0.28 after correction of the anemia. The capillary/cardiac muscle fiber ratio derived from PAS-stained, noninjected, fixed sections from autopsy material was 0.56 ± 0.13 for 12 anemic patients (7.5 g per 100 ml or less) and 0.67 ± 0.18 for 12 nonanemic controls matched for age and sex. Neither change was statistically significant. The mean total blood volume (Cr^{51}-labeled erythrocyte mass/venous hematocrit $\times$ 0.9) of 17 chronically anemic patients (hemoglobin 4.0 to 6.3 g per 100 ml) was 51.5 ml per kg $\pm$ 18.2 compared to the expected normal of 63 ± 3.4 ml per kg. The difference is significant ($p < .02$). In conclusion, tissue hypoxia occurs in patients at rest with hemoglobin levels below 5 g per 100 ml. The decrease in total blood volume in anemia has been confirmed, and no increase in capillarity of cardiac or skeletal muscle occurred.

Evidence for Active Transport Regulation of Cerebrospinal Fluid pH and Its Effect on the Regulation of Respiration. J. W. SEVERINGHAUS* AND R. A. MITCHELL, San Francisco, Calif.

Lumbar CSF pH averaged 7.331 in 20 Peruvian Andean natives (at 3,800 to 4,820 m). In four sea-level residents, during acclimatization to 3,800 m (White Mt., Calif.) while blood pH rose from 7.42 to 7.48, CSF pH rose only from 7.328 to 7.336, remaining constant

for eight days. While blood buffer base fell only 1 mEq per L in 8 days, CSF bicarbonate fell 5 mEq per L in 24 to 48 hours and CSF Cl^- rose 5 mq per L. CSF lactate rose only 1 mEq per L. The data suggested active transport regulation of CSF pH by the blood CSF barrier in response to the initial mild alkalosis induced by hypoxic stimulus of peripheral chemoreceptors. We found CSF potential to be +3 mv to blood in four dogs under pentobarbital anesthesia and in five normal men. If bicarbonate distribution were controlled passively, the CSF/blood concentration ratio would be 1.12, whereas in the four subjects this ratio was 0.90 at sea level, and fell to 0.80 after 2 days at 3,800 m. Furthermore, CSF pH reported in metabolic acidosis and alkalosis has been strikingly more normal than blood pH, independent of duration of disease. This suggests that the CSF bicarbonate changes in all acid-base abnormalities may be due to active transport regulation of CSF pH, rather than gradual penetration of the blood CSF barrier. CSF pH regulation, coupled with Mitchell's evidence that medullary respiratory chemosensitivity responds primarily to CSF $[H^+]$, helps explain respiratory control in acid-base disturbances. From CO_2 response curve data during acclimatization, we computed the relationship of ventilation to CSF pH and found it unaltered from sea level. Bicarbonate reduction thus explains the "increased sensitivity" of the respiratory center to CO_2 at altitude.

Comparison of Pathways of Pressor Response to Noxious Stimuli. ALVIN P. SHAPIRO, SPERO E. MOUTSOS, AND EMANUEL KRIFCHER, Pittsburgh, Pa. (introduced by I. Arthur Mirsky).

Blood pressure responses to noxious stimuli are determined by several intrinsic factors, including arteriolar sensitivity to circulating humoral agents and the adequacy of the autonomic nervous system (ANS). To elucidate the roles of humoral and ANS reactivity and to study their interactions in different conditions, responses to a single iv injection of 0.03 μg per kg of angiotensin II and to a cold pressor test were compared in 112 subjects. The subjects also were tested with two previously described psychological stimuli, i.e., a venipuncture accompanied by mental arithmetic and a frustrating color-reading test. Automatic indirect blood pressure and pulse recording equipment was used. Subjects consisted of 32 normotensive (N), 35 hypertensive (H), 25 normotensive diabetic (N-D), and 20 hypertensive diabetic (H-D) subjects. Responses (expressed as average increases in calculated mean blood pressure in mm Hg and subjected to analysis of variance) to angiotensin, cold pressor, venipuncture, and "color," respectively, were: N—26.2, 19.6, 9.4, and 8.1; N-D—35.5, 16.1, 12.7, and 10.1; H—39.6, 30.1, 19.3, and 15.0; and H-D—45.4, 18.6, 14.5, and 11.2. Hypertensive subjects exhibited significantly greater reactivity to all stimuli. Diabetic subjects displayed a lesser cold pressor (ANS) reaction, but an enhanced angiotensin (humoral) response. Median angiotensin/cold pressor ratios were: N, 1.26; N-D, 2.57; H, 1.30; and H-D, 2.62. Probabilities of a ratio > 2.0 were 72% and 65% in N-D and H-D, respectively, and 26% and 14% in N and H ($p < .01$). Therapeutic administration of sympatholytic drugs in hypertensive subjects similarly raises angiotensin/cold pressor ratios above 2.0. Relative maintenance of responses to psychological stimuli with impairment of neurogenic mechanisms was noted. Data indicate: *1*) humoral and neurogenic responses both are enhanced in hypertensive subjects, implying an important arteriolar component to their hyperreactivity, and *2*) vasoactive polypeptides such as angiotensin may provide a humoral mechanism alternatively controlling acute vascular responsivity, particularly when neurogenic mechanisms are depressed.

Studies on the Biological Action of Aldosterone In Vitro. GEOFFREY W. G. SHARP AND ALEXANDER LEAF,* Boston, Mass.

Previous studies have shown that aldosterone stimulates active sodium transport by the isolated urinary bladder of the toad. A recent refinement in technique allows marked improvement in reproducibility and sensitivity. Responses are obtained with *d*-aldosterone (4.6×10^{-9} M), desoxycorticosterone (5×10^{-7} M), corticosterone (1×10^{-5} M), and cortisol (1×10^{-5} M) in the serosal bathing medium. Cortisone does not stimulate even at 10^{-4} M, but it antagonizes the stimulation by cortisol. The effect of aldosterone is antagonized by progesterone and spironolactone. Ouabain, which has little or no inhibitory effect on the unstimulated transport rate, also promptly antagonizes the aldosterone effect. The effect of aldosterone is apparent about 1 hour after application and reaches its maximum at about 2 hours. At high concentrations the effect is considerably prolonged. Exposure of the tissue to hormone for only 5 minutes suffices to elicit a qualitatively normal response after the usual delay. Aldosterone also produces an effect when applied to the urinary surface of the bladder, but a larger dose is required. In association with this stimulation of sodium transport, aldosterone significantly increased the tissue pool of sodium as measured isotopically after addition of Na^{24} to the mucosal medium ($n = 13$, $p < 0.001$). The site of action of aldosterone is, therefore, localized at (or near) the plasma membrane of the urinary surface of the single layer of mucosal cells of this tissue. This surface is also the site of action of vasopressin. This method, which provides a rapid and simple measurement of the physiological action of aldosterone in a tissue in which the transport system is well characterized, should facilitate the systematic investigation of the mechanisms of action of this important hormone.

On the Mechanism of Feminization in Laennec's Cirrhosis. JOYCE C. SHAVER, MARTIN S. ROGINSKY, AND NICHOLAS P. CHRISTY,* New York, N. Y.

The feminizing features of Laennec's cirrhosis have been ascribed to impaired degradation of endogenous

estrogen. Conflicting bioassay data have shown either normal or delayed metabolism of estrogen in hepatic disease. This study represents application of radioisotopic technics to the problem. Ten normal subjects and six patients with typical Laennec's cirrhosis were investigated as follows. Two to 3 μc of chromatographically pure 4-C^{14}-estradiol-17β (SA, 0.04 mc per mg) or 18 to 20 μc of 6,7-H^3-estradiol-17β (SA, 135 mc per mg) was rapidly administered intravenously; 25 to 50-ml blood samples were obtained at 15-minute intervals for the first 90 minutes, then at 30-minute intervals up to 3 hours; fractional urine collections were made from 0 to 120 hours. Plasma was extracted with chloroform (recovery of radioactivity 88 to 93%) and urine, with ethyl acetate (recovery $>$ 90%); radioactivity was assayed in a liquid scintillation spectrometer. Disappearance of radioactivity from plasma described a double exponential: $t_{\frac{1}{2}}$ of the first (method of least squares) averaged 22 minutes (range 17 to 29) for normal, 36 minutes (range 27 to 56) for cirrhotic subjects ($p < 0.01$); $t_{\frac{1}{2}}$ of the second exponential was the same for normal (96 minutes, range 91 to 135) and cirrhotic subjects (104 minutes, range 77 to 151, $p > 0.10$). Chromatographically isolated plasma estradiol was similarly cleared. Calculated apparent volume of distribution (AVD) of isolated estradiol was 3 to 15 times larger than that of aldosterone, cortisol, or corticosterone, indicating greater extravascular sequestration of estradiol. AVD of estradiol was 3 times larger than normal in cirrhosis, indicating impaired capacity of the cirrhotic liver to concentrate estrogen. Urinary excretion of total radioactivity was not reduced in cirrhosis: normal subjects excreted 11 to 16% of administered dose in 0 to 3 hours, 56 to 80% in 4 days; cirrhotic subjects excreted 17 to 28% in 0 to 3 hours, 60 to 90% in 4 days; excretion of free (unconjugated) radioactivity was 1 to 3% in 4 days in both groups. The demonstration of impaired estradiol degradation and distribution together with normal excretion and, by inference, secretion necessitates the conclusion that circulating level of estrogenic hormone is elevated in Laennec's cirrhosis.

Internal Conduction Plethysmography of the Dog Left Ventricle. L. THOMAS SHEFFIELD, Birmingham, Ala. (introduced by Tinsley R. Harrison).

A means of measuring a direct function of ventricular size has been sought that would be both clinically accurate and practical for use in either the experimental animal or the intact human subject. Several methods satisfy the first requirement, but not the latter. Nyboer has shown that the resistance of a blood-containing body segment, between two externally applied electrodes, is a compound function of the quantity of blood within the segment, tissue resistance, and skin resistance. Our experiments have shown that by placing the sensing electrodes within the blood itself and measuring in terms of conductivity, there is a direct relationship between electrical measurement and cavity size. This relationship was tested in the pulmonary artery, aorta, and left ventricle in the dog, and in the human pulmonary artery. Quantitation of the electrical measurement into units of radius or volume has involved a technic of measuring electrode conductivity in a graduated series of electrolyte-filled hollow models and, in either calibration or dynamic measurements, dividing the result by the measured specific conductivity of the electrolyte medium itself, to obtain that component of conductivity affected by size only. A series of experiments measuring the agreement between this internal conductance plethysmogram (ICP) and the inner ventricular radius as calculated from the mercury circumference gauge was undertaken. This showed good correlation between the two when the ICP interelectrode distance was such that a representative "sample" of ventricular cavity was included between the two electrodes. The time course of ventricular volume in the dog heart, as measured by the ICP, is described, and its potential use in estimating size and compliance of cardiac chambers and large vessels of the catheterized human subject is discussed.

Sandfly Fever in the New World. A. SHELOKOV, J. A. BRODY, AND R. V. MCCLOSKEY, Bethesda, Md., and Canal Zone, Panama (introduced by Charles L. Wisseman, Jr.).

Sandfly fever, an important epidemic disease of the Old World, has been regarded as being limited to the areas of Europe, Africa, and Asia that harbor *Phlebotomus papatasi.* A virus recovered in Panama from the blood of a febrile patient was shown to be antigenically related to a Brazilian virus isolated from a forest rodent (Shope), while this agent in turn is related to the prototype Naples virus of sandfly fever. The significance to public health of these findings for the New World is indicated by the demonstration of antibodies to the human blood isolated among the population of Panama and other Latin American countries.

Pathogenesis of Etiocholanolone Fever. JONAS A. SHULMAN AND WALTER HERRMANN, Seattle, Wash. (introduced by W. M. M. Kirby).

Etiocholanolone is known to produce fever in man, but the mechanism of pyrexia caused by this steroid is not understood. In this experiment, over 30 volunteers were injected with etiocholanolone, and the following observations were made. Etiocholanolone is pyrogenic only after intramuscular, but not after intravenous injection. Considerable inflammation occurs at the site of injection, and fever follows 8 hours later. The metabolism of tritiated etiocholanolone has been studied after im and iv injection. After iv injection, the half-life of free etiocholanolone is 10 minutes, and the fall in free etiocholanolone is associated with rapid conjugation to glucuronide and sulfate. These fractions are cleared within another 30 to 50 minutes. After im injection, peak

absorption occurs between 90 and 150 minutes, and free etiocholanolone, etiocholanolone sulfate, and etiocholanolone glucuronide are completely cleared within 4 hours. At the height of the fever, 8 hours after injection, there was only minimal radioactivity in the plasma. This was limited to etiocholanolone sulfate, which is not pyrogenic. After im injection of 50 mg of etiocholanolone, fever and leukocytosis occurred in 70% of subjects. The evaluation of local inflammation by measurement of the skin temperature at the site of injection made it possible to predict the onset and the magnitude of systemic fever and of leukocytosis. Of 4 leukopenic patients injected, 3 had no fever or rise in peripheral leukocyte counts. One leukopenic patient who responded with fever had a sixfold increase in leukocytes. The data suggest that experimental etiocholanolone fever does not correlate with the plasma level of free or conjugated etiocholanolone and that inflammation, rather than a specific pyrogenic effect, may be the most important factor in the pathogenesis of etiocholanolone fever.

