ABSTRACTS

An Investigation of Purine and Pyrimidine Excretion in Normal and Leukemic Subjects Utilizing Ion Exchange Column and Paper Chromatographic Techniques. WILLIAM S. ADAMS, WILLIAM A. SKOOG, and FRANCES W. DAVIS, Los Angeles, Calif. (Introduced by William N. Valentine).

Although extensive studies of uric acid excretion in leukemic subjects have been reported in the past, very little is known of the urinary excretion of other purines and pyrimidines in this disease. Earlier workers have been hampered by the lack of suitable methods for investigating these substances. Our laboratory has developed a method which consists of the absorption of an aliquot of a 24 hour urine on a Dowex 2 anion exchange column and fractional collection of the effluent using a pH gradient elution technique. The elution properties, R_f values obtained from conventional two phase paper chromatographic methods, and ultraviolet absorption spectra have been compared with known standards. This method permits the separation, identification and quantitation of products of nucleoprotein degradation in urine.

The elution patterns from 4 normal and 40 leukemic patients have been studied. Results indicate that normal urine contains cytosine, uracil, uridine, guanine, inosine, adenine and uric acid. Marked quantitative differences in the excretion of some of these substances have been noted in all leukemic subjects studied to date. Moreover, cytidylic acid, thymine and guanosine have been detected only in leukemic urine. Increased inosine excretion has been found in the urine of patients with acute leukemia and chronic lymphocytic leukemia, whereas guanosine has only been found in urine of patients with chronic granulocytic leukemia.

Insufficient data are as yet available to enable one to relate a pattern of excretion to a particular morphological type of leukemia. However, it is possible that the results of these investigations will permit a more accurate differentiation of the acute leukemias than is presently possible on the basis of existing morphologic or tissue culture techniques. Furthermore, if specific biochemical abnormalities are demonstrated, it may be possible to design more suitable antimetabolite therapy in the treatment of these diseases.

The Effect of Anesthetic Agents on Water Diuresis. ARTO H. APRAHAMIAN, JAMES L. VANDERVEEN, JOHN P. BUNKER, ANNE P. MURPHY, and JOHN D. CRAWFORD,* Boston, Mass.

The commonly observed failure of intra- and postoperative patients to develop water diuresis in circumstances which would normally so predispose and where such a response would be of homeostatic value has prompted an investigation of the role of anesthetic agents in diuresis inhibition. Nineteen young adult female patients scheduled for elective surgery were studied. All were in good health. On arrival in the operating suite, fasting but not premedicated, a brisk diuresis was induced by rapid intravenous infusion of 5 per cent dextrose and water. There was no delay in the development of diuresis ascribable to apprehension or other factors. Atropine was then given without effect on the diuresis and finally anesthesia was induced using ether, nitrous oxide, cyclopropane or thiopental. Measurements were made of urine flow, urine and plasma osmolality and of the arterial blood levels of the anesthetic agents.

Induction with ether, nitrous oxide or cyclopropane regularly caused abrupt and long lasting inhibition of the established water diuresis. The responses were similar but not strictly comparable to the inhibition resulting from intravenous vasopressin. Induction with thiopental was without effect on the diuresis. Furthermore, patients anesthetized with thiopental continued diuresis during the subsequent addition of any one of the three inhalation agents. During thiopental anesthesia water diuresis could be inhibited by exogenous intravenous vasopressin and was noted to subside spontaneously when water loading was terminated.

It is concluded that thiopental has no antidiuretic effect of its own and, at anesthetic levels, it prevents the antidiuretic response to ether, nitrous oxide and cyclopropane. These data suggest the possibility that use of thiopental may serve to avoid for the surgical patient the intra- and postoperative period of high susceptibility to water intoxication which is due, at least in part, to the prolonged fixed antidiuresis caused by certain of the commonly used inhalation agents.

Studies of Glucuronide Synthesis and of Glucuronyl Transferase Activity in Liver and Serum. IRWIN M. ARIAS, BERTRAM A. LOWY, and IRVING M. LONDON,* New York, N. Y.

In studies on the mechanism of conjugation of glucuronic acid with bilirubin and other receptors, evidence has been obtained which is suggestive of a new pathway in glucuronide formation. On incubation of 4-methyl umbelliferone (4-MU) with *a*) glucuronic acid (GA), *b*) adenosine triphosphate (ATP), *c*) uridine triphosphate (UTP), and *d*) the soluble fraction of rat liver homogenate, 4-MU glucuronide is formed. Preliminary studies indicate that alpha-glucuronic acid-1-phosphate (GA-1-P) can substitute for GA and ATP. In this system, glucuronide synthesis occurs primarily with the soluble fraction of liver, whereas the formation of glucuronides from uridine diphosphate glucuronic acid (UDPGA) involves glucuronyl transferase which is present in microsomes. This capacity of liver to synthesize glucuronides from glucuronic acid is pertinent to the observation of Danoff and Holt that the administration of GA to

* Member.

infants with erythroblastosis or physiologic jaundice results in reduction of the serum concentration of indirect-reacting bilirubin, presumably by promoting the formation of bilirubin glucuronide.

Previous studies have established that glucuronyl transferase, which is present in liver microsomes of man and several animal species, transfers GA from UDPGA to various receptors including bilirubin, and have shown a specific deficiency of this enzyme in Gilbert's disease. To investigate further the role of this enzyme in this and other clinical states, a highly sensative assay which employs 4-MU, a fluorescent compound, as the receptor has been developed and applied to liver obtained on needle biopsy and to serum. Marked diminution in glucuronyl transferase activity (GTA) was observed in liver biopsy specimens of six patients with nonhemolytic acholuric jaundice of varying severity (serum bilirubin, 1.8 to 22 mg. per cent). In the serum of normal rats, rabbits, dogs or humans, GTA was not detected. But in animals with hepatic necrosis produced by administration of carbon tetrachloride, in three patients with massive hepatic necrosis and in five of seven patients with viral hepatitis, GTA was demonstrable in the serum. To effect glucuronide synthesis in serum, UDPGA was required.

Quantitative Variations in the Nucleohistone Binding Properties of Human Serum. JOHN ARNOLD and CHARLES PAK, Chicago, Ill. (Introduced by Richard L. Landau).

The serum of patients with systemic lupus erythematosus (SLE) has been shown to contain factor(s) with a special affinity for nucleohistone (NH). These factors are difficult to estimate quantitatively and probably escape detection entirely when they occur in low concentrations. The customary determination of these factors by the lupus erythematosus cell test depends in part on the process of phagocytosis. A new quantitative and sensitive method for measuring the affinity of serum components to NH was devised to explore the variations of this property of serum in SLE and in the serum of patients with other dysproteinemias. This method is based on the ability of a nucleohistone film at the air-water interface to react to a test substance by changes in the surface area of the NH film when serum components either bind or penetrate the film.

Under prescribed conditions of pH, temperature, and ionic strength of substrate solution, source and state of polymerization of the NH film and lateral pressure on the film, this method will reveal the presence of factors with an affinity for NH when the serum concentration is as low as one part serum per million parts of substrate solution. The method gives results reproducible to within ± 2 per cent.

The serum of 15 patients with histologically verified SLE was compared to the serum of normal people and to the serum from patients with a variety of plasma protein abnormalities. The nucleohistone films gave a several-fold greater increase in area than did the sera from other sources. However, under suitable conditions the sera from all sources tested gave evidence of a limited degree of NH binding.

By using different sources of NH it has been possible to show that the component of human serum responsible for the NH binding phenomenon is not a single molecular species.

Leukokinetics as Measured with Radioactive Diisopropylfluorophosphate (DFP^{32}). JOHN W. ATHENS, ALVIN M. MAUER, HELEN ASHENBRUCKER, and GEORGE E. CARTWRIGHT,* Salt Lake City, Utah.

Previous work in this laboratory has demonstrated that leukocytes can be labeled with diisopropylfluorophosphate containing radioactive phosphate (DFP^{32}).

Two mg. of DFP^{32} containing 100 to 200 μc. per mg. of DFP is injected intravenously. The leukocytes are isolated from 20 ml. of blood by dextran sedimentation of the red cells followed by gramicidin-lysolecithin hemolysis of the red cells remaining in the supernatant suspension. Platelets are removed by differential centrifugation. The resulting leukocyte sample is plated between two scintillating plastic squares and the radioactivity is determined in a scintillation counter.

The shape of the radioactivity curve obtained in 50 normal subjects has been consistent and reproducible. The curve consists of three phases: a rapid exponential decrease in the first 48 hours after injection of the label (Phase 1); a second period of almost constant radioactivity (Phase 2); and a final exponential decrease in activity (Phase 3). The mean $T_{1/2}$ (± 1 S.D.) of Phase 1 was 5.8 ± 2.2 hours. Phase 2 terminated 11.5 days after labeling (range, 9 to 16 days). The $T_{1/2}$ of Phase 3 averaged 3 days (range, 2 to 6 days).

No evidence has been obtained that any one of the three phases can be explained by reutilization of the label, by elution of the label, or by removal of cells damaged by the label. Since most, if not all, of the leukocyte radioactivity is present in granulocytes, this method provides a means for studying the steady state kinetics of granulocytes. Several possible models for granulocyte kinetics will be presented and discussed.

Studies of the Properdin System in Normal Humans, in Infections and in Malignant Blood Dyscrasias, Using the Phage Technique for Its Measurement. ALDONA BALTCH and PAUL A. BUNN,* Syracuse, N. Y.

Properdin is a naturally occurring protein constituent of human serum. Its concentration varies under certain abnormal conditions of health, *e.g.*, in acute infections and in some blood dyscrasias. Its concentration in serum is measured by determining the degree of its virucidal activity against *E. coli* phage T_2r+. Serum from 30 normal adult subjects has been tested. In the majority (20 subjects) levels between 6 and 16 are found.

Serial determinations of levels of properdin have been made in 90 patients with acute and chronic infections. Serum from staphylococcal infections (20 with positive blood cultures, 12 with serious local infections) revealed

that early in the course of the disease, concentrations fell to below four units. Within four to six days levels rose to normal in those who recovered. Concentrations of properdin remained below two units in those who died. Similar experiences were recorded in acute infections caused by group A streptococci, in Gram-negative bacterial sepsis, in pneumococcal and mixed pneumonia, and in acute lung abscess and empyema. Patients with subacute bacterial endocarditis, Asian influenzal infections, and other subacute or chronic infections failed to demonstrate an initial fall of properdin from normal. All recovered.

Sixty-two patients with malignant blood dyscrasias have been studied (Hodgkin's disease, sarcoma, lymphocytic, myelogenous and monocytic leukemia, and multiple myeloma). Patients with active disease in each of the categories generally had levels below two units. In contrast, normal levels of properdin were observed in patients whose Hodgkin's disease, myelogenous leukemia, and sarcoma were quiescent. Reactivation of disease was associated with fall to zero levels. Patients with lymphocytic leukemia had levels below two units regardless of activity of disease.

Total complement determinations were made in 32 of the diseased patients. Serial changes in concentrations of properdin and total complement were unrelated.

Radioactive Sodium for the Measurement of Myocardial Blood Flow. BERNARD A. BERCU, WILLIAM H. DANFORTH, ERNEST E. PUND, JR., and GERALD A. DIETTERT, St. Louis, Mo. (Introduced by John R. Smith).