Inactivation of Factor VIII (AHF) by a Gamma Globulin Anticoagulant. N. RAPHAEL SHULMAN,* VICTOR J. MARDER, AND ALFRED LEITNER, Bethesda, Md.

The rare hemorrhagic disease of acquired hemophilia that occurs spontaneously in elderly men and women is caused by an anticoagulant that inactivates Factor VIII (AHF). Although the anticoagulant has been found in the gamma globulin fraction of plasma, it has not been proven to be an antibody, and recently has been considered to be an enzyme. The present work concerns the nature of *in vitro* and *in vivo* reactions of an anticoagulant of this type that arose in four previously normal persons. The anticoagulant in each case had chromatographic, electrophoretic, and sedimentation properties of 7 S gamma globulin. The reaction between Factor VIII and the anticoagulant was consistent in all respects with formation of an antigen-antibody complex; namely, the reaction proceeded at 0° C at a rate approximately $\frac{1}{4}$ the rate at 37° C, and the equilibrium between reactants obeyed the law of mass action. The anticoagulant-Factor VIII complex was soluble, did not form a line in agar gel, and did not fix complement. It was apparent from results of injecting the anticoagulant into animals and man, and from injecting Factor VIII into persons having the anticoagulant that the stoichiometry and rate of the reaction *in vivo* was the same as it was *in vitro*. These observations led to the following sensitive *in vivo* test for anticoagulant. Fifty ml of fresh normal plasma is injected into a patient who lacks Factor VIII and is suspected of having an anticoagulant. Anticoagulant is present if the clotting time, or the two-stage prothrombin consumption test, or both, are not normal for more than 1 hour after injection. This test can detect levels of anticoagulant that are too low to demonstrate by *in vitro* tests.

The Role of the Phrenicoesophageal Ligament in the Lower Esophageal Sphincter. CHARLES I. SIEGEL AND ELLIOTT MICHELSON, Baltimore, Md. (introduced by Leighton E. Cluff).

The lower esophageal sphincter is now generally recognized as the principal barrier to gastroesophageal reflux. The role of the several factors controlling the resting pressure within this sphincter has not been clearly defined. In order to dissect the influence of various structures on this mechanism, manometric studies were performed before and after surgical modification of individual structures in the region of the esophagastric junction. The study being reported describes the observations made on the effect of section and resuture of the phrenicoesophageal ligament. In 9 dogs, division of the phrenicoesophageal ligament was performed without modification of other structures. In 8 of 9 dogs, this resulted in a diminished resting pressure in the lower esophageal sphincter from 12.7 to 4.7 mm Hg above intragastric pressure. After subsequent surgical restoration of the phrenicoesophageal ligament, mean intrasphincteric pressure was restored to 11.2 mm Hg above intragastric pressure. The diaphragm did not play a role in these pressure changes as the hiatus itself was not altered during this study. The observations reported strongly suggest that the phrenicoesophageal ligament plays an important role in maintaining the tone of the lower esophageal sphincter. This may have important implications in the surgical repair of hiatus hernia, where restoration of the integrity of the phrenicoesophageal ligament may be of great importance in preventing gastroesophageal reflux.

Determination of Left Ventricular External Work and Power in Intact Man. ROBERT E. SNELL AND PETER C. LUCHSINGER, Washington, D. C. (introduced by Donald L. Fry).

Mechanical performance of the left ventricle in intact man has previously been described on the basis of mean pressure-flow measurements. In the present study, computations of left ventricular hydraulic power output (exclusive of the coronary circulation) have been made using instantaneous flow curves obtained in the ascending aorta by the computed pressure gradient technique. Investigations have been carried out in eight persons without cardiovascular disease to determine resting values for external ventricular work and power under various states of loading. Changes in ventricular input and output load were produced by the application of tourniquets to the extremities and by the administration of norepinephrine. Resting external stroke work of the left ventricle ranged from 90 to 150 g-m per beat. Less than 5% of this total was represented by kinetic energy. Power-time curves showed a sharp rise to a peak before mid-systole. During the remainder of the ejection period, power decreased gradually. Stroke work fell significantly during the application of tourniquets inflated to diastolic pressure levels. Minute work decreased to a

lesser extent. Power output was decreased throughout systole. Administration of norepinephrine sufficient to elevate pressure by about 40% resulted in a 60% increase in stroke work. Peak power remained similar to the peak control values. Most of the increased work resulted from maintenance of power output at a high level during late systole.

Fine Structural Basis of Starling's Law of the Heart. EDMUND H. SONNENBLICK, THOMAS S. COTTRELL, AND DAVID SPIRO, New York, N. Y. (introduced by John V. Taggart).

Cardiac output is matched to venous return from beat to beat by virtue of Starling's Law, which is based on the fact that, within certain limits, an increase in initial fiber length results in an increase in the force of the subsequent muscular contraction. In this study, the ultrastructure of heart muscle as seen in the electron microscope was related to contractile force developed at various muscle lengths. Preparations of cat, right ventricular, papillary muscle as well as strips of human right ventricle obtained during corrective heart surgery were used. Analysis of the fundamental unit of contraction, the sarcomere, at precisely defined points along the length-tension curve shows that: *1*) sarcomere length is a function of muscle length, increasing from 1.5 μ where resting and active tensions were zero to 2.2 μ at the apex of the active tension curve and *2*) sarcomere lengthening occurs entirely by widening of the I band with no change in the width of either A band or H zone. These results have been interpreted in terms of the previously described, partially overlapping, filamentous organization of the sarcomere. Accordingly, thin filaments (presumably actin) course from the Z line through I and A bands and terminate at the edge of the H zone, while thick filaments (presumably myosin) extend the length of the A band. Thus, with muscle lengthening, thin filaments are elongated while thicker filaments of the A band remain at constant length. Force of contraction is a function of sarcomere length, which in turn is a function of thin-filament length. These findings provide the first structural explanation for Starling's Law of the heart. They also lend support to a "folding" model for myocardial contraction, and are not consonant with a purely "sliding" model as proposed by Huxley.

Studies on the Formation of the Platelet Plug. THEODORE H. SPAET,* New York, N. Y.

It is likely that the first step in hemostasis is the aggregation of platelets on denuded connective tissue at the site of vascular injury. Data from other laboratories have suggested the validity of damaged mesentery as a model system to study the reaction between platelets and connective tissue; this system was used in the present studies. Rat omentum was traumatized by gentle scraping with a scalpel blade. A small portion of the membrane was then stretched on a frame of two, concentric, plastic rings, freed from the remaining omentum, and agitated briefly in blood or platelet-rich plasma (PRP). The specimen was rinsed in saline, mounted on a cover slip, and examined by phase microscopy. No aggregation of platelets occurred unless the omentum had been traumatized. With rat blood, the following findings were obtained. Exposure of the tissue to PRP resulted in *ad*hesion of numerous platelets to connective tissue fibers, but there was no demonstrable *co*hesion. Platelets stuck to the fibers, not to each other. Additional *co*hesion with formation of platelet "plugs" developed with PRP from heparinized animals, with citrated whole blood, and in PRP from warfarinized animals with no added anticoagulant. When EDTA was the anticoagulant, only *ad*hesion occurred either in PRP or whole blood. Human PRP gave similar reactions with rat mesentery, except that citrated whole blood failed to yield *co*hesion. Addition of ADP to citrated PRP during the process of agitation induced striking *co*hesion with human PRP, but gave equivocal results with rat PRP. The data suggest that platelet *ad*hesion and *co*hesion are separate reactions. Possibly the former is an immediate physical reaction between platelets and connective tissue, and the latter is mediated through ADP. Neither reaction appears to require blood coagulation.

Studies on the Mechanism of Diet-induced Alterations of Plasma Cholesterol. NORTON SPRITZ, SCOTT GRUNDY, AND EDWARD H. AHRENS, JR.,* New York, N. Y.

The mechanisms by which serum cholesterol concentration is altered by exchanges of dietary fat are still unclear, mainly because of methodologic limitations arising from the large number of contaminants with which fecal sterols are excreted and the multiple forms in which they appear. A specific method was therefore developed for the quantitative isolation and identification of acidic and neutral sterols of feces that permits an estimation in man of rates of cholesterol synthesis, excretion, and compartment exchange. In order to determine the effects of dietary fat on sterol balance, seven hospitalized patients were fed liquid formulas containing corn or coconut oils, or similar glycerides free of nonsaponifiable material (three normocholesteremic, two hypercholesteremic, and two hyperlipemic patients). Cholesterol-4-C^{14} was administered orally in some experiments. The following observations were made. *1*) Corn oil glycerides produced lower plasma cholesterol concentrations, whether or not nonsaponifiables had been removed. *2*) Absorption of ingested labeled cholesterol varied from 62 to 86% and was not affected by the type of dietary fat. *3*) During steady states, the excretion of neutral sterols averaged 451 ± 26 mg per day, and of bile acids 283 ± 22 mg per day ($n = 12$), and was not affected by exchange of dietary fats. Slopes of plasma cholesterol specific-activity decay curves were also similar on the two diets. These findings suggest that synthesis was not significantly affected by the quality of dietary fat. *4*) During transition periods, when plasma cholesterol concentrations were altered by exchanges of dietary fat, there were no significant effects on total fecal sterol ex-

cretion rates. There were, however, transient alterations in specific-activity decay curves of plasma cholesterol and fecal bile acids that suggested a temporary increase in the sterol pool from which bile acids are synthesized. These studies indicate, by exclusion, that changes in plasma cholesterol induced by dietary fat result from redistribution of cholesterol between plasma and other body pools.

Phospholipid Content and "Distilled Water Test" in Congenital and Acquired Thrombocytopathias. MARIO STEFANINI* AND MUSTAFA KARACA, Toledo, Ohio, and Izmir, Turkey.

Thromobocytopathia is a hemorrhagic disorder with a bleeding tendency usually discovered at menarche, at surgery, however minor, or after trauma. It is characterized by abnormal size and morphology of the platelets, by prolongation of the bleeding time, and by poor generation of thromboplastin during blood clotting because of deficiency of platelet thromboplastic factor. Thrombocytopathia may be a congenital disorder transmitted through a dominant nonsex-linked gene, or may appear as an acquired abnormality in a number of disease states. A study of the total content and partition of phospholipids in human platelets was conducted with standard procedures of chemical isolation and chromatographic analysis in five documented cases of congenital and in eighteen cases of acquired thrombocytopathia, including three cases where acquired thrombocytopathia appeared related to ovarian dysfunction. The clotting activity of the platelets and of their fractions was studied by use of prothrombin consumption and thromboplastin and thrombin generation systems. The content and partition of platelet phospholipids were normal in congenital thrombocytopathia. The clotting abnormality of these platelets, in fact, could be corrected by treatment with distilled water. On the other hand, platelets from patients with acquired thrombocytopathia showed low content of phopholipids and their fractions. Their thromboplastic activity could not be improved by treatment with distilled water. In conclusion, *1*) the clotting defect of the platelets in congenital thrombocytopathia is primarily one of lack of release of phospholipids exhibiting thromboplastic activity, while a true depletion of phospholipids and of thromboplastic activity is found in acquired thrombocytopathia, and *2*) treatment of platelets with distilled water releases their normal thromboplastic activity in congenital thrombocytopathia.

Humoral Factors in Experimental Pulmonary Embolism. MYRON STEIN, DUNCAN THOMAS, VISHRAM REGE, AND GENZO TANABE, Boston, Mass. (introduced by Stanford Wessler).

The cause of the bronchoconstriction associated with pulmonary embolism in man and the experimental animal has not been fully elucidated. Autologous peripheral venous thrombi were released to the lungs of anesthetized, spontaneously breathing dogs. Embolization produced significant increases in total lung resistance, lung elastance, respiratory rate, and parallel dead-space ventilation. The increases in lung resistance and elastance occurred within 1 minute, lasted from 4 to 30 minutes, and were unrelated to changes in lung volume. Intravenous heparin (5,000 U) given 30 minutes before release of preformed thrombi completely blocked the increases in lung resistance and elastance. Postembolic tachypnea and increase in parallel dead-space ventilation were not altered by heparin. The bronchoconstriction produced by intravenous histamine, acetylcholine, and serotonin was not influenced by prior injection of heparin. The intravenous administration, however, of an antiserotonin agent (1-methyl lysergic acid butanolamide) before release of thrombi prevented an increase in lung resistance except with massive embolization. When the antiserotonin agent was incorporated in the thrombus before release to the lung, increases in lung resistance and elastance did not occur, even in the presence of massive embolization. The effect of heparin demonstrates that the physical presence of emboli in the lungs is not responsible for bronchoconstriction. The inability of heparin to prevent intravenously injected serotonin from causing bronchoconstriction indicates that heparin does not block this action of serotonin. It is suggested that thrombin on fresh emboli induces serotonin release from circulating platelets, and that this release is prevented by the antithrombic action of heparin.