Na^{24} was injected into the myocardium of the open chest dog to determine if the disappearance curve could be used as a measure of myocardial blood flow. This project was predicated on the fact demonstrated by Kety that a small deposit of isotonic sodium chloride injected into striated muscle is removed at a rate that is proportional to the local blood flow. An amount of 2.5 to 3.75 microcuries in a volume of 0.1 to 0.15 ml. was injected. The radioactivity of the Na^{24} was measured at 30 second intervals with a collimated scintillation counter placed over the radioactive sodium depot. Counts per minute were plotted against time on semilogarithmic paper. Coronary blood flow was measured with a flowmeter connected between the left carotid artery and the cannulated left coronary artery. The myocardium supplied by the left coronary artery was determined by the postmortem injection of Evans blue dye into the cannula in the left coronary artery. The muscle colored by the dye was then weighed.

The slope of the decrease in the local radioactivity of the injected Na^{24}, k, was determined by the formula:

$$k = \frac{C_0 - C_1}{0.434\ (t_0 - t_1)}$$

where C_0 and C_1 are the logarithms of the radioactive counts at the times t_0 and t_1, respectively.

It was found that the slope of the disappearance curve, k, was a measure of myocardial blood flow. This value changed rapidly if blood flow was altered. With no coronary blood flow, as in the fibrillating heart, the k value is zero. Studies of the effect of work load and heart rate on the k value are underway, but these do not seem to alter it.

The Anabolic Effect of Sheep Prolactin in Man. DELBERT M. BERGENSTAL* and MORTIMER B. LIPSETT, Bethesda, Md.

Growth hormone prepared from the pituitaries of animals other than primates has been shown to be inactive in man. In contrast to this finding, we wish to report the anabolic effects of sheep prolactin in man. The preparation used was provided by the Endocrinology Study Section of the Public Health Service and had a potency of 20 International Units per milligram as determined by the pigeon crop-sac assay. There was only slight contamination with adrenocorticotrophic hormone, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone.

This preparation was given in 25 mg. doses twice daily subcutaneously to four women who had been subjected to hypophysectomy. The urinary nitrogen excretion decreased from 1 to 3 grams daily without significant change in fecal nitrogen. This effect took two to three days to develop and remained two to three days after discontinuing the prolactin injections. The urinary amino acid nitrogen was elevated during the periods of prolactin administration. Potassium and phosphorus balances were generally positive during periods of positive nitrogen balance.

Prolactin was ineffective in causing nitrogen retention in one man and in one woman who had had a pituitary stalk section and was lactating. Lactation did not increase in this patient nor was it noted in any of the other patients. There were no changes in the urinary excretion of 17-ketosteroids and 17-hydroxy corticoids throughout the studies.

In order to rule out the possible effect of the small amount of sheep growth hormone contaminating the prolactin, a purified sheep growth hormone was given to one of the patients who responded to prolactin. At a dose 10 times the maximum contaminating amount, the nitrogen balance and urinary amino acid nitrogen were unchanged.

These experiments demonstrate that a purified fraction of sheep pituitary which is active in stimulating the pigeon crop-sac can cause nitrogen retention in the hypophysectomized woman.

Granulocytic Activity of Human Plasma. HOWARD R. BIERMAN,* G. JUNE MARSHALL, TADASHI MAEKAWA, KEITH H. KELLY, and PAULINE PLANTE, Duarte, Calif.

The uniform response of leukopoiesis in all parts of the widely distributed red marrow indicates control by circulating substances. If humoral control of granulocytopoiesis exists, the plasma obtained during marrow regeneration following a nondestructive period of leukocyte depletion should exhibit granulocytic activity.

The intraperitoneal injection of 1 ml. of plasma per 100 gram of rat (Sprague-Dawley or Wistar) causes a characteristic absolute granulocytosis during the first 10 hours. Employing this rat bioassay system to measure the granulocytic potency of plasma, 30 subjects were studied on 71 occasions.

Fourteen plasma samples from 10 apparently healthy laboratory personnel induced an absolute granulocytosis of 215 to 663 per cent of the control level. Heating to 56° C. for 195 minutes or freezing at 4° C. for 6 weeks resulted in loss of activity. Nineteen plasma samples of 7 patients with nonhematologic neoplastic diseases induced granulocytosis of 88 to 893 per cent of the control level.

Leukopheresis in dog and man is followed by an increased granulocytopoiesis within 72 hours. Following 10 leukophereses in 6 patients, the plasma obtained 48 to 96 hours thereafter induced an absolute granulocytosis of 392 to 1,460 per cent of the control level and was distinctly higher than the granulocytic activity of plasma obtained before or 24 hours after leukopheresis.

Sixteen plasma samples from 10 patients with granulocytic and lymphocytic leukemia exhibited granulocytic activity (278 to 1,073 per cent). Four of the five patients whose plasma showed the highest granulocytic activity had acute myeloblastic leukemia. The granulocytic response to leukemic plasma was usually more prolonged than observed with nonleukemic plasma.

Further studies characterizing the granulocytic activity of human plasma indicate that it is related to the state of granulocytopoiesis in the marrow, is variable in its potency from day to day, and is thermolabile.

"Low Pressure" Kidney and the Water Concentrating Mechanism. WILLIAM D. BLAKE,* Portland, Ore.

A number of investigators have observed that moderate reduction in glomerular filtration rate (GFR) may be associated with either increased or decreased osmotic concentration of the urine. The direction of change apparently depends on whether flow is partially decreased in all nephrons or completely blocked in only a fraction of the nephron population. The above observations have been confirmed and extended, particularly with respect to complete cessation of flow in a portion of the nephrons. Studies were carried out on anesthetized, laparotomized dogs in which urine was collected separately from the two kidneys by ureteral catheterization. Creatinine clearance was used to estimate GFR. Urinary osmotic pressure (freezing point depression) was established above or below that of plasma by the intravenous infusion of hyper- or hypotonic solutions. After two or three "control" collection periods GFR was acutely decreased in one kidney either by partial occlusion of the main renal artery or by complete occlusion of a branch thereof. Partial occlusion of the main artery increased osmotic pressure of urine whether control urine samples were hyper- or hypotonic to plasma. When complete cessation of flow to a portion of the nephrons was induced by clamping a branch of the renal artery, there was an increase in GFR/TmG, urine flow/GFR and osmolar clearance/GFR ratios. The osmotic pressure of urine decreased when control samples were hypertonic to plasma and showed inconsistent and probably insignificant changes when control samples were hypotonic. Presumably complete cessation of blood flow to part of the kidney led to increased GFR in remaining functional units. An increased solute load per tubule would diminish osmotic concentration of a hypertonic urine. Physiological significance of this means for diminishing GFR remains to be explored.

Hypothalamic Irradiation in the Rat. P. BLANQUET and C. A. TOBIAS, Berkeley, Calif. (Introduced by John H. Lawrence).

The hypothalamus has been found to exert a control over thyroid metabolism. This important fact has usually been shown by the use of stereotaxic lesions produced in the median eminence. The possibility of irradiating strictly localized sections of the rat hypothalamus by means of a focused beam of high energy particles (from the Berkeley 184 inch cyclotron) has recently been demonstrated, and we have been able to focus the beam on different areas of the median eminence.

All rats (15 animals) whose median eminences were irradiated manifested localized cleancut damage at this level. The uptake of I^{131}, determined *in vivo* and after dissection of the thyroid, was normal or slightly elevated compared with controls. However, the amino acid content of the gland was found to be greatly altered. The intrathyroidal concentrations of monoiodotyrosine and diiodotyrosine, precursors of the hormone, were normal, but thyroxine was very low in all our experiments (between one-third and one-tenth of its mean value in blanks). This finding indicates that it is possible to obtain selective effects on thyroid metabolism even in the absence of pituitary lesions and seems to suggest a precise control by the median eminence area over the synthesis of thyroxine.

Acid Mucopolysaccharide Content of Thyroid Gland in Thyroid Disease and After Thyrotropin. ALFRED J. BOLLET and WILLIAM H. BEIERWALTES, Detroit and Ann Arbor, Mich. (Introduced by Stefan S. Fajans).

Thyroid tissue has been reported to be rich in glycoprotein and poor in mucopolysaccharide. Acid mucopolysaccharide analyses were done by a new chemical method on human and dog thyroid and the skin of patients with pretibial myxedema. Thyroids of untreated dogs showed an average mucopolysaccharide content of 64.6 μg. per cent dry weight measured as uronic acid (carbazole method). After the administration of 20 to 30 units of thyrotropin (TSH) to 3 dogs, the concentration increased 1.6- to 2.3-fold compared to control lobes. It is of interest that the concentration of mucopolysaccharide in the thyroid increased under TSH stimulation, when cell mass increased and intrafollicular colloid decreased.

These observations on the dog thyroid after increased TSH activity prompted us to perform mucopolysaccha-

ride assays on skin and thyroid tissues in human thyroid diseases allegedly associated with varying degrees of TSH activity. Biopsy tissue from areas of pretibial myxedema in three patients contained 2.4 to 3.7 times as much mucopolysaccharide as clinically uninvolved areas of skin from the same patients. The mucopolysaccharide content of surgically removed thyroid tissue from five patients with colloid adenomatous goiters ranged from 125 to 288 mg. per cent; two patients with exophthalmic goiter and one with Hurthle cell adenoma had concentrations in the same range. The highest value obtained, 421 mg. per cent, was in a patient with Hashimoto's struma. Approximately 60 per cent of the mucopolysaccharide in these glands was digested by testicular hyaluronidase. Chromatographically at least two components were present. One had electrophoretic and chromatographic characteristics of chondroitin sulfate and the other of hyaluronic acid. Further specimens are under assay at present to add statistical significance. Appropriate histology correlation will be made.

A Measurement of the Effect of Psychic Stimulation on Peripheral Venous Tone. STUART BONDURANT, SANFORD I. COHEN, and ALBERT J. SILVERMAN, Wright-Patterson AFB, Ohio, and Durham, N. C. (Introduced by William M. Nicholson).

Evidence of psychogenic changes in peripheral vascular tone is often observed. The present study was designed to measure these changes in the tone of peripheral veins.

Pressure was recorded from a temporarily occluded, tributary-free forearm vein segment. Pressure change in this system is caused by neurogenic change in the tone of the vein. This pressure was measured as each of 10 subjects heard a randomized series of four "charged" words, four "bland" words and four equivalent periods of silence. The subjects were alone in a dark, soundproof room throughout the experiment. The words were delivered at four minute intervals through earphones. Venous occlusions were released for two minutes between measurements. The "charged" words (words which were expected to shock the subject) and the "bland" words (words to which the subject was expected to be indifferent) were matched in number of syllables and were delivered at equal volumes.

The group mean maximal pressure changes were: following a period of silence, +2.7 cm. H_2O (range, −3 to +17); following a "bland" word, +6.9 (−1 to +24); following a "charged" word, +11.6 (+1 to +32). The large ranges of segment pressure change chiefly reflect differences among individuals in the magnitude of the response. The difference between the response of the group to each of the three conditions is significant at the $p < 0.002$ level (nonparametric analysis). The pressure changes occurred slowly, reaching a peak 10 to 15 seconds after the stimulus was given, and persisted, under the experimental conditions, for 30 to 60 seconds. The pressure change following a "charged" word was of the same order of magnitude as that observed in the forearm vein segment following Valsalva maneuver.

Such psychogenic change in peripheral venous tone would be expected to influence circulatory dynamics in health and disease and would probably modify various clinical and experimental tests of cardiovascular function.

Ventricular Rate and Response to L-epinephrine and Levarterenol in Experimental Heart Block: The Influence of Pentobarbital, Hydrocortisone, and Acid-Base Changes. SAMUEL H. BOYER and ARTHUR W. CHISHOLM, Baltimore, Md. (Introduced by Victor A. McKusick).