Altered Tubular Sodium and Water Reabsorption during a Saline Infusion: Effects of a Unilateral Reduction in Glomerular Filtration Rate. RICHARD M. STEIN, D. DANNY BERCOVITCH, AND MARVIN F. LEVITT,* New York, N. Y.

The administration of iso- or hypertonic saline to hydropenic dogs at increasing rates for 4 to 6 hours produced a progressive rise in Na excretion that eventually exceeded 20% of the filtered load. During the course of the infusion, as filtered load rose an average of 53%, an increase was noted in $T^c_{H_2O}$ formation and net Na reabsorption. It appears, therefore, that the rate of Na transport at the ascending limb and presumably elsewhere in the tubule is enhanced by a rise in filtered load. Late in the course of the infusion, or occasionally when filtered load failed to rise, a fall in net Na reabsorption was noted. When a unilateral reduction in GFR (10 to 50%) was produced by sustained renal artery constriction, a reduction in $U_{Na}V$, somewhat greater than the percentage fall in GFR, was observed. Thereafter, as the rate of infusion was increased, filtered load tended to rise in parallel fashion on both sides. Filtered load on the constricted side often attained levels observed earlier in the course of the infusion on the nonconstricted side. At equivalent filtered loads, however, Na reabsorption was significantly lower ($U_{Na}V$ significantly greater) on the constricted side. Despite this difference in Na reabsorption, $T^c_{H_2O}$ at comparable filtered loads was similar on the two sides. Hypotonic urine was not formed by either kidney throughout a

wide range of solute clearance. It is concluded that in dogs a saline infusion progressively decreases tubular Na reabsorption, but this phenomenon may be masked by a simultaneous increase in reabsorption provoked by a rise in filtered load (each stimulus possibly producing an opposite effect at separate sites). The observation that $T^c_{H_2O}$ at comparable filtered loads was similar on the two sides despite an apparent inhibition of a reabsorption suggests that this inhibitory effect appears earliest and is most pronounced at some site distal to the ascending limb.

Relation between Fat-Mobilization and Hypermetabolism Induced by Norepinephrine and by Triiodothyronine. DANIEL STEINBERG,* PAUL J. NESTEL, ELSWORTH R. BUSKIRK, AND RONALD H. THOMPSON, Bethesda, Md.

Intravenous infusion of norepinephrine (0.2 to 0.4 μg per kg per minute) into 11, fasting, young, healthy volunteers for periods of 10 to 26 minutes increased rates of O_2 consumption ($\dot{V}_{O_2}$) by 21 ± 8.5% (SD). Concomitant with the increase in $\dot{V}_{O_2}$, serum FFA levels rose by 127 ± 57%. In 7 studies, plasma FFA turnover was measured by using a continuous intravenous infusion of palmitate-1-C^{14}. Calculated turnovers increased by 74 ± 53% (range 11 to 154%). In 3 of these subjects, the minimal rate of conversion of plasma labeled FFA to $C^{14}O_2$ was also measured (total cpm in CO_2 divided by plasma FFA specific radioactivity measured concurrently) and increased by 25, 44, and 94% during norepinephrine infusion. When 75 to 100 mg of pronethalol, a β-adrenergic blocking agent, was given intravenously just before infusion of norepinephrine, the FFA response was partially or completely blocked, FFA turnover and oxidation failed to increase, and there was no increase in $\dot{V}_{O_2}$. Blood pressure and pulse changes were no different from those seen in absence of the blocker. Five triiodothyronine-treated subjects were given intravenous hexamethonium (3 to 9 mg per minute) while in the metabolic chamber. Plasma FFA levels fell by 13 to 33%, and $\dot{V}_{O_2}$ fell by 4.3 to 12.9% despite increased rates of peripheral heat loss. These results suggest that the "calorigenic" effect of norepinephrine in man may be secondary to its FFA-mobilizing action, i.e., the higher concentrations of available substrate may "drive" metabolism in the liver and in peripheral tissues. The present results are compatible with the hypothesis that the hypermetabolism in hyperthyroidism may be secondary to the demonstrated sensitivity of adipose tissue to catecholamine stimulation and the elevated FFA levels seen in this condition.

Localization of the Renal Tubular Defect for Sodium Excretion in Human Hypertension. PHILIP R. STEINMETZ, ERVIN A. GOMBOS, ROBERT P. EISINGER, AND DAVID S. BALDWIN,* New York, N. Y.

Reduced sodium excretion ($U_{Na}V$) in one kidney compared to its mate has been observed in unilateral renal disease associated with hypertension and in essential hypertension. Various mechanisms could be responsible for the reduced $U_{Na}V$, but a particular one might characterize the pressor kidney. Since elaboration of solute-free water (C_{H_2O}) depends upon abstraction of sodium from tubular fluid at the diluting site, the diluting mechanism was examined in hypertensive subjects in an attempt to localize the defect in Na reabsorption. During mannitol diuresis superimposed on maximal water diuresis in 7 normotensive subjects, urine volume increased 2 ml and C_{H_2O} 1 ml per minute for each ml increase in solute clearance (C_{osm}). These increments of C_{H_2O} require an increase in Na delivery to the diluting site, which can be estimated by assuming additional reabsorption of approximately 140 μEq of Na for each ml increment of C_{H_2O}. In 8 of 20 hypertensive subjects, C_{H_2O} was disparate in the separate kidneys. In 3, proportional disparities in GFR and $U_{Na}V$ were also observed, suggesting loss of total nephron function. In 5, decreases in C_{H_2O} ranging from 30 to 60% were associated with reduced $U_{Na}V$ despite essentially equal GFR. Simultaneous C_{H_2O} values in the separate kidneys (with disparate C_{osm}) resembled successive C_{H_2O} values in a single normal kidney during mannitol diuresis. This suggests that the kidney retaining Na dilutes normally, and that the decrease in C_{H_2O} formation reflects reduced Na delivery to the diluting site. These results indicate: *1*) excessive Na reabsorption in some hypertensive subjects is due to a tubular defect proximal to the diluting site, and *2*) the magnitude of disparity between the separate kidneys in tubular reabsorption of Na can be estimated as 140 μEq for each ml difference in C_{H_2O}. The present study provides more precise characterization of the defect than does measurement of $U_{Na}V$.

Studies of the Mechanism of Binding of Thyroxine by Human Serum Albumin. KENNETH STERLING,* New York, N. Y.

Previous equilibrium dialysis studies of the interaction between thyroxine and human serum albumin have indicated that the protein has four primary binding sites per molecule with an association constant of approximately 100,000. The evidence suggested that binding requires an interaction between the dissociated (anionic) phenolic hydroxyl group of thyroxine and cationic groups on the protein molecule, probably epsilon-amino groups of lysine residues. Diiodotyrosine has been found to be very weakly bound by albumin, which supports the concept that the diphenyl ether (thyronine) structure is essential for firm binding. Competitive binding studies with thyroxine analogues were undertaken to evaluate the role of the various reactive groups of the thyroxine molecule. The importance of the dissociable hydroxyl (phenolate) group in the interaction was explored by use of a compound with a nonionizable substituted group, namely, the O-methyl ether of thyroxine (methoxythyroxine), which was studied as a competing ligand. Computations revealed the methyl ether derivative had an association constant approximating 16,000, or only one-sixth that of thyroxine. Modifications of the alanine side chain

resulted in variable reductions in affinity. Tetraiodothyropropionic acid, which differs from thyroxine only in lacking the $-NH_2$ group, showed a relatively slight reduction of binding (association constant of 80,000). This suggests that the carboxyl group may be more important than the amino group. Experiments with the decarboxylated derivative, thyroxamine, were difficult due to limited solubility, but tended to confirm the role of the carboxyl group. The importance of the three-carbon side chain was further suggested by a lower association constant (50,000) for the interaction between albumin and tetraiodothyroacetic acid, and a still lower constant (36,000) when the ligand was tetraiodothyroformic acid. Corroborative information was provided by studies with a variety of analogues and organic compounds. In summary, *1*) the diphenyl ether structure of thyroxine is required for firm binding by human serum albumin, and *2*) it appears likely that the interaction between the anionic phenolate group of thyroxine and cationic groups on the protein molecule is accompanied by an additional interaction of the alanine side chain.

Reflex Pulmonary Vasoconstriction Induced by Aortic Body Stimulation. SHLOMO STERN, RICHARD E. FERGUSON, AND ELLIOTT RAPAPORT,* San Francisco, Calif.

We studied the effect of aortic and carotid chemoreceptor stimulation on the pulmonary circulation by injection of nicotine (5 to 20 μg per kg) into the ascending aorta in 31 experiments on 8, anesthetized, open-chest dogs. Pulmonary arterial, left atrial, and systemic pressures were measured. Right ventricular stroke output was obtained by an electromagnetic flowmeter placed around the main pulmonary artery, without dissection of the area between the pulmonary artery and ascending aorta. The effects of injection were observed after an average latent period of 3 seconds. During the first 10 seconds of response, the average fall in heart rate from control was 9.8 beats (SEM 0.9); the average increase in systemic mean pressure was 35.2 mm Hg (SEM 5.0); the average increase in pulmonary arterial systolic, diastolic, and mean pressures was 5.2 (SEM 1.04), 2.1 (SEM 0.55), and 3.4 mm Hg (SEM 0.34), respectively; the left atrial mean pressure rose an average of 0.7 mm Hg (SEM 0.11); the total 10-second pulmonary blood flow decreased an average of 54 ml (SEM 13.3); and pulmonary vascular resistance (PVR) increased by 0.013 mm Hg per ml per 10 seconds (SEM 0.002). These responses were essentially unaltered by administration of succinylcholine, which eliminated the respiratory effort induced by nicotine. The rise in PVR could still be demonstrated after injection of atropine in an amount sufficient to prevent bronchoconstriction and changes in heart rate. There was no rise in PVR when we injected nicotine at the same point in the ascending aorta after the aortic body was inactivated, or when we injected it into the bracheocephalic artery so that it stimulated only the carotid bodies. This would appear to localize the origin of the reflex to the aortic chemoreceptors.

Copper Absorption in Malabsorption Syndromes. IRMIN STERNLIEB, New York, N. Y. (introduced by I. Herbert Scheinberg).

Clinically, manifest copper deficiency never occurs in normal man because of the surfeit of copper present in almost any diet. However, some patients with steatorrhea exhibit a decrease in the concentration of ceruloplasmin, the principal copper protein of serum. The present investigation is an attempt to determine whether this decrease is due to impairment of absorption of copper, impairment of synthesis, or loss of the protein. Forty-six patients with steatorrhea of various etiologies were studied. In 36 of them with either distal intestinal resection, localized small bowel disease, or mild or remittent sprue, the concentration of ceruloplasmin in serum was normal or increased. The remaining 10 patients had active nontropical or tropical sprue, or scleroderma, and in them there was moderate to marked deficiency of ceruloplasmin that was roughly proportional to the severity of the primary disease. Greatly decreased intestinal absorption of copper was demonstrable in 2 of these 10 patients after oral administration of Cu^{64}. Intravenous administration of this isotope to the same two patients was followed by a normal, or higher than normal rate of synthesis of ceruloplasmin. We concluded that impaired absorption of copper underlay the ceruloplasmin deficiency, a situation exactly opposite that existing in patients with hereditary deficiency of ceruloplasmin.

Mechanism of Macrocytic Response to Erythropoietin. FREDERICK STOHLMAN, JR.,* ANTOINETTE BELAND, AND DONALD HOWARD, Boston, Mass.

Studies from a number of laboratories have established that erythropoietin differentiates stem cells. There is a question, however, whether erythropoietin directly affects differentiated erythroid precursors. Arguments for such a peripheral effect center about the rapidity of response in normal animals. High doses of erythropoietin produce a shortening of maturation time and skipping of divisions with resultant short-lived macrocytes. The shortening of stem-cell to emergence time might account for the rapid response in normal animals. The relationship of macrocytic response to the dose of erythropoietin was studied in rats in which the number of erythroid precursors had been substantially reduced by hypertransfusion. A single dose of 35 U of erythropoietin failed to induce macrocytosis even though it produced a sevenfold increase in reticulocytes. Fractionation of this dose, 7 U at 0 hours and 28 U at 24 hours, produced a macrocytic response at 48 hours. When 35 U of erythropoietin was given in a single injection to normal animals, macrocytes were seen at 48 hours. Fractionation of the dose did not increase the degree of macrocytosis. The maximal response, however, occurred at 72 hours. In animals receiving 35 U of erythropoietin at 0 and 24 hours, the degree of macrocytosis and the absolute reticulocyte response appeared to be greater in normal than in transfused animals. From the above it appears that macro-

cytes are not produced after the injection of erythropoietin unless differentiated erythroid elements are present in the bone marrow. Further, the qualitative difference in the response of normal and hypertransfused animals points to either a second effect of erythropietin or the presence of another regulator in urinary erythropoietin.

Natural Opsonins to Group A Streptococci and to Staphylococci in the Sera of Germ-free Mice and Colostrum-deprived Piglets. GENE H. STOLLERMAN,* RICHARD EKSTEDT, RALPH FRIEDENBURG, AND IRUN COHEN.

In preliminary studies, 6-week-old germ-free (GF) and conventional mice (CM) were found to be equally resistant to intraperitoneal challenge with variants of Group A *Streptococci* lacking capsules and M protein, and with coagulase-positive *Staphylococci*. To explore further the mechanisms of natural resistance in these hypogammaglobulinemic animals, studies of opsonization were made. Bloods of GF and CM mice were equally effective in opsonizing avirulent Group A *Streptococci* and all strains of *Staphylococci* studied. Encapsulated streptococcal variants resisted phagocytosis equally in both groups of mice. We were unable to identify these opsonins as specific antibodies because they could not be absorbed at 0° C by large numbers of homologous organisms. The serum of a new-born piglet, before suckling, is known to contain only minute amounts of γ-globulin. It was considered of interest to determine the opsonic power of such sera. Colostrum-deprived piglets were bled immediately after delivery by cesarian section. Sera from these animals contained 0 to 1 U per ml of complement compared with 21 U per ml in sow serum and 0.076 g per 100 ml γ-globulin compared with 1.37 g per 100 ml in the sow. Immunoelectrophoresis confirmed the presence of a small amount of γ-globulin in the piglet serum. M-negative, capsule-negative Group A *Streptococci* and all strains of *Staphylococci* studied were opsonized equally well by bloods of colostrum-deprived piglet and sow serum. The opsonins cannot be absorbed at 0° C by homologous strains. The presence of capsules markedly reduced opsonic activity. The results suggest that native resistance to unencapsulated Group A *Streptococci* and to coagulase-positive *Staphylococci* may be related to natural opsonins that are not specific antibodies.

An Unusually Low Death Rate from Myocardial Infarction in an Italian-American Community in Pennsylvania. CLARKE STOUT, JERRY MORROW, EDWARD BRANDT, AND STEWART WOLF,* Oklahoma City, Okla.

Five communities in Pennsylvania were surveyed to determine mortality rate from myocardial infarction. Data from death certificates covering a 7-year period were verified against hospital records and the records of private physicians. In the town of Roseto, the death rate per 1,000 per year from myocardial infarction in men of all age groups was 1 as compared to 4.3 for Nazareth, 4.1 for Bangor, 4.5 for Stroudsburg, and 3.4 for East Stroudsburg. For the women of Roseto, the figure was 0.6 as compared to 2.2 for Nazareth, 2.4 for Bangor, 1.5 for Stroudsburg, and 1 for East Stroudsburg. There were no deaths in Roseto from myocardial infarction before age 47, but deaths from myocardial infarction below the age of 40 were recorded among members of Rosetan families who had moved away from the community. In the age groups 55 to 65 and 65 and above, more men than women were alive in Roseto. Roseto, originally settled in 1882 by Italian immigrants from Roseto, Italy, has grown to a town of 1,630 inhabitants, still exclusively Italian. Despite the low incidence of death from myocardial infarction, the Rosetans eat large amounts of fat (average 111 g for males and 92 g for females). They are generally overweight (average for adult males: height 67 inches, weight 178 pounds; females: height 63 inches, weight 146 pounds). Serum cholesterol, determined on one-third of the adult population, was similar to other United States figures. The reasons for the low incidence of death from myocardial infarction in this relatively obese Italian community where a large amount of animal fat is eaten are not immediately apparent. The stability of the community was striking, however, and it was evident that the people were fun-loving, vigorous, unpretentious, and mutually supporting. As a group they were found to work hard, play hard, and drink considerable alcohol, especially red wine.

Suppression of Hematopoiesis by Ethanol. LOUIS W. SULLIVAN AND VICTOR HERBERT,* Boston, Mass.

Several lines of evidence suggest ethanol may suppress hematopoiesis. Since ethanol may be only a weak hematosuppressive agent, it was reasoned that a patient with nutritional anemia, on the brink of response to minimal dose therapy, might be an appropriate subject to demonstrate such suppression. A 61-year-old chronic alcoholic woman with megaloblastic anemia due to folate deficiency was maintained on a diet containing approximately 5 μg total folate a day. In addition, she was given, during successive periods of 14 days or more of uniform daily administration, increasing amounts of pteroylglutamic acid (PGA). After periods of 25 and 50 μg, and while on 75 μg PGA daily, a small reticulocyte response began, and it slowly subsided when whiskey and wine ad libitum were added to the regimen. After 104 days, and while the patient was still receiving 75 μg PGA daily, ethanol was stopped. The patient had brisk reticulocytosis with a peak of 21.7%. The cycle of reticulocyte suppression by ethanol and reticulocytosis on withdrawal was repeated 3 more times, with reticulocyte peaks of 18.0, 26.3, and 12%, respectively. On each occasion, sternal marrow converted to normoblastic on withdrawal of ethanol, and reverted to megaloblastic when ethanol was readministered. Meanwhile, hematocrit rose from 18 to 42%, and serum and erythrocyte folate also slowly rose, suggesting 75 μg PGA was above the minimal daily folate requirement for this 110-pound woman. During ethanol ingestion, Cr^{51}-labeled

red cells survived normally ($t_{\frac{1}{2}} = 27$ days), intravenous Fe^{59} disappeared with $t_{\frac{1}{2}} = 55$ minutes, and plasma iron turnover was 36.2 mg per day. Serum iron rose during ethanol administration and fell on withdrawal. Preliminary observations suggest a similar sequence of events may be produced in vitamin B_{12} deficiency. It is concluded that ethanol suppresses hematopoiesis, perhaps by directly affecting folate metabolism.

Nickel Inhalation and Pulmonary Carcinogenesis. F. WILLIAM SUNDERMAN, JR., AND F. WILLIAM SUNDERMAN,* Philadelphia, Pa.

Previous studies from this laboratory have emphasized the prevalence of cancer of the lung among industrial workers exposed to nickel carbonyl, $Ni(CO)_4$. Nickel carbonyl is a volatile, highly toxic compound that is formed whenever reactive nickel comes into contact with carbon monoxide. Mainstream tobacco smoke contains nickel in mean concentration of 0.14 ppm. Metastasizing pulmonary carcinomas have been induced in rats by chronic exposure to $Ni(CO)_4$ in concentration of 4 ppm for 30 minutes 3 times weekly for 1 year, and also by single massive exposure to $Ni(CO)_4$ in concentration of 80 ppm for 30 minutes. In present studies, lungs and livers of normal rats and of rats exposed to $Ni(CO)_4$ have been subjected to ultrasonic disintegration and ultracentrifugal fractionation. In tissue homogenates of normal rats, principal localization of nickel was in microsomal and supernatant fractions. After acute and chronic exposure of rats to $Ni(CO)_4$, nickel was increased in microsomal and supernatant fractions of lung and in the microsomal fraction of liver. Part of the nickel in lung and liver was bound to RNA and was not released from RNA by prolonged dialysis. Concentrations of nickel in NaCl-precipitable RNA of normal rat lung and liver averaged 110 and 59 μg Ni per g RNA, respectively. Twenty-four hours after inhalation of $Ni(CO)_4$ in concentration of 80 ppm for 30 minutes, nickel in NaCl-precipitable RNA of lung and liver averaged 270 and 160 μg Ni per g RNA. The increased nickel-binding of RNA was associated with characteristic alterations in thermal phase-transition curves and magnetic resonance properties of RNA. These demonstrations of subcellular localization of nickel and of *in vivo* interaction between nickel and RNA contribute to elucidation of nickel carcinogenesis.

A Method for the Study of Uremia in Primates. PAUL E. TESCHAN, JOSEPH C. SHARP, AND GERALD P. MURPHY, Washington, D. C. (introduced by J. Russell Elkinton).

Among the characteristic early clinical findings in uremia referable to the brain is impaired capacity for sustained mental effort. Accordingly, a method utilizing quantitative expressions of certain cerebral functions has been developed to permit direct, detailed study of the uremic syndrome. Monkeys (*Macaca mulatta*) were trained to a behavioral schedule requiring sustained vigilance and repetitive, accurate performance. A reversible uremic state was then induced by continuous intravenous reinfusion of urine. Behavioral deficits (a tenfold increase in average rate of error) together with mild anorexia and lassitude abruptly occurred after 20 to 60 hours of urine infusion when plasma urea nitrogen (PUN) concentrations exceeded 120 mg per 100 ml (peak levels, 130 to 170 mg per 100 ml), and as abruptly disappeared during the early hours of recovery diuresis after cessation of the infusion. Behavioral deficits were not correlated with variations in plasma potassium (range, 2.0 to 8.5 mEq per L) or sodium (range, 130 to 170 mEq per L) concentrations. In further observations utilizing a more refined performance schedule, infusion of a concentrated urea solution was associated with abrupt behavioral deterioration at a PUN concentration of 120 mg per 100 ml. Forty-five minutes after discontinuing the infusion (PUN, 62 mg per 100 ml), normal behavior had been re-established. The results suggest that *1*) a reversible uremic state may be produced in monkeys by this urinary reinfusion method, *2*) objectively measurable, quantitative behavioral deficits may ensue at significant levels of azotemia, *3*) these deficits are not directly related to changes in plasma sodium or potassium concentrations in the ranges noted, nor to the process of infusion itself, and *4*) elevated urea concentration may contribute to the genesis of the uremic syndrome.

Reversible Contraction of an Actomyosin Preparation Induced by an Electrical Current and Its Association with Calcium. LOUIS TOBIAN * AND STEPHEN MARTIN, Minneapolis, Minn.

Propagated action potentials plus calcium somehow induce actomyosin contractions in living muscle. Moreover, calcium prevents relaxing factor activity. Studies were done to determine whether the electrical event itself can induce contractions in an actomyosin preparation with depolarized, nonfunctioning cell membranes. Rabbit psoas strips were refrigerated 48 hours at 0° C in 50% glycerine. Each strip was then washed and mounted in a bath with muscle extract to provide relaxing factor plus 140 mM KCl, 6 mM $MgCl_2$, 2 mM $CaCl_2$, 6 mM ATP, and pH 6.8. Current of 150 mamp was then passed longitudinally through the strip, inducing an isometric contraction during the 10 seconds of current passage. Seven such strips had an average contraction of 176 mg per mm^2 cross-section. Eight other strips were treated similarly except that no calcium was added to the bath other than that coming from the muscle extract. These eight strips had an average contraction of 52 mg during current passage, significantly less than the contractions associated with added $CaCl_2$ ($p < .02$). When placed in oxygenated Krebs solution at 37° C, all strips lost as much potassium as similarly treated, dead muscle strips, suggesting that the cell membranes were not pumping cations. Any bit of membrane that was intact would be completely depolarized in the 140 mM KCl. These data suggest a hypothesis: calcium is bound

in a molecular "pocket" in resting muscle, thereby allowing relaxing factor to prevent an ATP-induced contraction of actomyosin. An action potential sends a small current longitudinally along the interior of the muscle fiber, which acts on the charged groups of a special protein and momentarily changes its configuration, thereby exposing the previously sheathed calcium. The exposed calcium then reverses relaxing activity, allowing ATP to induce contraction. Thus, this small electrical current itself may couple excitation and contraction.

Stop-Flow Study of Renal Excretion of Tritiated Vasopressin. EUGENE J. TOWBIN AND CARL B. FERRELL, Little Rock, Ark. (introduced by Richard V. Ebert).

Stop-flow techniques were utilized to study renal excretion of tritiated antidiuretic hormone in 30 anesthetized dogs. Arginine vasopressin was tritiated by catalytic exchange and repurified by column chromatography. Fifty μg of biologically active peptide with a specific activity of 46 μc per mg was injected intravenously. The antidiuretic activity of the urine correlated well with its radioactivity, which validates the use of urinary radioactivity as a measure of its hormone content. After the induction of a brisk solute diuresis, two protocols were used. *Stop inject:* The ureteral catheter was clamped, and after 5 to 12 minutes, a dose of radioactive hormone, along with a filtration marker (i.e., C^{14} inulin or creatinine), was rapidly injected intravenously. The ureteral clamp was released 3 to 5 minutes later, and ½-ml serial samples of urine were collected. The concentration of vasopressin was proportional to the concentration of the filtration marker, indicating the hormone is only filtered. *Inject stop:* After priming doses, a continuous infusion of tritiated vasopressin and creatinine was maintained in 12 experiments. After 5 minutes of urinary stasis, creatinine clearance ratios, (U/P tritium)/(U/P creatinine), were constant throughout the serial urine samples which represent the anatomic sites of the nephron. This constancy means that vasopressin is neither secreted nor reabsorbed by the renal tubule. Although constant in any one experiment, clearance ratios varied from 0.2 to 0.8 with a mean of 0.6. Plasma protein binding of tritiated arginine vasopressin would account for the clearance ratio being less than one. Seventy clearance periods showed a mean clearance per kidney of 25.4 ml per minute for creatinine and 15.3 ml per minute for tritiated vasopressin.

Free and Conjugated Estriol in Arterial and Venous Umbilical Cord Blood. PHILIP TROEN,* Boston, Mass.