Mortality in Stokes-Adams disease remains high, death occurring when an adequate myocardium might respond to proper stimulation. Some factors which may influence instability of the ventricular pacemaker and response to drug therapy have been investigated. Levarterenol and *l*-epinephrine (0.0045 micromoles per kilogram) were rapidly injected intracavally in dogs with surgically induced chronic complete heart block. In trained animals the change produced in ventricular rate was inversely related to resting rate. The absolute rate, however, did not depend on resting rate and was fairly fixed for a given drug dosage in each dog. Therefore the modification of resting rate and response to sympathomimetic amines could be studied independently and simultaneously.

Intravenous pentobarbital was followed by transient increase in resting rate and significant ($p < 0.02$) persistent increased drug sensitivity. Intravenous hydrocortisone had no acute effect on either parameter. Acidosis ($pH \leqq 7.1$) produced marked slowing of resting rate and refractoriness to sympathomimetic amines. CO_2 was a much more powerful depressant of resting rate than was 0.6 N HCl for the same arterial pH. Drug responsiveness was depressed equally by both respiratory and metabolic acidosis. Alkalosis ($pH \geqq 7.5$), however induced, was accompanied by roughly parallel increases in resting rate and drug sensitivity. Ectopic beats following sympathomimetic amine injection were observed principally during periods of decreasing alkalosis.

These observations suggest a few ways in which ventricular rate and response to sympathomimetic amines may be altered. Response to *l*-epinephrine and levarterenol is apparently dependent upon extracellular pH while resting rate, depressed more by CO_2 than by mineral acid, is possibly dependent upon intracellular pH. Increased ventricular rate with elevated pH may be due to hypersensitivity toward endogenous sympathetic discharge. Acidosis, particularly of respiratory origin, should be promptly recognized and treated in the patient with complete heart block. When no initial acid-base derangement is present, alkalinization is hazardous.

Saluretic Effect of Antidiuretic Hormone (ADH) Inhibition in the Hydropenic State. JOHN W. BOYLAN and DOROTHY E. ANTKOWIAK, Buffalo, N. Y. (Introduced by David K. Miller).

When conditions known to enhance water diuresis are imposed during hydropenia, we have found that a different pattern of solute excretion is observed.

The methods employed were *1*) negative pressure breathing (NPB), and *2*) alcohol, both of which have been shown previously to increase urine flow by an increment of free water, solute excretion being relatively unaffected. In NPB the postulated mechanism is one of reflex inhibition of ADH secretion initiated by stretch volume receptors within the thorax. Alcohol is believed to inhibit ADH secretion by direct action on the neurohypophysis.

The subjects were male medical students, known to respond by diuresis to NPB. They remained supine, urine being collected by an indwelling catheter. Calculations of osmolar clearance (C_{osm}) and free water excretion (C_{H_2O}) were made in the usual way from the determined osmolar concentrations of urine and plasma.

When three consecutive half-hour periods revealed a steady state of urine formation, the subjects breathed at a continuous pressure of -15 cm. H_2O for one-half hour. A period of recovery followed.

Under these conditions the initial increase in urine flow is associated with an increase in solute excretion in such a way that the free water excretion actually falls below control levels.

This finding was duplicated in each subject when alcohol ingestion was substituted for NPB. The alcohol was given as 100 proof bourbon whiskey, 45 to 60 ml. by mouth, the amount found to produce a diuresis of comparable magnitude to that following NPB.

These observations suggest a diphasic consequence of ADH inhibition at low urine flows, the initial action being one of saluresis. Alternate mechanisms will be discussed, but the data indicate that volume receptor activity in the usual physiological state of water conservation is more effective than studies carried out during hydration have indicated.

The Uptake of Isotopic Magnesium in Different Portions of the Heart. J. LEONARD BRANDT, WARREN GLASER, and AUDREY JONES, Brooklyn, N. Y. (Introduced by R. Janet Watson).

Previous studies from this laboratory have shown that the uptake of Mg^{28} (half-life, 21.3 hours) per gram dry tissue is from 3 to 10 times greater in heart muscle than in skeletal muscle over a 48 hour period of observation. The studies focused attention on the unusual "avidity" of heart muscle for isotopic magnesium as compared to skeletal muscle since the reported magnesium content of each is about the same. The present study is an extension of these observations.

In a series of 4 dogs and 6 rabbits which received isotopic magnesium intravenously and were sacrificed at intervals over a 48 hour period, samples of muscle from the cardiac septum, left ventricle and right ventricle were taken and the concentration of Mg^{28} per gram dry weight determined. The concentration was highest in the septum and least in the right ventricle in 8 of the 10 animals. According to the binomial distribution, the probability of any sequence of 3 out of a possible 6 occurring 8 out of 10 times by chance alone is about 1 in 8,000. Combining the data for all 10 animals, the t test for the significance of the differences between the means of related samples is significant at $p < 0.001$ for comparison of septum with left ventricle, septum with right ventricle and left ventricle with right ventricle. Thus, the sequence septum, left ventricle, right ventricle, as regards the order of concentration of Mg^{28} in the heart, has very high statistical significance.

The mean values of the per gram dry weight concentration of Mg^{28} remain different in the samples over the 48 hours, and are 0.0295, 0.0269, and 0.0207 μc. for septum, left ventricle, and right ventricle, respectively. Reported differences in the per cent muscle and connective tissue in the right and left ventricles are in the same direction (more muscle per gram on the left) as the concentration differences of Mg^{28} in the right and left ventricles. However, the anatomical differences between right and left ventricles are of a greater order of magnitude than the observed chemical differences, and therefore do not completely explain the findings. It is concluded that the finding of the highest Mg^{28} concentration in the cardiac septum, least in the right ventricle with the left ventricle intermediate, has biological significance.

The Mechanism of Tolerance to Fever. ABRAHAM I. BRAUDE,* MARGARET ZALESKY, and HERNDON DOUGLAS, Pittsburgh, Pa.

The state of diminished febrile reactivity, or tolerance, which follows repeated injections of bacterial pyrogens has been associated with accelerated clearance of circulating pyrogen as measured by the transferable-pyrogen technique of Beeson, or by our method employing massive doses of radioactive endotoxin. As high-activity Cr^{51} became available, however, we repeated our clearance studies with smaller doses approaching those used in fever studies, and injected 0.1 mg. Cr^{51}-labeled *E. coli* endotoxin intravenously. Tolerant and nontolerant rabbits cleared this dose of endotoxin at equal rates with circulating concentrations falling 90 per cent within 15 minutes. To confirm these results with another pyrogen, tuberculous rabbits were made tolerant to tuberculin-fever by repeated intravenous injections of 1.0 mg. tuberculin (PPD). After intravenous injection of 5.0 mg. Cr^{51}-labeled PPD, circulating tuberculin disappeared at identical rates in nontolerant and tolerant tuberculous rabbits, with 80 per cent cleared at 30 minutes. This dissociation of enhanced clearance from febrile tolerance also occurred in rabbits whose tolerance to the pyrogenic action of endotoxin disappeared; such animals cleared massive doses of endotoxin much faster than controls despite equal febrile responses.

Turning elsewhere for explanations of tolerance, we studied febrile responses after two successive injections of endotoxin at various intervals. At two hours, a second injection was met by complete refractoriness; but this refractory state gradually diminished until a monophasic febrile response appeared at 12 hours that was identical to the classical tolerant fever curves ordinarily requiring

a week to induce by daily injection. Rabbits injected repeatedly with 0.001 mg. endotoxin every 12 hours maintained the refractory monophasic response and the tolerant pattern. This refractoriness at 12 hours was unaccompanied by accelerated removal of massive doses of circulating endotoxin.

These results indicate that febrile tolerance is independent of accelerated removal of pyrogens by reticuloendothelial cells and may represent, instead, perpetuation of the refractory state of the heat centers.

The Nature of the "Defect" in Concentrating and Diluting Mechanisms of the Diseased Kidney. NEAL S. BRICKER, RICHARD R. DEWEY, HERBERT LUBOWITZ, and JAMES M. STOKES, St. Louis, Mo. (Introduced by Herman N. Eisen).

Patients with chronic renal disease are characteristically unable to elaborate concentrated urine. Ability to dilute the urine persists longer, but ultimately diminishes and permanent isosthenuria supervenes. Although this sequence has never been adequately explained, it has been widely held that specific tubular sites for concentration and dilution are damaged by the pathologic processes. The present studies do not support this thesis.

The concentrating and diluting mechanisms were investigated in dogs with experimentally induced chronic renal disease. The lesions were either: *a*) contracted kidney histologically resembling advanced Bright's disease (produced by perfusing the anoxic kidney with an aminonucleoside solution); or *b*) progressive pyelonephritis. Employing a bladder-splitting technique, the function of the diseased kidney was compared with that of the contralateral intact kidney in serial studies.

Despite marked decrease in renal mass, both concentrating ability (solute-free water reabsorbed per 100 ml. of glomerular filtrate [$T^c_{H_2O}$/GFR]) and diluting ability (free-water clearance per 100 ml. of glomerular filtrate; [C_{H_2O}/GFR]) were consistently normal for the diseased kidney. Simultaneous values for diseased versus intact kidneys revealed $T^c_{H_2O}$/GFR to be slightly greater for the intact kidney, and unexpectedly C_{H_2O}/GFR was slightly greater for the diseased kidney. Hence, from maximum concentration through maximum dilution, urine from the diseased kidney contained relatively more free-water per unit volume of glomerular filtrate. Accordingly, maximum osmolar U/P ratios were less for the diseased kidney.

From the experimental data, it is postulated that patients with chronic bilateral renal disease develop isosthenuria because of an adaptive change in nephron function (increased GFR per functioning nephron) evoked by the decreased total nephron population, rather than from specific impairment of tubular transport systems. Under these conditions, *normal concentrating and diluting mechanisms* brought to bear on increasing volumes of filtrate would produce progressively smaller modifications of final urine osmolarity. The emerging concept furnishes a rational explanation for the observation that concentrating ability disappears before diluting ability.

Evidence for the Presence of More Than One Mechanism of Acid Production in the Kidney. WILLIAM A. BRODSKY * and JOHN T. KAIM, Louisville, Ky.

If secretion of hydrogen ions were the sole mechanism for acidification of urine, one would predict that pCO_2 of urine would be greater than or equal to that of plasma during formation of acidic urine. However, such a prediction was not verified experimentally in dogs under conditions of both metabolic and respiratory acidosis. In short, urine pCO_2 was less than plasma pCO_2 by 6 to 40 mm. of Hg in 25 per cent of over 200 periods of production of acidic urine, and was greater than plasma pCO_2 in 60 per cent of such periods. Moreover, injection of large amounts of a potent preparation of carbonic anhydrase failed to obliterate gradients in acidic urine. Similar injections did obliterate gradients in alkaline urine. The validity of calculated values of pCO_2 in several specimens of acidic urine and plasma was confirmed by tonometric determinations of α CO_2 and pK'_1 of these fluids. Implications of these data are: *1*) There are at least two tubular mechanisms for acidifying urine —one which tends to increase the urinary pCO_2, like H^+ ion secretion, and another which decreases urine pCO_2, like reabsorption of bicarbonate ion as such. *2*) The existence of pCO_2 gradients suggests that the permeability coefficient of certain distal tubule walls for CO_2 must be less than the value usually assumed on the basis of Krogh's datum on a rubber membrane. *3*) "Delayed" reaction rates of $CO_2 \leftrightharpoons H_2CO_3$ in lumen fluid can account for pCO_2 gradients in alkaline, but not in acidic urine. *4*) Mechanisms responsible for production of acidic urine (H^+ ion secretion or HCO_3^- ion reabsorption) are not active during production of alkaline urine.

Comparative Hemodynamic Effects of Isoproterenol and Exercise (Walking) in Cardiac Patients. ROBERT A. BRUCE,* LEONARD A. COBB, SHIGEAKI KATSURA, and JOHN MORLEDGE, Seattle, Wash.

Preliminary studies on hemodynamic effects of isoproterenol and exercises have been made on eight nonsedated but symptomatic ambulatory patients with either congenital or rheumatic heart disease. Both radial and pulmonary arteries were catheterized for pressure recording and determination of cardiac output by direct Fick principle; indicator dilution curves were obtained in many patients. Observations were made during steady states of rest, both recumbent and sitting, during intravenous infusion of isoproterenol, usually 1 to 3 gamma per minute, and during exercise while walking on a treadmill at 10 per cent grade and 1.7 mph. This exercise increased oxygen consumption about fourfold.

In each case, heart rate and cardiac index increased, as systemic resistance fell, with either exercise or isoproterenol. In confirmation of positive inotropic effect reported by Dodge and Murdough, stroke index increased with isoproterenol in all but two patients. This increase exceeded that observed with exercise. Stroke index fell with either isoproterenol or exercise in two individuals

(complete A-V block in one, mitral stenosis in the other), presumably due to shortened diastolic filling period associated with marked acceleration of heart rate. As appearance and mean transit times shortened, central volume increased in certain patients with isoproterenol. Total pulmonary resistance decreased significantly in all with isoproterenol; from the available data this reduction was chiefly postcapillary.

During recumbency the positive inotropic effect of isoproterenol, when not precluded by tachycardia, exceeds the increase in stroke index observed with moderately severe exercise in the upright posture in cardiac patients. Mechanisms of inotropic action are not known, but changes in vascular resistance and venous reservoirs may be contributory.

Coarctation of the Abdominal Aorta with Stenosis of the Renal Arteries and Hypertension: Clinical and Pathological Study of Two Cases. ALBERT A. BRUST,* JOHN M. HOWARD, MILTON BRYANT, and JOHN GODWIN, Cincinnati, Ohio, and Atlanta, Ga.

Two youthful hypertensive patients with stenosis of the abdominal aorta and renal arteries, both of whom came to necropsy, have afforded an opportunity for detailed study of the associated clinical and pathologic findings. Because of the experiences with the first patient, a correct diagnosis was made antemortem in the second.

Although these patients bore striking similarity to each other their clinical manifestations were clearly distinguishable from those of thoracic coarctation. In both, symptomatic hypertension appeared at a youthful age, systolic and diastolic blood pressure elevation in the legs equaled or exceeded that in the arms, a systolic thrill and bruit were present over the upper abdomen and lumbar spine, and pulses in the lower extremeties were only slightly diminished in volume. The ribs were not notched and collateral circulatory channels were not apparent.

Urinalyses, concentrating capacity and phenolsulphonephthalein excretion tests were normal. Aortography clarified the diagnosis in both instances but a complication of the procedure caused the death of one of the patients. The other died of cerebral hemorrhage.

Pathologic features included stenosis of the abdominal aorta at the renal artery level and partial obstruction of both renal arteries from intimal thickening, apparently occurring as a "jet stream" phenomenon. There was no evidence of hypertensive vascular or parenchymal renal damage distal to the constricted arteries. In addition, the aorta of one patient contained an anomalous valve-like structure 2 cm. in length which produced partial aortic obstruction above the renal arteries.

Twelve reports of coarctation limited to the abdominal aorta have appeared in the medical literature since 1861. Their manifestations plus those of these two additional patients facilitate the delineation of certain characteristic physical signs whose clinical recognition is essential to accurate diagnosis and the planning of appropriate surgical therapy.

Factors Influencing the Diuretic Response to Ingested Water. MAURICE B. BURG, SOLOMON PAPPER, and JACK D. ROSENBAUM,* Boston, Mass.

Water diuresis was established in normal human subjects by the institution and maintenance of a large oral water load (20 ml. per kilogram body weight). Total urine flow and free-water clearance were found to vary with osmolal clearance and sodium excretion. Thus in the seated subject, each fell proportionately, urinary osmolality remaining constant. The assumption of recumbency caused an increase in each, again proportionately, without change in urinary osmolality. Similarly a proportionate rise in free-water clearance and osmolal clearance was produced in the seated subject by the hypotonic expansion of the extracellular fluid volume. The rate of excretion of urea and potassium was unrelated to these changes. The fact that sodium excretion and osmolal clearance always paralleled each other made it impossible to correlate urine flow and free-water clearance more closely with one than with the other. Accordingly experiments were performed in which osmolal clearance was increased under conditions such that sodium excretion was not increased (seated posture, previous dietary restriction). This was accomplished either by the ingestion of urea or the infusion of mannitol, sufficient water being administered to prevent a rise in serum osmolality and antidiuretic hormone release. Under these circumstances free-water clearance did not rise despite an increase in osmolal clearance. On the other hand a comparable or lesser increase in osmolal clearance, accompanied by a rise in sodium excretion, was always associated with a rise in free-water clearance, suggesting that the renal handling of sodium is the prime mover.

Chemical Composition of Amyloid. EVAN CALKINS and ALAN S. COHEN, Boston, Mass. (Introduced by Walter Bauer).

Present studies have attempted to obtain a clear concept of the chemical nature of amyloid, and to isolate this substance in a form suitable for more precise definition.

Livers and spleens, obtained immediately post mortem from eight patients with various types of amyloidosis, and from individuals without amyloidosis, were frozen and subsequently homogenized and analyzed for dry weight, nitrogen, sulfur, phosphorus, ash, hexosamine, hexose, uronic acid, lipid, proline, hydroxyline and glycine content. Concentrated preparations of amyloid were obtained by differential centrifugation with sucrose and polyvinylpyrollidone, and were similarly characterized. Extraction of amyloid with 0.02 N NAOH yielded a fraction suitable for ultracentrifugal and electrophoretic characterization.

The results, taken together, indicate that amyloid consists of carbohydrate (5 per cent), ether soluble material (15 per cent), and protein, and has a high water content. The carbohydrate consists of hexosamine (2 per cent chiefly glucosamine) and hexoses (galactose, glucose, mannose, fucose). Sialic acid is also present. Uronic acid is less than 0.1 per cent (by orcinol and carbazole

techniques), indicating that amyloid does not contain quantitatively significant amounts of polyuronides, such as hyaluronic acid or chondroitin sulfate. The low hydroxyproline content (less than 1 per cent) suggests that the protein component is not collagen. This was substantiated by proline and glycine analyses.

Physical characterization of the extracts showed considerable homogeniety in the ultracentrifuge (sedimentation constant 6 S) and by electrophoresis (an α globulin). These values are also characteristic of serum α-2 glycoprotein, which was markedly elevated in three patients with secondary amyloidosis. The serum of one of these patients was fractionated (by Dr. Karl Schmid), and the purified α-2 glycoprotein was found to contain 4 per cent hexosamine, 5 per cent sialic acid, and 4 per cent hexose (galactose, mannose).

Renal and Cellular Responses to Acute and Chronic Respiratory Acidosis. NORMAN W. CARTER, DONALD W. SELDIN,* and H. C. TENG, Dallas, Texas.

Renal and cellular compensations for respiratory acidosis were studied in tube-fed rats kept in a metabolic cage constructed to maintain a given atmospheric CO_2 tension constant. Blood and urine electrolytes and acid-base patterns were measured; renal carbonic anhydrase and glutaminase activities were assayed. Rats were sacrificed in groups at intervals from one hour to 14 days to distinguish acute and chronic effects.

After one hour in 10 per cent CO_2 there was a rise in plasma pCO_2 (35.4 ± 2.6 mm. Hg to 91.4 ± 3.1 mm. Hg) and CO_2 content (24.7 ± 1.8 mEq. per L. to 34.4 ± 1.8 mEq. per L.) while there was a fall in plasma chloride (108.8 ± 2.8 mEq. per L. to 96.0 ± 2.5 mEq. per L.) and blood pH (7.43 ± 0.02 to 7.17 ± 0.04). By 24 hours, at the same plasma pCO_2, CO_2 content rose to 42.0 ± 3.0 mEq. per L. while plasma chloride fell to 88.1 ± 4.0 mEq. per L. No further significant changes supervened thereafter.

Urine ammonia, titratable acid and chloride excretion were increased during the first day only, while potassium excretion remained elevated throughout. Sodium excretion was unchanged. Despite profound acidosis, activities of renal glutaminase and carbonic anhydrase did not increase. Muscle analysis disclosed slight, but significant, decrease in potassium and a more marked decrease in sodium. Acetazolamide (Diamox®) administration, sufficient to suppress measurable renal carbonic anhydrase activity completely, resulted in high plasma pCO_2 (180.7 ± 31.8 mm. Hg) and a CO_2 content of 51.3 ± 4.40 mEq. per L.

From these studies it is concluded that: *1*) Elevation of bicarbonate and reduction of chloride in plasma is maximal at 24 hours; these changes are principally a consequence of cellular buffering. *2*) Renal glutaminase and carbonic anhydrase are not activated by intracellular acidosis. *3*) Increased bicarbonate reabsorption can occur as a result of hypercapnea in complete absence of renal carbonic anhydrase activity.

Left Ventricular Function at Rest and During Exercise. CARLETON B. CHAPMAN,* ORLAND BAKER, and JERE H. MITCHELL, Dallas, Texas.

Simultaneous left ventricular volume and pressure curves were recorded in five anesthetized dogs at rest and during exercise, using a biplane cinefluorographic technique at 30 frames per second. Volume was calculated from tracings from each pair of frames using a modification of Simpson's parabolic rule. The error of the method, as determined from ventricular model experiments, is less than 10 per cent.

Resting end-diastolic volume was 76.1 ± 7 cc. and that during (not after) exercise was 64.8 ± 7 cc. The difference was consistent throughout the studies and is statistically significant.

Exercise produced slight increase in stroke work but total power production per minute rose from 37 ± 8 to 55 ± 0.6 thousand Gm.–cm. per Kg. Kinetic energy, calculated from ejection and velocity curves, was 2 per cent of total work (resting) and 4 per cent during exercise. The underestimate of potential (PV) work as a result of using mean instead of integrated values was negligible at rest but was 13 per cent during exercise. The corresponding figures for kinetic energy were 35 and 51 per cent, respectively.

Variation in the heart's application of force from stroke to stroke, and the dynamic effect of bigeminy and bradycardia, are easily discernible in the volume-pressure records.

The study demonstrates the value of biplane cinefluorographic methods for obtaining "instantaneous" ventricular volume curves. It shows conclusively that ventricular end-diastolic volume decreases during exercise, that failure to calculate kinetic energy at high flows may lead to significant error, and that use of mean values for calculating ventricular power production is inadmissable where high flows are involved.

Erythropoietic Response to Repeated Phlebotomy with Iron Replacement in Man. ROBERT G. CHAPMAN and MATTHEW BLOCK,* Denver, Colo.

Measurements in the adult human with hemolytic anemia indicate the ability to increase the rate of erythropoiesis to seven times normal. What increase may be produced in the adult who is without hematologic disorder? To answer this, two adult human males were subjected to phlebotomy two to three times a week for periods of 56 and 130 days. In order to avoid erythropoietic limitation secondary to iron deficiency, these men received intramuscular iron (Imferon®) in amounts equal to that removed by phlebotomy.