Estriol is the major classical estrogen of the placenta and fetus in human pregnancy. As a possible indication of the relative contribution of the placenta and fetus to estriol metabolism, free and conjugated estriol circulating between the placenta and fetus was measured. Multiple pools of separated umbilical artery blood and umbilical vein blood were analyzed. A further separation was made between blood obtained at repeated cesarean section and blood obtained at normal vaginal delivery. Mean concentrations of estriol ranged from 90 to 96% of the total estriol concentration. There was a significantly lower concentration of conjugated estriol in cesarean section blood than in vaginal delivery blood. Mean values for free estriol concentration and for free estriol as the percentage of total estriol were also significantly less in cord blood from cesarean section than from vaginal delivery. These results suggest that changes in umbilical cord blood concentration of both free and conjugated estriol may take place in association with labor, or delivery, or both. There was no significant difference in mean values for conjugated estriol between artery and vein in either cesarean section or vaginal delivery material. Of the conjugated estriol in cesarean section cord blood, 84 to 88% was estriol sulfate with no significant difference in estriol sulfate concentrations between arterial and venous blood, respectively. These results are of interest in view of the ability of both fetal and placental tissue to sulfurylate estriol and the ability of placental tissue to hydrolyze estrogen sulfate. There was a significant difference in the arteriovenous ratio of free estriol concentration in cord blood from cesarean section ($A/V = 0.49$) compared to blood from vaginal delivery ($A/V = 1.23$). This finding may be related to estriol biosynthesis by the placenta.

Glucagon-producing Tumors of the Islets of Langerhans. ROGER H. UNGER, ANNA M. EISENTRAUT, JAN D'V. LOCHNER, Dallas, Tex. (introduced by Elias Strauss).

The possibility of glucagon secretion by alphacytoid neoplasms of the islets of Langerhans has been suspected but never proven. In the following study, specific evidence for the existence of glucagon-producing tumors is presented. Specimens were obtained surgically or at necropsy from 4 patients with non-β-cell islet tumors and diabetes mellitus. In one, a 62-year-old man requiring 40 U of NPH for 10 years, a 5.5-g benign alphacytoid adenoma was an incidental autopsy finding. In the 3 others, middle-aged women with diabetes of recent onset, severe gastrointestinal, or bizarre endocrinologic disease, or both, and evidence of malignancy, islet-cell carcinomatosis with extensive hepatic metastases was found. After acid-alcohol extraction, specimens were assayed for glucagon and insulin by specific radioimmunochemical technics. All 4 tumors contained physiologically significant quantities of glucagon (51.6, 64.7, 2.6, and 10.1 μg per g wet wt) and negligible traces of insulin (2.1 to 16 mU per g). To ascertain hormone source, concentration of glucagon and insulin in a tumor was compared with that of its surrounding pancreas. The tumor/pancreas ratio for glucagon was 1.19 (64.7 mμg per g/54.2 mμg per g) and for insulin < 0.04 (2.1 mU per g/$<$ 60.0 mU per g), indicating the tumor to be the source of its glucagon, and surrounding pancreas of its insulin. For further evidence of glucagon

production by tumor, metastases to a nonglucagon-producing tissue, the liver, were assayed in 2 patients. Tumor nodules contained 2.6 and 10.1 mμg per g of glucagon, and the surrounding liver, none. These results provide the first specific evidence of benign and malignant glucagon-producing islet-cell tumors. Although neither glucagon hypersecretion nor a casual relationship to the coexisting diabetes was established, physiologically significant augmentation of total body glucagon, estimated at 100 to 300% in cases with metastases, was demonstrated. If freely available and devoid of normal secretory restraints, such increases of glucagon could, depending on rate of release, hepatic responsiveness, and compensatory response of β-cells, influence blood glucose hemostasis in the direction of hyperglycemia.

Augmented Natriuretic Response to Acute Sodium Infusion Following Blood Pressure Elevation with Metaraminol in Normotensive Subjects. CARLOS A. VAAMONDE, I. NORMAN SPORN, RUBEN G. LANCESTREMERE, JOSEPH L. BELSKY, AND SOLOMON PAPPER,* Boston, Mass., Richmond, Va., and Albuquerque, N. Mex.

It is well-established that patients with essential hypertension have an exaggerated natriuresis in response to acute intravenous administration of sodium. While the mechanism for this remains unknown, one of the possibilities is that elevation of blood pressure per se may cause this enhanced natriuresis. Five normotensive male subjects were studied. Three received a diet containing 150 mEq of sodium daily, and 2 a diet containing 10 mEq daily. They remained recumbent from 8:00 a.m. until 3:00 p.m., and voided spontaneously at 30-minute intervals. The subjects were studied on 4 separate occasions. *1*) "Blank day": no manipulation was undertaken other than venipuncture to collect blood. *2*) "Saline-control day": 2 L of hypotonic (125 mEq per L) sodium chloride-lactate solution was infused intravenously from 10:30 a.m. to 12 noon. *3*) "Metaraminol-control day": metaraminol was infused from 9:30 a.m. to 1:00 p.m. at a rate sufficient to increase the mean blood pressure an average of 27 mm Hg. *4*) "Metaraminol-saline day": metaraminol was infused as in *3*) above, and the hypotonic sodium solution was given from 10:30 a.m. to 12 noon. The maximal rate of sodium excretion on "metaraminol-saline day" in 4 subjects was between 51 and 94% greater than on the "saline-control day." In the other subject, the difference was sevenfold. The maximal rate of sodium excretion on "metaraminol-saline day" was 142 to 260% greater than on "metaraminol-control day" and 108 to 258% greater than on "blank day." Metaraminol alone did not produce exaggerated natriuresis. The data demonstrate that elevation of the blood pressure with metaraminol results in an augmented natriuretic response to the acute administration of sodium in normotensive subjects. The mechanism whereby increased blood pressure with metaraminol results in enhanced natriuresis is not known.

The Nature of the Antibody in Penicillin-induced Hemolytic Anemia. PAUL P. VANARSDEL, JR., AND KAY B. FRANZ, Seattle, Wash. (introduced by Wade Volwiler).

Most patients whose sera contain hemagglutinating (HA) antibodies to penicillin give a history of systemic allergic reactions. Other patients, who develop HA antibodies during penicillin therapy, may have concurrent hemolytic anemia. We have studied two such patients, both of whom developed hemolytic anemia, but no other signs of hypersensitivity, during treatment for bacterial endocarditis. Their direct Coombs antiglobulin test was positive only during treatment. Each serum was tested for HA activity by a standard technique in which group O, Rh-negative human erythrocytes were incubated with benzylpenicillin, stored overnight, washed just before use, and added to serial twofold dilutions of each serum. The HA titers of the two sera were 1:256 and 1:1,024. Further studies were designed to compare these sera with selected high-titered sera from allergic patients. The HA titer of all allergic sera which were preincubated with benzylpenicillin, 6-aminopenicillanic acid, penicilloyl polysine, or penicilloyl ε-aminocaproate was inhibited in proportion to the concentration of the agent used. In similar studies with the two hemolytic anemia sera, concentrations of the first two agents up to 1,200 μM were not inhibitory. The two penicilloyl agents, which inhibited allergic sera at 1 μM concentration, also had little effect. A thousandfold increase in the concentration of penicilloyl ε-aminocaproate produced partial inhibition of one hemolytic anemia serum, but had no effect on the other. Antibody was absorbed, however, by penicillin erythrocytes from both groups of sera to the same degree. Thus, the antibody associated with penicillin hemolytic anemia is unique in that it shows no affinity for free penicillin or the more reactive penicilloyl group, yet reacts with erythrocytes preincubated with penicillin. These studies suggest that it has a specificity and significance quite different from the HA antibody associated with penicillin allergy.

Quantitation and Implications of Human Fecal Iodine. L. VAN MIDDLESWORTH,* NORMA J. BOONE, A. WHITEHEAD, S. DE LAMERENS, J. N. ETTELDORF, Memphis, Tenn.

Fecal iodine has been shown to be predominantly organic iodine. We have measured urine and fecal iodine of 24 normal adults and children, 3 patients administered NaI, and 8 patients given thyroid medication. All excreta were collected separately over 24-hour periods for 3 to 10 consecutive days. Fecal specimens were collected on aluminum foil and dried at 100° C. Radioiodine tracer showed no loss of I^{131} during the drying process. The dried material was ground to a fine dust, and 20 to 200 mg was analyzed for total iodine by the chloric acid digestion. The iodine concentration of dried feces in newborn infants was similar to that in children and adults. The sum of urine and fecal iodine excretion of newborn

infants exceeded their daily intake, and the fecal excretion of older children was up to 50% of their daily intake. Large amounts of NaI medication resulted in less than 0.1% of the administered iodine being excreted in the feces of adults. The iodine of USP thyroid was excreted in the feces at a variable rate amounting to 20 to 90% of the administered dose. The micrograms of iodine per gram of dry feces were: newborn infant, 1 to 3; normal child and adult, 1 to 4; hypothyroidism, <1; after replacement therapy with USP thyroid, 5 to 9; and replacement thyroxine therapy, 8 to 10. Abdominal scans after oral I^{131}-thyroxine show a large part of the tracer dose is probably not absorbed, and it is rapidly transferred to the colon; 30 to 50% of the absorbed thyroxine was concentrated in the liver. It is suggested that fecal iodine may usually reflect thyroid function, and under some conditions it may represent an important loss of thyroid hormone from the human being.

Studies on the Fate of Tritiated Busulfan in Man. HELEN A. VODOPICK, HENRY E. HAMILTON, HERBERT B. JACKSON, C. T. PENG, AND RAYMOND F. SHEETS, Iowa City, Iowa (introduced by Elmer L. DeGowin).

Although busulfan (Myleran) has been used therapeutically in man for over a decade, its action remains unknown. The purpose of this report is to present our studies on the fate of tritium-labeled busulfan in human beings. Tritiated busulfan with specific activity of 6.9 mc per mmole was given orally to five patients: one control, two with polycythemia vera, and two with chronic granulocytic leukemia. The total dose ranged between 72 and 231 mg in 21 days. Two patients with chronic granulocytic leukemia received 10 to 100 mg intravenously as single injections. One man with primary hemorrhagic thrombocythemia was given 315 mg intravenously in 35 days. We used a beta liquid scintillation spectrometer for radioassay of all samples to measure the plasma clearance ($t_{\frac{1}{2}}$), plasma specific activity, rate of absorption from the gastrointestinal tract, and urinary excretion. The therapeutic response to tritiated and unlabeled busulfan was similar. After an initial dose, plasma clearance varied between $1\frac{1}{2}$ and $4\frac{1}{2}$ hours. An initial fall and then gradual rise in plasma radioactivity during the first 24 hours was interpreted as a plasma "re-entry phenomenon." Maximal excretion of urinary radioactivity lagged several days after the initial dose. During prolonged administration of a constant daily dose of tritiated busulfan, a plateau of plasma radioactivity occurred. After the last dose of labeled drug, two exponential rates of loss from plasma were apparent, with equal exponential rates of urinary excretion. The biologic half-life after a single intravenous dose was 5 days. Radioautography did not reveal labeling of myeloid cells despite detectable plasma radioactivity. With decline to low levels of plasma radioactivity, a sudden increase in the peripheral leukocyte count from 23,000 to 120,000 per mm^3 occurred in 4 days, suggesting significant therapeutic and pharmacologic implications.

Serogrouping of Escherichia Coli from Patients with Infections of the Urinary Tract. KENNETH L. VOSTI, LEONARD M. GOLDBERG, AND LOWELL A. RANTZ,* Palo Alto, Calif.

The ability to classify serologically *E. coli* has revealed that a relatively few serographs account for most infections of the urinary tract. Their occurrence in different populations has not been defined, nor has the frequency of recurrence with the same or different 0 groups. It is the purpose of this communication to report such observations gained from a study of 74 obstetrical patients with 83 instances and 58 nonobstetrical patients with 107 instances of significant bacteriuria. *E. coli* accounted for approximately 85% of the infections in each category of patients. Eighty per cent of the *E. coli* could be serologically classified. Fifteen different 0 groups were identified in the urine of obstetrical and 11 groups in nonobstetrical patients. Groups 01, 025, 050, and 075 accounted for 14 and 30%, and 04 and 06 for 41 and 52% of the groupable strains among these patients. No difference in the frequency of occurrence of these 0 groups was noted among patients whether they were infected during the ante- or postpartum periods. Serogrouping of *E. coli* permitted the recognition of 25 instances of recurrent infection with a different serogroup among 15 patients and 24 instances of recurrence with the same serogroup among 17 patients. Groups 04 and 06 accounted for 75% of the recurrences with the same serogroup. These data reveal: *1*) 04 and 06 are the *E. coli* serogroups most frequently associated with infections of the urinary tract in obstetrical and nonobstetrical patients, *2*) recurrence with the same or different serogroups has been observed with equal frequency, and *3*) 0 groups 4 and 6 account for a disproportionate number of recurrences with the same serogroup.

Comparative Effectiveness and Toxicity of Two Influenza Vaccines in Elderly Persons. DOUGLAS W. VOTH AND HARRY A. FELDMAN,* Syracuse, N. Y.