Initially a fall in hemoglobin and hematocrit occurred, but after two to three weeks, these indices stabilized at two-thirds of their original values. The rate of hemoglobin synthesis, assuming negligible *in vivo* breakdown of young red cells, reached amounts of 16.4 and 13.1 grams per day (0.27 grams per kilogram body weight per day and 0.21 grams per kilogram body weight per

day, respectively). This represents only a two- to threefold increase in erythropoiesis over that required for normal replacement of aging cells prior to the phlebotomies. The average reticulocyte counts during phlebotomy, correcting for a decreased red count, were 3.6 per cent and 4.2 per cent, respectively. The plasma iron turnover measured at the end of the experiment in the subject with the greater erythropoiesis was 165 mg. per day or five to six times normal, indicating that most of the turnover was unrelated to erythropoiesis. His red cells had taken up 85 per cent of the iron tracer by the fourth day. At this time the bone marrow contained iron and showed only a 10 to 20 per cent increase in erythroid activity in comparison with the prephlebotomy marrow.

Serum Cholesterol Esters, Cholesterol Esterase, and Intestinal Cholesterol Exchange in Patients with Malignant Obstructive Jaundice. SAMUEL CHENG, ESTEBAN PINEDA, and MALCOLM STANLEY,* Louisville, Ky., and Boston, Mass.

Total and ester serum cholesterols were determined in 85 patients with malignancy. Of 39 patients severely jaundiced (mean serum bilirubin, 21.5 mg.) the mean serum cholesterol ester percentage was 22.2 (pancreatic cancer without metastases), 25.1 (with hepatic metastases), and 21.4 (hepatic metastases from extrapancreatic sites). Of 20 mildly jaundiced patients (mean, 3.1 mg.), lesser abnormalities in respective groups were: 67.4 per cent, 64.1 per cent, and 62.1 per cent. Twenty-six non-jaundiced patients (all three groups) had normal esters (mean, 71 per cent). Decreased ester percentages were proportional to increased serum bilirubins. Absolute quantities of serum ester cholesterol were reduced in jaundice. Jaundiced patients also had increased free (absolute quantities, percentages), total cholesterols to five to six times normal. In jaundice, decreased ester does not favor diagnosis of hepatocellular disease; this decrease is also usual in malignant biliary obstruction.

In serum of a patient with localized pancreatic carcinoma (serum bilirubin, 24.6 mg.; total serum cholesterol, 900 mg.; 2 per cent esters) no synthetic cholesterol esterase activity was demonstrable.

In two patients (obstructions *complete,* one above) intestinal cholesterol exchange was: *"secretion"* (normal, 2 to 2.5 Gm. per day) 403, 392 mg. per day; *absorption* (normal, 1.5 Gm. per day; 75 per cent) 171 (37 per cent), 37 (8.5 per cent) mg. per day. Exclusion from the gut of pancreatic secretions, bile with endogenous free cholesterol and bile salts markedly reduced cholesterol absorption. Proportions of cholesterol "secretion" originating from bile intestinal mucosa are unknown. Mucosal "secretions" of 400 mg. per day may be high (hypercholesteremia, other consequences of biliary obstruction, abnormally shedding mucosa). Hence, bile probably contributes 1.6 to 2.1 Gm. per day or more normally to total secretion.

Cholesterol is absorbed largely as ester; decreases in intestinal absorption were probably mainly responsible for decreased serum esters. Free biliary cholesterol regurgitation into blood was likely an important factor in increasing serum free cholesterol. Absent cholesterol esterase probably contributed to extreme serum cholesterol ester decrease in above patient.

The Effects of Aminophylline on the Coronary and Peripheral Circulation in Patients with Emphysema. RAYMOND C. CHRISTENSEN, MURRAY GRAY, HARRY ABRAMSON, CARL KOBELT, LESEM J. BAER, and HARPER K. HELLEMS,* Detroit, Mich.

Since aminophylline increases coronary blood flow and cardiac output in the experimental animal, it is generally accepted that similar effects occur with its therapeutic use in patients with cardiac and pulmonary diseases. To determine its cardiovascular effects in 11 patients with moderately severe pulmonary emphysema, coronary and peripheral circulatory dynamics were measured using cardiac catheterization techniques before and 10 to 19 minutes after the intravenous administration of 0.5 grams of aminophylline. None of the patients had angina, and they were not in heart failure at the time of study.

The drug caused the expected pulmonary response with an increase in minute ventilation, a decrease in arterial CO_2 and no change in the resting reduced arterial oxygen saturation (88.6 ± 1.59 to 89.13 ± 1.96 per cent S.E.M., $p > 0.1$). There was a slight increase in total body oxygen consumption, a widening of the systemic oxygen A-V difference from 49.3 ± 0.7 to 62.9 ± 1.2 ml. per L., $p < 0.001$, and a decrease in cardiac output from 4.31 ± 0.28 to 3.85 ± 0.30 L. per minute, $p < 0.001$. Cardiac rate increased from 92 ± 3.0 to 113 ± 5 beats per minute, $p < 0.05$. Mean brachial artery pressure decreased from 101.4 ± 4.9 to 93.7 ± 5.6 mm. Hg, $p < 0.05$. There was a decrease in stroke volume from 47.9 ± 3.1 to 34.7 ± 2.7 ml. per beat, $p < 0.001$, and left ventricular stroke work from 67.6 ± 4.4 to 45.3 ± 4.2 Gm. M. per beat, $p < 0.001$. Peripheral resistance did not change.

Coronary blood flow decreased from 92.4 ± 4.2 to 79.5 ± 3.3 ml. per 100 Gm. left ventricle per minute, $p < 0.001$. Myocardial oxygen A-V difference widened from 11.72 ± 0.57 to 13.13 ± 0.63 volumes per cent, $p < 0.001$, chiefly due to a fall in coronary sinus O_2 content from 5.44 ± 0.40 to 4.48 ± 0.30 volumes per cent, $p < 0.05$. There was no change in myocardial oxygen consumption, myocardial R. Q., or in coronary vascular resistence. Left ventricular efficiency decreased, as indicated by a fall in efficiency index from 0.35 ± 0.02 to 0.29 ± 0.02, $p < 0.05$.

It is concluded that while aminophylline improves the ventilatory status of the patient with emphysema, it simultaneously causes potential deleterious effects on the cardiovascular system.

Effect of Histamine on Nocturnal Gastric Secretion During Sleeping and Waking States. JULES COHEN, JACK M. COLWILL, NANCY DAVIS, WILLIAM KESSLER, CHARLES R. SHEPARDSON, FRANZ REICHSMAN, and GEORGE L. ENGEL,* Rochester, N. Y.

We have reported decreased gastric secretion, including decreased responsivity to histamine, during states of

withdrawal and sleep in a depressed infant with a gastric fistula. It seemed of interest to study the secretory response to histamine during sleep in healthy subjects.

Nocturnal gastric secretory patterns were studied in 28 healthy young adults by continuous Wangensteen suction. Twelve subjects were studied once; and 16 from two to six times. Sleep or wakefulness was evaluated by frequent electroencephalograms and by continuous observation. Because of difficulty sleeping with an indwelling nasal tube, 100 to 200 mg. of pentobarbital (Nembutal®) was administered intramuscularly. Histamine diphosphate (0.1 mg. per 10 Kg. body weight) or betazole (Histalog®) (1.8 mg. per 10 Kg. body weight) was injected subcutaneously once during sleep and once when awake in each experiment.

The gastric specimens were titrated electrometrically with 0.1 N NaOH, the free and total acid end points being read at pH 3.5 and pH 8.2, respectively. We determined several physiological variables but report only on total HCl secretory rates which we consider the most reliable measurement of parietal cell activity.

For each experiment the range among subjects of the mean total acid secretory rate (excluding periods of histamine stimulation) was from 0.54 to 9.12 mEq. per hour. The greatest variation from night to night in the same subject was from 2.50 to 9.12 mEq. per hour.

The secretory response to histamine (or Histalog®) was marked in both sleeping and waking states. Mean total acid secretion in a 55 minute period of maximum stimulation was 11.70 ± 1.36 mEq. (sleeping) and 9.73 ± 0.76 mEq. (waking). The difference is not statistically significant.

Thus, parietal cell responsivity in healthy sleeping subjects remains high, suggesting that sleep in such subjects is a biological state differing from the sleep-withdrawal state in certain depressive conditions.

The Effect of Induced Anxiety and Hostility on Cardiovascular Functions. J. J. COMBS, JR., G. N. BRYANT, M. D. BOGDONOFF, and J. V. WARREN,* Durham, N. C.

Although it is recognized that affect states may influence cardiovascular function, there is little information regarding the pattern of response and its mechanism of production. It may well be that such factors play an important role in determining the manifestations or even presence of organic cardiovascular disease. To study this problem, we have observed the response of 23 normal male volunteers to induced variation in affect state produced by verbal and electrical stimuli. Heart rate, respiratory rate, arterial and central venous pressure were monitored on a photographic recorder. Cardiac output was determined by the dye technique. Following control determinations, change in affect state was attempted by suggesting an endangering personal situation or by a noxious stimulus (electric shock). Further circulatory observations were made at the point of apparent maximum emotional response. Conversational material was tape recorded, including a focused interview of the subject by an objective examiner immediately after the maximum response observations. These data were then reviewed for evaluation of affect state and only later correlated with the hemodynamic response.

In 12 subjects with evidence of induced anxiety, cardiac output increased 47 per cent. Mean arterial pressure rose 11 per cent; stroke volume, 22 per cent; heart rate, 23 per cent; and peripheral vascular resistance decreased 27 per cent. In six subjects whose poststimulatory state reflected hostility, cardiac output rose 15 per cent; arterial pressure, 9 per cent; and heart rate, 17 per cent. Insignificant changes in stroke volume and peripheral resistance occurred. Five subjects were evaluated as having no change in affect state. In these individuals, cardiac output and other hemodynamic measurements failed to change significantly.

The magnitude of cardiovascular response varied directly with the evaluated intensity of the affect state, but could not be correlated with personality patterns derived from personal history. In both affect states the pattern of response was similar, and in some respects resembled that associated with epinephrine administration.

Cation Exchanges in the Heart: Relation to the Cardiac Action Potential. HADLEY L. CONN, JR.* and JOHN C. WOOD, Philadelphia, Pa.

One hypothesis relating ion transfers to the cardiac action potential assumes that the spike phase of this potential is due to sodium influx into cells, the plateau phase to potassium outflux, and the terminal fast component a result of reversal of ion flow by a "sodium pump." We have attempted to secure information pertinent to these matters through measurement of sodium and potassium exchanges in the isolated dog heart, between cardiac cells and their environment. Tracer (K^{42} and Na^{24}) techniques were used for this purpose. With arterial blood perfusing the myocardium at constant rate, "build-up" or "washout" type tracer studies were carried on for 30 to 150 minutes. Appropriate arterial and venous blood and/or myocardial samples were analyzed for K, K^{42}, Na, Na^{24}, Cl, H_2O, pH, O_2, CO_2, and hematocrit. The potassium results were best fitted to a model representing the heart as a two compartment open, series system. The sodium results also best fit this framework with an additional parallel compartment apparently representing connective tissue. Both sodium and potassium exchange per heart beat were essentially constant over the ranges of rate studied (50 to 180 per minute), with the sodium exchange rate being twice that of potassium, 0.04 and 0.02 mEq. per Kg. per beat, respectively. When the sodium rate and normal spike potential values are considered in relation to the initial hypothesis, membrane capacitance can be calculated. The value is 10 μfd per cm.^{2}, similar to values obtained by direct measurement. Assuming unchanged capacitance through the spike and plateau phases, termination of the latter phase should occur at about -25 millivolts, another prediction close to that normally observed. The findings are further compatible with the concept of a pumping mechanism exchanging two sodiums for one potassium. Finally, the findings suggest equa-

tions quantitating the relationships between ion transfers, changes in potential, and membrane conductance (permeability).