One hundred twenty persons ranging in age from 40 to 105 years (average, approximately 70) and residing in a county home comprised the study population. The objectives of the study were to determine: *1*) the serological response of elderly people to polyvalent fluid and repository influenza vaccines, *2*) reactions to the vaccines, *3*) protective values of the vaccines, and *4*) the cardiopulmonary effect of influenza in elderly persons. In double-blind fashion, one-third received 0.25 ml of oil-emulsion, repository vaccine, another one-third, 0.5 ml of aqueous vaccine, and the remainder, 0.5 ml of normal saline. All injections were given in the deltoid muscle with the same size needle. Vaccine reactions, systemic and local, were adjudged to be very low and scarcely different in the three groups. No persistent local changes resulted from the depot vaccine. Antibody response, vaccine efficacy, and the possible deleterious effects of influenza infections on cardiopulmonary compensation are

being evaluated by regular bleedings and close daily surveillance of the population throughout this respiratory season.

Inhibition of the Phagocytic Capacity of the Human Reticuloendothelial System in Viral Infections. HENRY N. WAGNER, JR.,* MASAHIRO IIO, AND RICHARD B. HORNICK, Baltimore, Md.

Phagocytic capacity of the reticuloendothelial system (RES) in man can be measured safely and reasonably accurately by observing the rates at which various doses of radioisotopically labeled, colloidal, aggregated albumin particles are removed from the peripheral circulation. In addition, the sites of accumulation of the particles (primarily liver and spleen) can be determined by scintillation scanning. These methods have made possible studies of the effects of disease on the RES. Recently, we reported that in certain experimental human *bacterial* infections, phagocytic capacity was increased during the period of infection compared to pre- and postinfection values. We have now observed in two *viral* infections, dengue and sandfly fever, produced experimentally in man, that nonspecific phagocytosis, as determined by the maximal rate of clearance of aggregated albumin particles, is markedly impaired during the period of infection. During periods of normal and altered phagocytic capacity, the relationship between administered dose and clearance rate followed Michaelis-Menten enzyme-substrate kinetics, permitting calculation of V_{max}, the maximal rate of clearance of the particles from the blood. Changes observed during both bacterial and viral infections were not due to alteration in blood flow to the RES, but to changes in the efficiency of phagocytosis. The data are consistent with the concept that in certain bacterial infections, nonspecific phagocytosis is stimulated, whereas in certain viral infections, nonspecific phagocytosis is inhibited.

Renal Tubular Reabsorption of Halides. MACKENZIE WALSER* AND W. JOSEPH RAHILL, Baltimore, Md.

As reported previously, if two substances A and B are reabsorbed with rate constants K_A and K_B at any point in the tubule, and if the ratio K_A/K_B remains constant along the length of the tubule, then ratios R_A and R_B of quantities excreted/filtered will be related by $R_A/\log R_B = K_A/K_B$. Similar ions whose clearances are interrelated in this manner can thus be assigned values for relative permeance that are independent of plasma concentration, clearance, or filtration rate. Simultaneous ratios of excreted/filtered chloride and radioactive iodide, bromide, or fluoride were measured in dogs chloride depleted or infused with NaCl, NaI, or NaBr, or osmotic diuretics, or both. Halide clearances R_I, R_{Br}, and R_F, and also urinary discrimination, defined as R_I/R_{Cl}, R_{Br}/R_{Cl}, and R_F/R_{Cl}, varied greatly with R_{Cl}. Tubular discrimination, however, or relative permeance, calculated as above, was independent of R_{Cl} in the range 0.002 to 0.3: K_I/K_{Cl} $(= \log R_I/\log R_{Cl}) = .417 \pm .006$ (SE; $n = 148$); $K_{Br}/K_{Cl} = 1.135 \pm .010$ $(n = 77)$; and $K_F/K_{Cl} = .156 \pm .008$ $(n = 59)$. The correlation between observed clearances and those predicted from chloride clearances by these equations was 0.96 for iodide, 0.93 for bromide, and 0.83 for fluoride. No other variables appeared to affect these relationships including urine pH (5.5 to 7.8), urine flow (0.4 to 25 ml per minute), filtration rate (2 to 7 ml per minute per kg), plasma halide concentrations, loading with sulfate, bicarbonate, thiocyanate, perchlorate, or nitrate, or administration of probenecid. These results, and recalculation of other reported data, indicate that the major determinants of renal transport are common to most monovalent anions. The sequence $SCN > Br > Cl > I > NO_3 > F$ does not reflect ion size or mobility, but closely parallels the sequence of permeability in motor neurons.

Inhibition of Cerebral Ammonia Detoxication and Its Effect on Ammonia Metabolism and Toxicity. KENNETH S. WARREN AND STEVEN SCHENKER, Bethesda, Md. (introduced by Dieter Koch-Weser).

Glutamine formation is the main pathway of ammonia detoxication in tissues other than the liver. A competitive inhibitor of the glutamine synthetase reaction has made possible an *in vivo* study of the effect of inhibition of cerebral ammonia detoxication on ammonia metabolism and toxicity. After ip injection of 150 mg/kg methionine sulfoximine (MS) into Swiss albino mice, the brain ammonia concentration more than doubled. When a nonlethal dose of ammonium chloride was injected into groups of control and MS-treated mice, the brain ammonia concentration of the control group reached a peak at 20 seconds, and then decreased by 75% within 6 minutes. In contrast, the MS-treated mice reached a similar immediate peak in cerebral ammonia concentration, but demonstrated no decrease whatsoever by 6 minutes. While MS-treated mice thus exhibited a relative inability to detoxify ammonia, they also became markedly resistant to exogenous ammonia toxicity (LD_{50}). In addition, the peak brain ammonia concentration of the MS-treated mice, all of which survived, was higher than that of the controls, 50% of which died. This indicates that ammonia intoxication does not depend on the mere presence of high cerebral ammonia levels, but is related to the so-called mechanism of detoxication by which ammonia enters into cerebral metabolic cycles.

Potassium Reabsorption in the Proximal Tubule of the Dog Nephron. JOHN F. WATSON AND JAMES R. CLAPP, Bethesda, Md. (introduced by Robert W. Berliner).

Inulin and potassium concentrations were determined on the same samples of dog proximal tubular fluid and plasma. Proximal transtubular potential differences were determined in separate experiments. No correlation was found between tubular fluid to plasma (TF/P) potassium ratios and location along the proximal tubule. Twenty-seven TF/P potassium ratios from 5 hydropenic dogs (excreting 19% of the filtered potassium) ranged from 0.74 to 1.80 with a mean of 1.30 (SD ± 0.28). Twenty-

four TF/P potassium ratios from 4 potassium-infused dogs (excreting 92% of the filtered potassium) ranged from 1.00 to 1.90 with a mean of 1.40 (SD ± 0.32). Twenty-four TF/P potassium ratios from 4 potassium-depleted dogs (excreting 5% of the filtered potassium) ranged from 0.80 to 1.30 with a mean of 1.04 (SD ± 0.13) which is significantly lower ($p < 0.001$) than both normal and potassium-infused dogs. Transtubular potential differences obtained from similarly prepared groups of animals did not differ significantly. One hundred thirteen measurements ranged from −8 to −41 mv with a mean of −21 mv. The mean TF/P potassium ratio was lower than predicted from the Nernst equation for the corresponding mean transtubular potential difference, implying that potassium reabsorption occurs against its electrochemical gradient. Additional experiments performed in 4 dogs undergoing mannitol diuresis yielded twenty TF/P potassium ratios that ranged from 0.31 to 1.30 with a mean of 0.81 (SD ± 0.27). Simultaneous TF/P inulin ratios in all experiments demonstrated net potassium reabsorption that was progressive along the proximal tubule. These data suggest that potassium reabsorption is accomplished by an active transport mechanism.

Studies on Lysosomes: A Mechanism for Tissue Damage by Streptolysins. GERALD WEISSMANN, HAROLD KEISER, AND ALAN BERNHEIMER, New York, N. Y. (introduced by Lewis Thomas).

Hydrolytic enzymes released from lysosomes appear to mediate the damage to tissues induced by such agents as endotoxin or excess vitamin A. To test the hypothesis that release of these enzymes might be a factor in the pathogenesis of late sequelae of hemolytic streptococcal infections, purified streptococcal products were tested for their action on lysosomes. Lysosome-rich fractions in 0.25 M sucrose were prepared from homogenates of rabbit heart, liver, and lymph nodes. Suspensions incubated with buffer alone released 10 to 14% of the granules' total cathepsin, β-glucuronidase, and acid phosphatase activity into the suspending medium in 60 minutes. In contrast, 18 to 95% of total activity was released by 5 to 500 μg per ml of streptolysin O (SLO) or streptolysin S (SLS). These lysosomal effects were concentration dependent and paralleled hemolytic activity: cysteine activated SLO but not SLS, specific antibody or 10^{-4} M cholesterol inhibited SLO but not SLS, the pH optimum of SLO was 6.5, and SLS activity was greatest at 7.5. Release of malic dehydrogenase, a mitochondrial enzyme, from streptolysin-treated suspensions did not differ significantly from controls. Erythrogenic toxin, deoxyribonuclease, diphosphopyridine nucleotidase, streptokinase, protease precursor, and filtrates from mutants lacking SLS were inactive on lysosomes; cysteine-activated streptococcal protease was minimally active. One hour after intravenous SLO in rabbits, serum β-glucuronidase and acid phosphatase activity quadrupled. There was no increase in alkaline phosphatase activity, suggesting that increases in serum enzymes did not reflect leukocyte damage. Supernatant fluids from SLO- or SLS-treated lysosomal suspensions added *in vitro* to a proteinpolysaccharide from bovine connective tissue caused significant changes in the viscosity, sedimentability, and electrophoretic mobility of this material. These studies indicate that hydrolytic enzymes released from lysosomes by streptolysins can alter appropriate substrates in connective tissue, and suggest that the similar effects of streptolysins on erythrocytes and on lysosomes may reflect common properties of the membranes.

Effects of Plasma Proteins upon the Rheological Character of Blood in the Microcirculation. ROE E. WELLS, JR., RICHARD D. PERERA, THOMAS GAWRONSKI, AND ALI A. SHAHRIARI, Boston, Mass. (introduced by E. C. Eppinger).

The mechanics of blood flow in the microcirculation depend upon hydraulic and viscous forces. The fluid and structural viscosity of blood under these flow conditions have been shown to be a function of not only erythrocyte concentration, but of the interaction between erythrocytes and plasma proteins as well. Studies were conducted to determine the relative contributions to the fluid viscosity made by each of the plasma protein fractions and the various globulin subfractions. The fluid structure and viscosity of red cell suspensions mixed with physiologic concentrations of the plasma proteins and their subfractions were measured. Solutions of freshly prepared protein fractions were mixed with type O red cells twice washed with buffered saline. The resultant suspensions were made up to physiologic concentrations of the proteins fractions and hematocrit values to 40. The final supernatant "plasma" was analyzed for total protein content, electrophoretic distribution, osmolality, and pH. The greatest viscosity effect was found to be due to globulin of which the alpha and beta subfractions had the greatest effects. Albumin with the greatest wt/vol concentration (3.5 g per 100 ml) had the least effect on viscosity, differing insignificantly from the viscosity of the red cell saline suspension. Fibrinogen had an intermediate effect between globulin and albumin. Although fibrinogen had the lowest wt/vol concentration (0.5 g per 100 ml), it was the only suspension exhibiting a yield value, i.e., a fluid structure capable of sustaining a yield stress without flow deformation. Increasing fibrinogen levels to 1.0 and 1.5 g per 100 ml produced not only greater yield values, but also increased aggregates that resisted disaggregation with increasing rates of shear. Albumin appeared to prevent or reverse these aggregation phenomena and reduced the viscosity of the suspensions.

Effect of Lead, In Vivo and In Vitro, on Radiophosphorus Incorporation into Erythrocyte Phosphatides. M. P. WESTERMAN AND W. N. JENSEN,* Pittsburgh, Pa.

The *in vitro* rate of P^{32} incorporation into red cell phosphatides of 5 patients and 6 rabbits with hemolytic

anemia of chronic plumbism was determined. Similar measurements were made in normal human blood after preincubation with various concentrations of lead. The samples had erythrocyte : leukocyte : platelet ratios of approximately 4,000 : 1 : 1. Phosphatide separation of lipid extracts was obtained by the use of silicic-acid-impregnated paper and silicic-acid column chromatography. P^{32} uptake *in vivo* and *in vitro* leaded blood was limited to the phosphatidic acidlike fraction. Phosphatidic acid P^{32} in leaded human erythrocytes and in leaded rabbit erythrocytes was, respectively, 64 to 114 and 35 to 132 cpm per μg. Normal value for humans was 163 ± 40 and for rabbits was 236 ± 71 cpm per μg of red cell phosphatidic acid. Blood preincubated with lead (0.01 to 20 μM, PbAC) showed increased P^{32} incorporation into phosphatidic acid at 0.06 μM, a sixfold increment, and complete inhibition at 20 μM. Contribution of platelets and leukocytes to the P^{32} incorporation into phosphatidic acid failed to explain the findings. These studies show a suppression of P^{32} incorporation into red cell phosphatidic acid in plumbism that might contribute to the hemolytic anemia of that disorder. High concentrations of lead added *in vitro* to whole blood cause inhibition of P^{32} incorporation into phosphatidic acid, but lower concentrations differ in that enhancement occurs.