Determination of Endogenous Water Production in Renal Failure Based on Measurement of Oxygen Consumption. E. J. COWSERT, A. A. YOUNES, and Y. MORITA, Detroit, Mich. (Introduced by G. B. Myers).

Water is produced internally by oxidation of the three major foodstuffs and release of "preformed" water by tissue protein breakdown. This endogenously formed water was measured over a 24 hour period in each of six renal failure patients. Administered food consisted of about 150 grams carbohydrate only, a similar amount being given on each of several preceding days. Carbohydrate utilized was assumed to equal carbohydrate retained. Protein catabolized was calculated from external nitrogen balance corrected for plasma NPN change. The open circuit method was used for determining total oxygen consumption, two to five minute samplings being taken every one to five hours. Oxygen consumed by carbohydrate and protein utilization, calculated from established constants, was subtracted from total oxygen utilization to obtain that used by fat. From this, the amount of fat catabolized was derived.

Carbohydrate burned ranged from 83 to 174 grams per 24 hours; protein, 24 to 208; fat, 30 to 108. Water of oxidation produced from each foodstuff and preformed water released were calculated, using accepted constants. These patients produced endogenously 236, 241, 295, 333, 358, and 930 grams of water per 24 hours. Generally, magnitude of endogenously formed water correlated positively with both body size and severity of illness.

In addition, total caloric output was simultaneously measured by an established method which depends upon a reproducible relationship between insensible perspiration and total body heat production. From this, endogenously produced water was calculated, respective values being 242, 238, 294, 374, 414, and 1,045 grams per 24 hours.

The oxygen consumption method seems suitable for metabolic study in renal failure. It is felt that it is easier to use and gives somewhat more valid results than the method based on insensible perspiration. Endogenously produced water is of sufficient magnitude to necessitate inclusion in calculating water requirements in oliguria.

Growth and Form of Leukemic White Cells in Cultures Having Varying Amounts of the Natural Radioisotope of Potassium. WAYNE A. CROCKETT and REX L. HUFF,* Seattle, Wash.

Potassium-40 occurs with a natural abundance of about 0.01 per cent and of the isotopes present in the body contributes most of the ionizing radiation. To observe its effect, leukemic white cell cultures maintained by the techniques of Osgood and Brooke have been grown in replicate preparations; the controls having potassium with a natural abundance of K^{40} while the experimental group contained only one-fifth as much K^{40} but the same amount of potassium.

The cells grown in the reduced radioisotopic concentration were more abundant and their nuclei larger. The colonies showed a lesser tendency for central pyknosis and degeneration. Cells of control preparations tended to be evenly distributed between the slide and the supernatant fluid, while the media of the experimental group contained only a small fraction of the total cells in the preparation. In the latter instance most of the cells were adherent to the slide. There was a tendency for less pleomorphism in the experimental group, suggested objectively by a lesser standard deviation of nuclear diameter.

All changes are present in the earliest harvest and examination (24 hours); however, they are significantly different at 72 hours and more pronounced thereafter. At 96 hours the diameter of nuclei of the isotopically reduced preparation is greater by 30 per cent ($p \lessapprox 0.01$) than the control group.

The effect of increased K^{40} abundance is being studied along with the use of other intracellular radioisotopes. Tissue cultures of cells derived from nonmalignant sources are being used to evaluate any unusual property of the leukemic cells.

Demonstration of the Specific Metabolic Defect in Primary Essential Hyperlipemia with Secondary Diabetes. O. B. CROFFORD, J. G. CONIGLIO, H. C. MENG, and E. V. NEWMAN,* Nashville, Tenn.

A metabolic study of 120 days duration was conducted on a patient with primary essential hyperlipemia with xanthomatosis and hyperglycemia. While ingesting a low calorie, low fat diet, the patient's fasting serum lipids gradually decreased to normal and the xanthomata were resorbed. Even then, however, the patient continued to exhibit an abnormally "lipemic" response to orally administered fat. Keeping the fat intake constant, but increasing the total caloric intake of the diet resulted in a return of the patient's hyperlipemia. The patient's elevated fasting blood sugar disappeared while ingesting the low calorie diet and did not return while ingesting the high calorie diet until after the hyperlipemia had become well established. Upon resuming the original low calorie, low fat diet, and in the absence of the resorbable xanthomata, the hyperlipemia decreased much more rapidly. I^{131}-labeled triolein was given orally to the hyperlipemic patient and to normal subjects. The level of the lipid-bound I^{131} reached in the hyperlipemic patient's serum was both higher and more prolonged than normal. By comparing results when the fasting serum lipids were normal and when they were exceedingly high, we could demonstrate that the problem was truly a decreased rate of removing the labeled fat from the serum rather than a pool size artifact. Furthermore, the hyperlipemic patient was shown to have a normal rate of removal from the serum of intravenously administered I^{131}-labeled albumin bound oleic acid. Supported by a failure to exhibit a normal plasma clearing reaction in response to intravenous heparin, these data demonstrated the metabolic defect to be an inability of the hyperlipemic patient to transform

efficiently the complex glycerides into a form which is readily accessible to the tissue cells.

Dynamics of Proliferating Cell Systems of Man Studied with Tritiated Thymidine. EUGENE P. CRONKITE,* THEODOR M. FLIEDNER, JOSEPH R. RUBINI, VICTOR P. BOND, and WALTER L. HUGHES, Upton, N. Y.

Tritium labeled thymidine (T Th) is incorporated into new DNA of proliferating cells prior to mitosis affording a nuclear label ideal for autoradiographic studies. DNA labeling was employed to study the dynamics of cell proliferation in man. A selected terminal patient received 9 mc. of T Th (0.86 mc. per mg.) intravenously. Serial autoradiographic studies of bone marrow and blood were then performed. Labeled neutrophiles appeared in the peripheral blood on the third day after injection, reached a maximum in six to eight days and were rare by the twelfth day. Labeled medium and large lymphocytes, and classical monocytes were present one day after injection, reached maximum between the third and fifth day and were rare after the sixth day. Despite the reputed high turnover rate of small lymphocytes, virtually none were found labeled. In the bone marrow intense labeling of erythroid, myeloid and primitive mesenchymal elements was noted 19 minutes after injection. There was an orderly progression of maturation with dilution of the label in the more adult forms. Erythroid labeling became minimal by the third day. Myeloid labeling persisted longer. *In vitro* studies have demonstrated the presence of labeled mononuclear cells in the peripheral blood of normal men. These apparently have the capacity to synthesize new DNA and are presumed destined to divide when seeded upon the appropriate soil. The enigma of the failure of the small lymphocyte to label *in vivo* and *in vitro* has not been explained. Further application of these techniques and the development of appropriate mathematical models should make it possible to describe fully cell turnover rate, life span and fate in man.

The Capacity for Bilirubin Production as Reflected by the Concentration of Plasma Bilirubin. WILLIAM H. CROSBY,* Washington, D. C.

When severely injured patients were given massive transfusions of whole blood, a large amount of the red cells were extravasated or phagocytosed and eventually destroyed. The hemoglobin of such cells is degraded and the plasma bilirubin is increased, reflecting the conversion of hemoglobin to bile pigment. In these patients, unless there was damage to the liver or paralytic ileus, the concentration of bilirubin did not exceed 3 to 4 mg. per 100 ml. The plasma bilirubin is normally turned over about 10 times daily. At this rate the level of 3 to 4 mg. represented a clearance of about 1.5 Gm. of bilirubin, the amount derived from the degradation of about 45 Gm. of hemoglobin. Although massive amounts of hemoglobin were available in the extravasated red cells, the rate of bilirubin formation did not appear to exceed 1.5 Gm. per day; however, the process continued for several weeks as reflected by the elevation of the plasma bilirubin. This suggested that there might be a limit to the capacity of the mechanism for converting hemoglobin to bilirubin.

The problem was studied further in healthy subjects by intravenous injections of autologous hemoglobin solutions or rapid infusions of distilled water to produce intravascular hemolysis. Plasma hemoglobin concentrations were achieved which varied initially from 50 to 500 mg. per 100 ml. The rate of clearance of hemoglobin was determined by periodic sampling and at the same time the plasma bilirubin was measured. Regardless of the dose of hemoglobin the peak concentration of bilirubin did not exceed 3.5 mg. The time of the peak was at 200 to 250 minutes after the injection.

Computations based on these results indicate that there is a limit to the capacity of the mechanism for bilirubin production, and that regardless of the quantity of hemoglobin presented the amount converted to bilirubin does not exceed 2 Gm. per hour or about 50 Gm. per day.

The Acute Effects of Carbonic Anhydrase Inhibitors on Systemic Hemodynamics. ARCHER P. CROSLEY, JR.,* CESAR CASTILLO, D. JOSEPH FREEMAN, DOUGLAS H. WHITE, JR., and GEORGE G. ROWE, Madison, Wis.

Recently, interest has been shown in the etiology of the significant reduction in glomerular filtration rate as well as the hypotension which may occur following the use of newer carbonic anhydrase inhibitors. Although chronic observations have demonstrated that such changes may occur as the result of a depletion of plasma volume, certain hemodynamic observations have occurred on such an acute basis as to preclude the latter mechanism as an etiological factor.

For these reasons combined renal and systemic hemodynamic measurements were made in eleven patients with cardiac and/or renal disease before and 60 minutes after the intravenous administration of sodium *p*-sulfamyl benzoate, dichlorphenamide or chlorothiazide. The results demonstrated that these agents are capable of producing a significant rise in urinary pH and a fall in glomerular filtration rate. Associated with the latter was a significant reduction in cardiac output which in turn appeared to be related to a decrease in venous return as evidenced by significant declines in right auricular, right ventricular end diastolic, and pulmonary artery pressures. In one patient cardiac output returned to control values following elevation of the legs.

These results are interpreted as indicating a possible causal relationship between the decline in cardiac output and glomerular filtration rate. Furthermore, the fall in the former function was related to a decrease in venous return and is therefore similar to that observed following the use of ganglionic blocking agents. Such observations may provide a clue to the acute and enhanced reductions of blood pressure which may occur following the combined use of such agents over and above that due to reduced plasma volume.

Chemical Constituents of Pine Pollen and Their Possible Relationship to Sarcoidosis. MARTIN M. CUMMINGS * and PAUL C. HUDGINS, Washington, D. C.

The geographic distribution of the birthplaces of 1,800 veterans with sarcoidosis suggested that the cause of this disease might be related to some aspect of the forest environment. In the course of laboratory studies it has been found that the pollen of pine trees in the "endemic area" has acid-fast characteristics similar to those of the tubercle bacillus. In addition it has been possible to isolate a wax containing mycolic acid and diaminopimelic acid from the pine pollen. These constituents, in combination, previously thought to be characteristic of tubercle bacilli have been demonstrated within sarcoid granuloma from clinical sources by British investigators. It is suggested (by us) that their presence within sarcoid lesions may be related to exposure to pine pollen rather than to the tubercle bacillus.

Using the methods of Anderson for fractionation of tubercle bacilli it has also been possible to demonstrate in pine pollen the presence of phospholipids, a firmly bound lipid, and "A," "B," "C," and "D" waxes similar to those found in tubercle bacilli.

Tuberculin hypersensitive guinea pigs injected intradermally with pine pollen developed epithelioidal tubercles after prolonged incubation periods in preliminary tests. Further biological studies with fractions of pine pollen are in progress.

The Measurement of Serum Sulfation Factor as an Index of Somatotropin Activity in Man. WILLIAM H. DAUGHADAY,* WILLIAM D. SALMON, JR., and FRANCE ALEXANDER, St, Louis, Mo.

It has been previously reported that sulfation factor (SF) is a somatotropin-dependent component of normal rat serum that promotes sulfate-S^{35} uptake by cartilage from hypophysectomized rats *in vitro*. SF disappears from the sera of hypophysectomized rats but is present in sera of hypophysectomized rats treated with somatotropin. *In vitro* addition of somatotropin to cartilage incubates is without comparable effect.

SF activity of normal human serum can be assayed *in vitro* by incubation of segments of costal cartilage from hypophysectomized rats in a medium containing 0.1 ml. of serum. The mean sulfate uptakes with dilutions of a normal human reference serum (obtained from a 23 year old student) are proportional to the logarithm of the amount of serum added. This relationship makes possible an estimation of SF activity of unknown sera simultaneously incubated, relative to the reference serum.

SF activity has been found at all ages. The mean and standard error for three different age groups were: eleven children (7 to 11 years), 0.84 ± 0.12; 14 adults (20 to 40 years), 0.78 ± 0.13; and 6 adults older than 50 years, 0.75 ± 0.10. The mean SF activity of sera from eight surgically hypophysectomized patients, kindly provided by Dr. O. H. Pearson, was only 0.11. Three untreated patients with active acromegaly had increased levels of SF (3.7, 4.4, and 8.2) but in five clinically inactive or extensively treated patients elevated values were observed in only two cases. SF activities below 0.35 were observed in three of six patients with nonfunctioning pituitary tumors, three patients with pituitary dwarfism and two patients with Sheehan's syndrome.

This initial experience indicates that measurement of the serum SF activity is a promising index of pituitary growth promoting activity in man.

Changes in the Impedance Locus Diagram of Swine Skin Produced by Thermal Injury. WARREN H. DENNIS and JAMES D. TELFER, Louisville, Ky. (Introduced by J. Murray Kinsman).

At the present time, there is no method for the accurate determination of the depth of thermal injury soon after injury. The electrical properties of normal and thermally injured swine skin have been studied in an attempt to develop a reasonably objective measure of the depth of burns. Measurements of potential differences and direct current resistance are subject to such wide variation that interpretation is difficult. However, changes of the impedance locus diagrams (*i.e.*, reactance versus resistance) for normal swine skin and for skin injured by applying a heated brass plate for varying periods of time were found to be consistent. The impedance of the skin as a function of frequency (5 to 20×10^3 cycles per second) was determined from lissajou patterns of the current flowing in response to a voltage of 0.5 volts p-p applied across the skin. The normal skin yielded an impedance locus diagram essentially the same as that found by others for human skin. One sq. cm. of skin can be represented by an equivalent circuit of a small resistance, 1,200 ohms, in series with a second resistance, 150,000 ohms, shunted by a polarization element. The polarization element has a phase angle of 73°. Following an incomplete thickness injury, the series resistance is unchanged while that of the parallel element is reduced to 22,000 ohms. An unexpected change occurred in the phase angle of the polarization element which became 90°. Following complete thickness injury, the capacitative reactance is completely abolished, whence the impedance locus diagram reduces to a single point on the resistance axis. By inspection of the impedance locus diagram, one can easily differentiate a complete thickness injury from an incomplete thickness injury.

Delay of Absorption of Radiolabeled Cyanocobalamin in the Intestinal Wall in the Presence of Intrinsic Factor. ALFRED DOSCHERHOLMEN and PAUL S. HAGEN, Minneapolis, Minn. (Introduced by Edmund B. Flink).

Plasma absorption curves following the oral administration of small test doses of radiolabeled vitamin B_{12} to normal subjects and patients with pernicious anemia with the addition of intrinsic factor concentrate have shown peak concentrations delayed until the eight to twelve hour interval while no or negligible activity was detected during the first three to four hours of the tests. Because the

blood samples were drawn from antecubital veins, there was a question whether the curves really reflected the rate of absorption from the intestine or whether some alteration had taken place during the passage of the blood through the liver, lungs or peripheral tissues. In order to circumvent the possible role of the liver, absorption curves were obtained in a similar manner from several patients with cirrhosis of the liver who had functional portacaval shunts. All of them exhibited absorption curves similar to those observed in the peripheral vein blood from control subjects. Because alteration of the plasma absorption curve by the passage of the blood through the lungs or peripheral tissues could not be excluded in these subjects, portal vein blood was obtained directly by catheter from another patient with cirrhosis of the liver but without portacaval shunt. A portogram immediately before and after the conclusion of the study ensured the proper location of the tube in the portal vein. The absorption pattern was similar to that described above. Therefore, the delay of plasma absorption of vitamin B_{12} in the presence of intrinsic factor apparently occurs in the intestinal wall.

Regulation of the Serum Amylase: The Effects of Alterations in Carbohydrate Metabolism in Normals, Diabetics and Some Patients with Liver Disease. DAVID A. DREILING, WILLIAM S. ROSENTHAL, and HENRY D. JANOWITZ,* New York, N. Y.

Serum amylase is generally held to be derived from the pancreas. There is reason to believe, however, that the liver may be a more important source since we have observed that glucagon, which is without effect on the exocrine secretion of the human pancreas, induces a rapid, significant, although transient depression of the levels of serum amylase. Glucagon (2 mg.) was administered intravenously to 30 normal subjects and 21 diabetic patients, and the concentrations of blood glucose and amylase determined by modifications of the Somogyi methods before and at 20 to 30 minute intervals afterwards for 2 to 4 hours. The normal subjects and the diabetic patients without evidence of exocrine pancreatic disease experienced on the average a 50 per cent fall in the level of serum amylase within 60 minutes, which paralleled the expected rise in blood glucose. Similar results were elicited by intravenous administration of 25.0 Gm. of glucose or fructose. Utilization of glucose rather than the absolute level of blood sugar appeared to be involved since depression of blood glucose produced by 15 mg. of insulin or 2.0 Gm. of tolbutamide given intravenously to six normals and five diabetic patients was accompanied by a similar significant depression of the serum amylase values, while subcutaneous administration of 2.0 mg. of epinephrine to two normal subjects induced a significant elevation of both blood glucose and amylase concentrations.

In five patients with varying degrees of hepatocellular necrosis associated with either hepatitis or portal cirrhosis, glucagon induced on the average no significant elevation of blood glucose nor any fall in serum amylase.

From these and other published studies it appears that changes in blood amylase levels may be correlated with states of altered carbohydrate utilization. The present studies suggest that the liver is the important source of the amylase normally appearing in the serum.

The Chemical Quantitation of the Daily Fecal Hemoglobin Excretion in Normals and Patients with Bleeding Alimentary Canal Lesions. FRANKLIN G. EBAUGH, JR. and WARREN BEEKEN, Hanover, N. H. (Introduced by Joseph F. Ross).

Qualitative tests for occult blood in the feces are often misleading and quantitation of fecal blood excretion by radioactive sodium chromate, though reliable, is impractical for large scale application. A simple photospectrometric test employing 1 ml. of 1 Gm. per cent of benzidine base solution, 1 ml. of 1 per cent H_2O_2, and 0.002 ml. of 1/50 aqueous homogenate of feces read at 500 mμ has been developed. Forty-six normal males excreted 1.6 $\pm$ 1.2, and 17 females 1.5 $\pm$ 1.1, ml. of whole blood per 24 hours. Ninety-nine $\pm$ 18 per cent of blood added to 73 fecal suspension *in vitro* was recovered. A recovery of 106 $\pm$ 13 per cent by the benzidine and 105 $\pm$ 10 per cent by radioactive $Na_2Cr^{51}O_4$ of 70 to 80 ml. ingested blood labeled with radioactive $Na_2Cr^{51}O_4$ was observed in 13 volunteers. Additions of $FeSO_4$ to the diet resulted in the detection of 2.3 $\pm$ 2.7 ml. more of blood in 18 24-hour fecal collections by the chemical technique than by radioactive Cr^{51} counting. $FeSO_4$ *in vitro* gave less than the equivalent of 0.1 ml. blood by the benzidine test. Heme alone was 77 per cent as reactive as when present in equivalent amounts in hemoglobin. Boiling fecal suspensions for 10 minutes decreased the amount of blood detected by 31 per cent. Of the 142 24-hour fecal collections which contained less than 3.8 ml. of blood (upper limits of normal), results of the *qualitative* benzidine dihydrochloride test were positive in 16 per cent, equivocal in 28 per cent, and negative in 56 per cent; for the 45 fecal samples which contained more than 3.8 ml. of blood, the results were positive in 42 per cent, equivocal in 36 per cent, and negative in 22 per cent. *Quantitation* of fecal hemoglobin is of potential value in the early detection of gastrointestinal bleeding lesions before X-ray changes occur. The *qualitative* benzidine dihydrochloride test is misleading 38 per cent of the time.

Steroid Metabolism in the "Salt-losing" Form of Congenital Adrenal Hyperplasia. WALTER R. EBERLEIN and ALFRED M. BONGIOVANNI,* Philadelphia, Pa.

Approximately one-third of infants with congenital adrenal hyperplasia develop hyponatremia, hyperkaliemia, and progressive dehydration. The electrolyte disturbance is often corrected only by massive doses of desoxycorticosterone and is aggravated by adrenocorticotrophic hormone (ACTH) administration. Normal or above-normal amounts of aldosterone have been found in the urine. It has been suggested that these infants secrete a salt-losing steroid or aldosterone antagonist. Failing to find such a steroid in the urine, we reinvestigated steroid excretion quantitatively.

A 24 hour collection of urine from 12 untreated patients with congenital adrenal hyperplasia and 8 control subjects was assayed for total 17-ketosteroids, for pregnanetriol by means of column chromatography, and for tetrahydrocortisone using paper chromatography. All 12 patients excreted large amounts of 17-ketosteroids and pregnanetriol, the 4 active salt-losers particularly so. Five of the 12 patients excreted "normal" quantities of tetrahydrocortisone in the urine; 3 excreted less than 25 per cent of the control; 4 excreted no detectable amount of the steroid. None of the first 5 showed any evidence of a salt-losing state; conversely, the 4 patients not excreting tetrahydrocortisone were active salt-losers. The 3 patients who excreted small amounts of this steroid had been clinically considered "potential salt-losers" because of slightly low serum sodium and moderately elevated serum potassium levels, which were corrected by cortisone treatment alone.

The present study demonstrates that in all patients with congenital adrenal hyperplasia there is inefficient synthesis of hydrocortisone. In those patients whose adrenals are able, by tremendous overactivity, to produce sufficient hydrocortisone, electrolyte disturbances do not occur. It is suggested that the salt-losing form of this disease results from an essentially complete failure of hydrocortisone synthesis due to lack of adrenal 21-hydroxylase. The present study also suggests that a minimal amount of hydrocortisone is required for aldosterone to exert its metabolic actions in man.

The Effect of In Vivo Dialysis upon Muscle Composition in Uremia. R. E. ECKEL, R. S. POST, and J. H. DAVIS, Cleveland, Ohio. (Introduced by H. S. Ginsberg).

The effect of six hours' *in vivo* dialysis upon the water, amino acid, Na, K, Cl, and acid-soluble P content of muscle from uremic oliguric patients has been studied by means of biopsy immediately before and immediately after the procedure. Five dialyses in four patients have been studied and compared with normal findings. Muscle water was normal initially and decreased in four cases (two to abnormal values) and was unchanged in one. In three cases, extracellular water (ECF) constituted 41 to 46 per cent of total muscle water initially and intracellular water (ICW) was abnormally law. In these cases ECF decreased to 33 per cent or less of total muscle water and ICW was returned to or toward normal. In the remaining cases, ECF was less than 33 per cent of total muscle water and ECF and ICW were unchanged by the procedure. The volume of distribution of Na was smaller than that of Cl in four cases before dialysis, and remained so after dialysis in two. The most consistent predialysis finding was a high intracellular K concentration, present even though only one serum K was above 6.0 mM per L. The intracellular K concentration fell in all cases with dialysis. In two cases this was due to loss of K in excess of cell water; in the remainder, to increase in cell water without change in muscle K. Total muscle P decreased in all cases.