Intermittent Peritoneal Dialysis in Patients in Chronic or Acute Renal Failure with Indwelling Abdominal "Button," Inserted via Paracentesis Trochar under Local Anesthesia. RAYMOND E. WESTON,* MARTIN ROBERTS, GEORGE COANDA, AND ERIC LEIBOVITCH, Beverly Hills and Los Angeles, Calif.

Intermittent peritoneal dialysis has been demonstrated to prolong active life in chronic intractable uremia. To facilitate intraperitoneal passage of the catheter, several investigators have surgically implanted into the lower abdominal wall a plastic "button" sealed by a cap between dialyses. To obviate the need for such surgery under general anesthesia in severely ill uremic patients, a new Teflon and nylon device has been developed that can be inserted at the bedside via Duke-type abdominal paracentesis trochar with only local procaine infiltration. After withdrawal of the trochar, the "button" is trimmed to proper length and fixed into semi-permanent position by double-threaded locknuts. It can be immediately used for passage of the catheter through a polypropylene screwcap. A soft plastic gasket maintains a fluid-tight seal between the catheter and the hole in the screwcap as the latter is tightened. After dialysis, the catheter is removed and the opening sealed with a solid polypropylene screwcap. Subsequently, no difficulty is encountered passing the catheter twice weekly for the 24-hour peritoneal dialyses required subsequently to maintain chronic uremic patients. The reduced urinary solute load may lead to virtual anuria. Thereafter, retained water and sodium are removed by dialyzing with more hypertonic glucose solutions until the desired negative fluid balance is achieved. Then, dialysis is continued with dialysis solution with a 1.5% glucose concentration. In patients with acute renal failure, the "button," once implanted, permits daily dialyses for shorter periods. By use of the more hypertonic solutions, water and electrolytes as well as protein metabolites are removed daily. Thus, not only is biochemical homeostasis maintained, but also the need for fluid restriction is reduced, permitting adequate intake of calories orally and eliminating intravenous infusions. Once the diuretic phase is over, the "button" is easily removed at the bedside.

Transport of Electrolytes and Water across the Wall of the Rabbit Gallbladder. HENRY O. WHEELER,* Copenhagen, Denmark.

Gallbladders of rabbits were mounted *in vitro* in an apparatus that permitted measurement of electrical potential difference, net flux of water, and changes in electrolyte concentrations in mucosal and serosal fluid. In Krebs solution, movements of sodium from mucosa to serosa (7 to 55 μEq per hour per 100 mg bladder) were associated with chloride and bicarbonate. Net potassium flux was negligible. Net water flux (mucosa to serosa) was directly proportional to net solute transport (measured as sodium flux), and the transported solution was slightly hypertonic (average [Na], 192 mEq per L in 11 bladders). When mannitol was added to the mucosal fluid, water movement occurred against gradients often exceeding 80 mOsm per kg. The lumen was electrically positive with respect to serosa (0.4 to 11 mv, average 2.8 in Krebs solution, 5.6 to 29 mv with mannitol added to lumen). The flux ratios of both sodium and chloride (using Na^{22} and Cl^{36}) were invariably greater than those predicted for passive diffusion, indicating active transport of both ions out of the lumen. Potassium flux ratios (K^{42}) were consistent with passive diffusion. When isethionate ion was substituted for chloride (4 studies), there was net transport of bicarbonate against an electrochemical gradient. Anion and cation transport were not independence, since no transport or electrical potential was observed when choline was substituted for sodium. Evidently, absorption depends upon interdependent active transport of sodium and the major anions. Water movement is dependent upon active solute transport. Since water movement, however, can occur against osmotic gradients, it cannot be attributed to simple osmotic equilibration between the bathing media, and the observed solute-solvent coupling must depend upon a mechanism located within the wall of the gallbladder.

The Antigenicity of the Proteinpolysaccharides of Human Cartilage. DAVID WHITE, JOHN SANDSON, LAWRENCE ROSENBERG, AND MAXWELL SCHUBERT, New York, N. Y. (introduced by David Hamerman).

Chondroitin sulfate can be isolated from cartilage as a product called chondromucoprotein (CMP) that can be fractionated into two further products, PP-L and PP-H. Both PP-L and PP-H contain chondroitin sulfate bound to protein. Although many studies have failed to demonstrate antigenicity of chondroitin sulfate, the isolation of CMP has renewed interest in this problem. There

have been no immunological studies of the CMP of human cartilage. Articular cartilage was obtained from normal knee joints immediately post-mortem and its CMP isolated. Rabbits were immunized with either CMP or PP-L mixed with Freund's complete adjuvant. Intradermal skin tests of the rabbits with either CMP or PP-L were positive within 4 weeks after the initial immunization. Biopsy of the skin reaction revealed changes consistent with an Arthus reaction. Booster injections of CMP or PP-L without adjuvant were then given, and by 6 weeks potent antisera (AS) were obtained. Antibodies to CMP were detected by two methods (all AS were absorbed with excess human serum). *1*) Hemagglutination. By use of tanned sheep red blood cells coated with CMP, agglutination was demonstrated in AS from all rabbits; some had titers $> 1:2{,}560$. This agglutination could not be inhibited by blood group substance, collagen, or hyaluronate, but was inhibited by CMP, PP-L, and PP-H. *2*) Immunoelectrophoresis. Only one precipitin arc developed when CMP was studied with AS by agar gel immunoelectrophoresis. The component giving this arc had a mobility consistent with an anionic proteinpolysaccharide. The above studies indicate that antibodies have been produced in rabbits to one or more components of human CMP.

Leukocyte Debranching Enzyme in Glycogen Storage Disease. HIBBARD E. WILLIAMS, ESTHER M. KENDIG, AND JAMES B. FIELD,* Bethesda, Md.

Diagnosis of type III glycogen storage disease has depended on the demonstration of a deficiency of amylo-1,6-glucosidase activity (debranching enzyme, DE) in liver. An assay for this enzyme in leukocytes has been developed based on the incorporation of glucose-C^{14} into the branch points of glycogen. DE was assayed in leukocyte sonicates from control subjects, a patient (age 3) with proven type III glycogenosis, and her family. Activity is expressed as counts per minute of glucose-C^{14} incorporated into glycogen per 10^8 leukocytes during a 2-hour incubation. Activity was demonstrated to be linear during this time and proportional to the number of leukocytes sonicated. Mean DE activity in sonicated leukocytes from 12 control subjects (age 16 months to 45 years) was $3{,}158 \pm 125$ (range 2,200 to 4,820). DE activity in the patient with type III glycogenosis was 121 (range 0 to 255); 1,555 (1,400 to 1,710) in her mother; 1,070 (500 to 1,560) in her father; 945 (618 to 1,140) in a sister; and 2,900 (2,510 to 3,300) in a brother. None of these other family members had any clinical evidence of type III glycogenosis. DE activity in a patient with glucose 6-phophatase deficiency was 3,460, while in two patients with hepatic phosphorylase deficiency, the values were 2,410 and 3,260, respectively. DE activity in a patient with muscle phosphorylase deficiency was 4,600. These results suggest that this assay may be useful for diagnosing type III glycogenosis as well as the heterozygote state. Decreased DE activity in both parents of the patient with this type of glycogen storage disease is consistent with an autosomal recessive type of inheritance.

The Mechanism of Action of Tissue Thromboplastin. WILLIAM J. WILLIAMS,* Philadelphia, Pa.

A study of the mechanism of action of bovine lung thromboplastin has been undertaken employing highly purified preparations obtained by a combination of differential centrifugation, density gradient centrifugation, and treatment with butanol-benzene. The purified thromboplastin sediments as a single, broad band on zone centrifugation in a sucrose density gradient. The density range is about 1.09 to 1.13 at 4° C as determined by isopycnic gradient centrifugation. Marked coagulant activity develops on incubation of mixtures of lung thromboplastin, calcium chloride, and a bovine serum fraction containing these proteins adsorbed on barium sulfate and eluted with trisodium citrate (serum fraction). The product is assayed by its ability to accelerate the coagulation of recalcified plasma in the presence of added phospholipid. Phospholipid was essential for full activity of the assay system. Studies of the effects of concentration of lung thromboplastin and serum fraction on the rate of formation and on the final yield of coagulant activity demonstrated that purified lung thromboplastin functions as enzyme and the serum fraction as substrate in the reactions leading to development of coagulant activity in this system. Most of the coagulant activity formed in mixtures of lung thromboplastin, serum fraction, and calcium is soluble, but about 20 to 40% can be sedimented with the lung thromboplastin. The sedimented activity can be removed by washing with calcium-free solutions. Lung thromboplastin recovered after incubation with serum fraction and calcium were centrifuged in a sucrose density gradient. A portion of the thromboplastin accumulated in a narrow band in the gradient at density approximately 1.13, in contrast to the homogeneous, broad band found with untreated thromboplastin. The nature of this material has not yet been ascertained, but it may represent a complex between lung thromboplastin, calcium, and one of the coagulation factors.

The Antigenic Specificity of Human Platelets. HENRY E. WILSON,* HOWARD M. JOHNSON, AND MATTHEW C. DODD, Columbus, Ohio (introduced by Charles A. Doan).

In earlier studies in this laboratory, we have noted a high coincidence of isoantibodies to platelets and leukocytes in the sera of transfused patients and multipara. It has also been observed that there is marked variation in the complement-fixation of the platelets of normal donors with these sera. This indicates variation in antigenicity and suggests antigenic specificity of normal human platelets. In an effort to elucidate this phenomenon, platelet antibodies have been induced in normal volunteers. These healthy males were arranged in donor "pairs" on the basis of dissimilarity in their platelet reactivity with sera of transfusion-immunized patients. Each volunteer received multiple intradermal, or intramuscular injections, or both, of washed platelet sus-

pensions. In the first group of 8 volunteers, each received injections totaling from 2.2 to 6.5×10^{11} platelets. Four of these developed platelet antibody in 144 to 204 days. Another group composed of volunteers who had previously received tumor antigens intradermally was organized in the same manner as that described above. These individuals exhibited a marked accelerated reaction to relatively small quantities of antigen. Of this latter group, 7 of 9 volunteers developed antibody within 18 to 131 days of initial injection, during which they had received antigen totaling 1 to 3.8×10^{11} platelets. Four of the 7 developed antibody within 36 days of the initial injection. None of these exhibited reappearance of a previously demonstrated antibody to the implanted tumor tissue. Sera of each of the 7 contained complement-fixing antibody to platelets of his own immunizing donor. Three of these sera contained antibody to the same 3 donor antigens. Platelets of these 3 donors exhibited a similar reaction pattern when tested against all 7 antisera of the panel. Absorption studies confirm the presence of an antigen common to these 3 donors. Similar absorption studies thus far suggest that 3 major platelet antigens are represented in this panel of donors. These specific immunization studies yield evidence of antigenic patterns not so clearly demonstrable in the platelet antisera of transfused patients and multipara.

Studies on the Mechanism of Enhancement of Protein Synthesis by Estradiol. JEAN D. WILSON, Dallas, Tex. (introduced by Marvin D. Siperstein).

Previous studies have demonstrated that testosterone and estradiol accelerate protein synthesis specifically by enhancing the complex reaction consisting of peptide bonding and protein release. This step in protein synthesis involves four discrete components: *1*) participation of soluble RNA-amino acids (sRNA-amino acids), *2*) participation of soluble cofactors including GTP, *3*) bonding of sRNA-amino acids on ribosome particles of the microsome, and *4*) release of completed protein. To localize the specific phase of this reaction that mediates the enhancement of protein synthesis by sex hormones, these various components were evaluated in oviduct homogenates from normal and estradiol-treated chickens. Formation of sRNA-amino acid-C^{14} complexes by oviduct homogenates was uninfluenced by estradiol. The soluble fractions of treated and untreated oviducts exhibited no difference in their abilities to enhance microsomal protein synthesis, indicating that soluble cofactors were not rate-limiting in the untreated preparation. Likewise, the rate of release of protein-C^{14} into the supernatant fluid from microsomes prelabeled with valine-C^{14} was uninfluenced by estradiol. These studies exclude components 1, 2, and 4 and point to component 3, the bonding reaction, as the site of estradiol stimulation. The mechanism of enhancement of this ribosomal reaction was then investigated. Theoretically, estradiol could augment the bonding reaction by accelerating the complexing of sRNA-amino acids to ribosomal template, by increasing the amount of template, or by increasing the number of ribosomes. An increase in the RNA content of the ribosome-template complex was demonstrated, and an enhancement in RNA synthesis occurs at the onset of augmented protein synthesis in estradiol-treated oviduct. This suggests that estradiol enhances synthesis of either template RNA or ribosomal structural RNA. Finally, pulse-labeling studies with adenine-C^{14} disclosed that the first RNA component influenced by estradiol administration is a cytoplasmic RNA fraction that has characteristics of template RNA. These findings suggest that estradiol accelerates protein synthesis by augmenting template RNA production.

Spectrophotometric Determination of Urine Copper. JOHN F. WILSON AND M. E. LAHEY, Salt Lake City, Utah (introduced by Paul D. Hoeprich).

Previous wet-ash digestion methods for urine copper analysis have employed colorimetric reagents that *1*) were relatively insensitive, *2*) necessitated rigid pH control for maximal color development, or *3*) required additional solvent extraction. Oxalyldihydrazide (ODH), a relatively new copper reagent, is not only highly specific for copper, but possesses 2½ to 3 times the sensitivity of diethyldithiocarbamate and has a suitably wide pH range (8.6 to 10.3) for optimal color development. A wet-ash digestion procedure employing ODH has been developed. After digestion of the urine with sulfuric, nitric, and perchloric acids, the clear solution is neutralized with NH_4OH, then colorized with a freshly prepared "reagent mix" of equal parts of saturated aqueous ODH, NH_4OH, and 40% acetaldehyde. The pH of the colorized solution is in the range of 9.2 to 9.4. Maximal color develops in 15 minutes and is stable at 25° C for at least 24 hours. Copper standards of 0.5 μg in a final volume of 10.0 ml have a mean absorbance of 0.099 (SD ± .011) mμ in a 5-cm pathway. Replicate analyses on urines containing 0.4 μg copper per sample show a coefficient of variation of 10%. Recoveries of 0.5, 1.0, and 2.0 μg of copper added to urine samples have been 98, 101, and 97%, respectively. One hundred μg of iron per sample, representing a urine ion concentration of 0.67 mg per 100 ml, does not interfere with the colorization of 0.5 μg of copper. Iron in excess of this concentration can be removed by an additional step. The mean normal values for urine copper of 20 adults and 12 children are 14 (range 8 to 22) and 12 (6 to 17) μg per day, respectively.

A Vasoconstrictor Effect of Plasma during Salt Depletion. BERTRAM M. WINER, Boston, Mass. (introduced by Milton W. Hamolsky).

Dialyzed plasma from patients with renal artery stenosis has a vasoconstrictor effect that appears related to the action of renin. To determine whether salt depletion gives rise to a similar effect, the vasoconstrictor activity of dialyzed plasma was examined by use of rabbit aortic strips before and during administration of a diuretic or low-sodium diet. Plasma was dialyzed for 24

hours against cold running tap water, restored to isotonicity by NaCl, adjusted to pH 5.5, and incubated 1 hour at 37° C before addition to the muscle chamber. Administration of chlorthalidone, hydrochlorothiazide, or mercaptomerin induced within 24 to 48 hours a marked increase in vasoconstrictor activity of dialyzed brachial vein plasma in 7 normotensive and 15 hypertensive subjects. The effect disappeared within a week after omission of the diuretic. It did not develop when water was restricted for 24 hours. Vasoconstrictor activity of dialyzed brachial vein plasma increased in each of 4 hypertensive patients given a low-sodium diet. Renal vein plasma had greater activity than brachial vein plasma. The vasoconstrictor effect was not reduced by addition of phentolamine, EDTA, or inactive plasma, but was lost when plasma was incubated with lysed red cells containing angiotensinase. Dialyzed plasma with vasoconstrictor activity was pressor in pithed nephrectomized cats. Thus vasoconstrictor activity similar to that found in patients with renal artery stenosis develops in plasma in response to sodium depletion. It appears to reflect the action of renin elaborating angiotensin from substrate during incubation after dialysis. These and other studies support the concept that the renin system subserves a homeostatic function.

Free Globin in Red Cells. KASPER H. WINTERHALTER AND ERNST R. HUEHNS, Seattle, Wash. (introduced by Clement A. Finch).

In hemoglobin formation, heme and globin are separately synthesized, but while several intermediates of heme synthesis have been identified in normal and pathological red cells, the presence of free globin has not been demonstrated. Since globin combines spontaneously with hemin, we lysed red cells with a dilute solution of Fe^{59}-hemin and subsequently demonstrated radioactivity in the isolated Hb-A. In order to exclude contamination by heme-carrying nonhemoglobin proteins, the radioactive Hb-A ($\alpha^{A}_{2}\beta^{A}_{2}$) was "hybridized" with canine hemoglobin ($\alpha^{Can}_{2}\beta^{Can}_{2}$). The two new hemoglobin species formed, $\alpha^{A}_{2}\beta^{Can}_{2}$ and $\alpha^{Can}_{2}\beta^{A}_{2}$, had equal specific activity, indicating that the radioactivity was carried by Hb-A. Exchange between free and bound hemin was ruled out by treating "globin-free hemolysate" (prepared by column chromatography) with radioactive hemin; only a relatively small amount was incorporated. Finally, to exclude nonspecific binding to hemoglobin, hemolysate was passed through a CM Sephadex column at pH 7.5. After elution of Hb-A, Fe^{59}-hemin was added to the eluent. Subsequent fractions contained a heme-protein of high specific activity (50% of that of the hemin added). This protein was found to be identical with Hb-A by electrophoresis and spectral analysis. The latter suggests that the heme-protein linkage is the same as in natural hemoglobin. These experiments indicate that normal red cells contain free globin. From the amount of radioactivity incorporated, a concentration of about 0.03 g globin per 100 ml packed red cells can be calculated. Measurement of free globin present in a variety of diseases with impaired hemoglobin synthesis may give further insight into the assembly of the hemoglobin molecule.

Biochemical Lesion of Diphtheria Toxin in the Heart. B. WITTELS AND R. BRESSLER, Durham, N. C. (introduced by G. P. Kerby).

Administration of diphtheria toxin to guinea pigs is classically associated with the development of fatty degeneration of the myocardium. To elucidate the biochemical mechanism of this pathology, cardiac homogenates were prepared from guinea pigs inoculated subcutaneously with toxin 4 to 6 days before sacrifice. Histologic sections of these hearts revealed fatty degeneration. Oxidation of palmitate-1-C^{14} by the myocardium was decreased from normal levels of 2.5 μmoles to .20 μmole per gram homogenate per hour, and incorporation of labeled palmitate into phospholipids (PL) was markedly depressed. Addition of carnitine (γ-amino-β-hydroxybutyric acid trimethylbetaine) consistently restored the impaired fatty acid oxidation (FAO) to normal. Carnitine increased the FAO as much as ten times and restored the incorporation of palmitate into PL to normal levels. Weight loss sustained by the animals after injection of the toxin raised the question of whether starvation plays a role in the metabolic defect. Total food deprivation for periods of up to 6 days, however, resulted in a twofold increase in FAO and of palmitate incorporation into PL. Moreover, hyperlipemia that occurred in the fasted animal did not occur in the toxin-treated animal. The work of Pappenheimer indicating an effect of diphtheria toxin on cytochrome B suggested that the impaired FAO might be secondary to a defect in the electron transport system. The toxin-treated preparation oxidized both succinate-2,3-C^{14} and glucose-U-C^{14}, normally demonstrating the integrity of the glycolytic pathway, tricarboxylic acid cycle, and electron transport system. These observations indicate that diphtheria toxin produces a discrete biochemical lesion in the heart, inhibition of FAO. Correction of this defect by carnitine, a known stimulant of FAO, suggests that the toxin interferes with its biosynthesis.

Experimental Production of Pyridoxine Deficiency in Rats. PETER C. Y. WONG AND SHU CHU SHEN,* Cambridge, Mass.

Anemia in rats on a diet deficient in pyridoxine has not been consistently shown nor clearly demonstrated heretofore. The present study established that a moderate to severe microcytic hypochromic anemia regularly appeared in rats on a pyridoxine-deficient diet. This anemia responded promptly to pyridoxine administration. Twelve male Wistar rats weighing approximately 250 to 350 g were fed a diet deficient in pyridoxine. After 40 to 50 weeks, they all exhibited a severe microcytic hypochromic anemia. The red cell counts, when averaged for the group, did not fall markedly (8.6×10^{6} per mm^{3}), but the range was wide (10.0 to 5.6×10^{6}

per mm^3). The average values for hemoglobin and hematocrit dropped to 7.4 g per 100 ml and 36%, respectively; mean corpuscular value and mean corpuscular hemoglobin concentration were significantly reduced to 42 μ and 26.4%, respectively. Upon parenteral administration of pyridoxine (pyridoxine hydrochloride 2 mg 3 times a week for 2 weeks), reticulocytes reached a peak (17 to 73%) between days 4 and 8 after the first injection. The hemoglobin level and red cell count rapidly rose toward normal levels less than 2 weeks after the initial dose of pyridoxine. The myeloid: erythroid ratio in the bone marrow of 6 severely anemic rats exceeded 4:1 before therapy, suggesting hypoplasia of the erythroid series. The ratio lessened to below 2:1 on day 7 of therapy, indicating active erythropoiesis. The ratio resumed its normal value when the hemoglobin level had improved.

Studies on the Anticoagulant Properties of Natural and Synthetic Polynucleotides. S. YACHNIN, Chicago, Ill. (introduced by A. Kappas).

The anticoagulant properties of heparin and other polysulfates are thought to depend largely on their polyanionic structure. Polynucleotides, by virtue of their phosphate groups, constitute another class of compounds behaving as strong polyanions. An extensive investigation of the effects of both natural and synthetic polynucleotides on coagulation was therefore undertaken. The polynucleotides studied included polyadenylic (Poly A), polyuridylic (Poly U), polycytidylic (Poly C), and polyinosinic (Poly I) acids, mammalian and bacterial ribonucleic acids, various desoxyribonucleic acids, and apurinic acid. Poly I prolonged the recalcification time of plasma in glass and silicone much more effectively than all other polynucleotides (silicone control, 4 minutes 15 seconds; + 0.4 μmole P Poly I, 14 minutes 15 seconds). Poly I had no effect on a purified thrombin-fibrinogen system, while Poly A and Poly U delayed clot formation. Only Poly I was capable of prolonging the one-stage prothrombin time (control, 14 seconds; + 0.4 μmole P Poly I, 41.5 seconds). In the two-stage prothrombin test, Poly I alone displayed significant effect, delaying the rate of thrombin generation and reducing the peak amount generated. The anticoagulant effect of Poly I was most marked in the thromboplastin generation test (TGT), where heat denatured calf thymus DNA and apurinic acid also inhibited to a lesser extent. Other polynucleotides were almost entirely inactive in amounts up to 0.8 μmole P. Poly I displayed discernible anti-TG activity in amounts as low as 0.025 μmole P (control, minimal substrate clotting time 12.5 seconds at 4 minutes; + 0.2 μmole P Poly I, substrate clotting time > 50 seconds at 15 minutes). The anticoagulant properties of Poly I could be reversed by increasing $[Ca^{++}]$ or $[Mg^{++}]$ owing to its insolubility in the presence of these cations. In addition, the anticoagulant properties of Poly I could be stoichiometrically reversed by Poly C and Poly A, which form hybrid hydrogen-bonded helices with Poly I (I + C, 2I + A). The anticoagulant action of certain polynucleotides represents a biological property of this class of compounds heretofore unrecognized.

Hemoglobin Zürich: Chemical and Kinetic Studies. WILLIAM H. ZINKHAM, RONALD F. RIEDER, AND NEIL A. HOLTZMAN, Baltimore, Md. (introduced by Dudley P. Jackson).

An abnormal hemoglobin in 5 members from 3 generations of a Caucasian family was associated with moderate reticulocytosis without anemia. Severe anemia occurred during infections and after administration of sulfonamides. Studies initiated 1 week after onset of anemia in two patients revealed normal erythrocyte morphology, no Heinz bodies or other red cell inclusions, and no methemoglobinemia. Severe hemolysis persisted for 3 weeks after discontinuation of medications. Exposure of patients' red cells or cleared hemolysates to oxygen, redox dyes, or sulfisoxazole at 37° C caused methemoglobin formation and precipitation of the abnormal hemoglobin. Initially, precipitates appeared as small spheroidal bodies that stained with crystal violet; when the granules attained a diameter of 3 to 4 μ, staining with crystal violet no longer occurred. Stability of reduced glutathione, utilization of glucose, and activity of several enzymes in the glycolytic and shunt pathways were normal or increased. Spectral analyses of oxy-, met-, and cyanmethemoglobin from hemolysates were normal. By starch block electrophoresis, 60 to 70% of the hemoglobin was Hb A, and 25 to 35% was a hemoglobin migrating between Hb A and S at pH 8.6. Fingerprint analysis showed that peptide β-Tp VII was replaced by two new peptides, Ala-Arg and Gly-Lys, identifying the abnormal hemoglobin as Hb Zürich. This substitution presumably accelerates oxidative denaturation of the molecule, which then permits irreversible oxidation of other cellular constituents and premature cell death. In 4 days after administration of Fe^{59} citrate during a hemolytic episode, radioactivity appeared in approximately equal amounts in the two hemoglobin fractions. Specific activity of Hb Zürich was more than twice that of Hb A. After incorporation of Fe^{59} *in vitro* by reticulocyte-rich erythrocytes, ratio of specific activity of Hb Zürich to Hb A was 4:1. These observations suggest preferential loss or destruction of Hb Zürich.