The Effect of Hyperventilation on EFFECTIVE Right Atrial Pressure in Man. JOHN W. ECKSTEIN and WILLIAM K. HAMILTON, Iowa City, Iowa. (Introduced by James W. Culbertson).

We demonstrated previously that active venous constriction shifts blood from the extremities during hyperventilation. This blood, if redistributed centrally, could increase atrial pressure and the availability of blood to the heart. Such a mechanism might partly explain the enhanced cardiac output observed with overbreathing.

Right atrial and intrapleural (measured with an open-ended water-filled intraesophageal catheter) pressures and the resultant (effective) pressure were registered simultaneously. Ventilation and end-expiratory CO_2 concentration were monitored in each experiment. After control values were established subjects hyperventilated for one to three minutes with maximal inspirations and passive expirations. The procedure was repeated with 5 per cent CO_2 in the inspired gas after control values returned.

Mean atrial pressure decreased in each of 12 experiments during air hyperventilation; the decrease averaged 2.9 mm. Hg. Mean intrapleural pressure fell more than atrial pressure (11 of the 12 tests) by a highly significant ($p = < 0.01$) average value of 1.4 mm. Hg. This change represents an *increase* in *effective* or distending pressure (atrial minus intrapleural pressure) within the atrium.

Mean *effective* atrial pressure also increased in seven of eight experiments during overbreathing CO_2. In each of these eight experiments the absolute level of *effective* atrial pressure was higher ($p = < 0.01$) while overbreathing CO_2 than while overbreathing air. This difference was not a function of ventilatory volume.

The *effective* atrial pressure increase while overbreathing air probably is not caused by increased atrial tone. The only other explanation is an increased volume of blood distending the chamber. The greater increase in cardiac output while overbreathing air than while overbreathing CO_2 (Burnum, Hickam, McIntosh), despite a greater *effective* atrial pressure in the latter case, may mean that diastolic distensibility of the ventricle is increased during hypocapnia.

Avoidable Errors in Selection of Patients for Mitral Valvulotomy. E. E. EDDLEMAN, JR., Birmingham, Ala. (Introduced by Tinsley R. Harrison).

The preoperative determination of the relative importance of murmurs in patients with mitral stenosis and other coexistent valvular defects often presents a difficult clinical problem. Some patients reveal at operation a high degree of stenosis with a minimal mitral regurgitant jet while other patients with similar clinical findings display the opposite.

It has been found that a study of precordial movements (kinetocardiograms) may be helpful in determining the relative degrees of mitral stenosis and insufficiency.

This report deals with a study of four patients in whom mitral valvulotomy might have been avoided had the pre-

cordial movement been properly evaluated. Some of the features in the precordial traces (kinetocardiograms) which have been found to be particularly valuable are as follows: *1*) A marked forward precordial movement (heave) throughout systole speaks for a right ventricular hypertrophy and hence for a predominant mitral stenosis. *2*) Exaggeration of a predominant late systolic forward movement which is ascribed to atrial filling speaks for significant mitral insufficiency. *3*) Absence of a systolic heave offers strong evidence against a functionally important mitral stenosis. *4*) A precordial heave that diminishes in magnitude in the V_3 as compared to the V_2 position associated with an isolated large apical thrust speaks for both right and left ventricular hypertrophy and hence for a significant degree of insufficiency as well as stenosis.

Induced Tolerance of Dogs to Endotoxin: Correlation of Febrile Response and Adrenal Function. RICHARD H. EGDAHL and JAMES C. MELBY, Minneapolis, Minn. (Introduced by Wesley W. Spink).

When adrenal cortical activity in dogs was determined by measuring the Porter-Silber chromogens in venous blood collected from a cannula in the adrenal vein, it was observed that endotoxin caused a marked increase in adrenal activity. Administered endotoxin causes fever and an increase in adrenal activity. Therefore, experiments were designed to determine whether dogs could be made tolerant to endotoxin so that there was no febrile response or no increase in adrenal cortical function, as determined by the minute output of 17-hydroxycorticoids in adrenal venous blood. The results were related to the dose of *E. coli* endotoxin that was used. When large doses of endotoxin (1 to 5 mg.) were administered repeatedly, only rarely did an animal fail to exhibit both fever and an increase in adrenal activity. Furthermore, these large doses given daily did not depress adrenal or pituitary function. When smaller doses of endotoxin (0.01 mg.) were given daily, tolerance quickly developed as far as the febrile response was concerned, but adrenal activity was not depressed. Tolerance to the fever-promoting effect of endotoxin lasted for only a few days after the daily injections of endotoxin were discontinued. In not a single experiment was it possible to induce an increase of adrenal activity without fever following a single dose of endotoxin. From these experiments it can be concluded that: *1*) The response of the adrenal cortex to endotoxin is not due to fever. *2*) Tolerance to the fever-promoting property of endotoxin is readily accomplished. *3*) Tolerance to the stimulating effect of endotoxin on adrenal cortical function is produced with difficulty. *4*) Repeated sublethal doses of endotoxin do not cause a measurable depression of pituitary-adrenal function.

Rates of Osteogenesis Measured by Nonradioactive Strontium in Subjects with Normal and Decreased Skeletal Mass. E. EISENBERG, T. RUSSELL FRASER, and G. S. GORDON,* with the technical assistance of JEAN MARIE SIMIEN, San Francisco, Calif., and London, England.

Others have measured rates of skeletal formation in man with bone-seeking radioisotopes and have shown that strontium moves from blood to bone at the same rate as calcium. An accurate spectrophotometric method permits use of *nonradioactive* strontium for this purpose. Absence of radiation hazards allows repeated measurements in the same person. Ten mEq. of strontium gluconate is infused, and serum and urine content are determined for four days. The size of the exchangeable calcium pool (Ca_e) and its rate of turnover are calculated from the usual dilution formulae. Fecal excretion is negligible, and tracer does not reenter the pool in this time. Subtraction of the rate of urinary loss from total daily turnover yields the rate at which calcium is incorporated into bone (Ca_b). The following values were obtained: In 12 normal subjects, Ca_e was 211 ± 11 mEq. and Ca_b 47.5 ± 2.2 mEq. per day. In 23 senile osteoporotics, Ca_e was 164 ± 5.7 mEq. and Ca_b 34.9 ± 1.7 mEq. per day. In 2 thyrotoxic osteoporotics, Ca_e was 339 mEq. and Ca_b 121 mEq. per day. In 1 osteoporotic acromegalic, Ca_e was 311 mEq. and Ca_b 126 mEq. per day. In 7 hyperparathyroid patients, Ca_e was 362 ± 28 mEq. and Ca_b 101 ± 14 mEq. per day. In repeated measurements in 7 persons, the second value for Ca_e was 99.1 ± 6.6 per cent of the initial value, and for Ca_b 93.7 ± 3.3 per cent.

Values obtained by this technique agree well with those obtained by isotopic methods, show little scatter among normal subjects, and demonstrate a reduced rate of osteogenesis in senile osteoporosis. Since osteogenesis is accelerated in thyrotoxicosis and acromegaly, the osteoporosis in these disorders probably results from increased osteolysis. Ca_b is retarded in spontaneous and induced hypercorticism. The action of estrogens and androgens in osteoporosis is under investigation.

The Influence of Blood Glucose on Renal Clearance of Phosphorus in Diabetic Patients. H. ELRICK, C. J. HLAD, JR., N. E. WHIPPLE, and Y. ARAI, Denver, Colo. (Introduced by G. Middlebrook).

Previous studies from this laboratory have shown that in normal subjects the level of blood glucose (below the renal threshold for glucose) exerts a powerful and sensitive influence on the renal clearance of inorganic phosphorus. This was demonstrated by the finding of a consistent positive correlation between blood glucose and the renal clearance of phosphorus during glucose infusion.

In the present experiments the effect of intravenous glucose on phosphorus clearance was studied in five patients with severe diabetes. Studies were carried out in the basal state 25 hours after the last dose of insulin. Renal clearance methods were used and 7 to 10 10-minute urine collection periods were obtained before and throughout the glucose infusion.

Endogenous creatinine clearance, used as a measure of

glomerular filtration rate, remained unchanged. In two patients it was normal (109 and 129 ml. per minute) and in three it was diminished (47 to 79 ml. per minute). The lowest phosphorus clearance in the control period was 31 per cent (expressed as per cent of filtered load excreted); the maximum was 50 per cent. These values exceeded the maximum (21 per cent) observed in normal subjects.

Following glucose, serum phosphorus showed a rise (mean, 16 per cent) rather than the fall commonly observed in normal subjects. Phosphorus clearance did not parallel blood glucose; indeed, in four of the five patients it decreased with increasing blood glucose levels. The remaining patient had the lowest fasting blood glucose (147 mg. per cent) and an increase in phosphorus clearance was observed.

The findings indicate that the regulatory influence of blood glucose on phosphorus clearance observed in normal subjects does not occur in the diabetic patient with markedly elevated blood sugar. This is consistent with previous observations in normal subjects with induced glycosuria. These data further support the concept that phosphorus and glucose compete in the process of renal reabsorption over a wide range of plasma levels.

Further Studies on the Effects of Estrogen and Testosterone on the Circulating Thyroid Hormone. NORMAN H. ENGBRING and WILLIAM W. ENGSTROM,* Milwaukee, Wisc.

The fact that the serum protein bound iodine (PBI) rises in normal pregnancy led to our observation that estrogen administered to persons with normal thyroid function causes an increase in PBI. Conversely, administered testosterone has been shown to decrease the PBI. The estrogen-effect is associated with an increased capacity of serum interalpha globulin to bind thyroxine. These observed phenomena prompted further investigation, since changes in PBI might result from altered thyroidal output, or altered rate of peripheral disposal of hormone.

The possible independence of estrogen-induced increase in PBI on production of hormone was assessed by administration of estrogen to athyreotic individuals on replacement therapy. In those maintained entirely eumetabolic on desiccated thyroid, a modest rise in PBI resulted; in those on pure L-thyroxine, the rise was more marked. Patients on inadequate replacement therapy exhibited no rise in PBI. This might be anticipated because the serum of hypothyroid individuals already showed an abnormally increased thyroxine-binding capacity. These observations indicate that a functioning thyroid gland is *not* essential for the estrogen-effect on PBI.

The rate of disposal of thyroxine was assessed by radioactive thyroxine survival studies in six normal individuals before and during estrogen administration. In each case, there was a prompt prolongation of survival time; the average control half-life of 6.7 days increased to 11.2 days with estrogen administration. Thus, the estrogen-effect on PBI is accompanied by initial decreased rate of utilization or turnover of thyroid hormone.

The effect of administered testosterone was studied in nine euthyroid males; in each the PBI fell. The thyroxine-binding capacity decreased in three, but remained unchanged in six. No alteration in thyroxine survival time was demonstrated in five studies. Thus, the testosterone-induced fall in PBI is not as easily interpreted as the estrogen-induced rise.

Studies on the Phylogenesis of Gamma Globulins and Plasma Cells. RALPH L. ENGLE, JR., KENNETH R. WOODS, and JAMES H. PERT, New York, N. Y. (Introduced by Paul Reznikoff).

There is evidence that the production of gamma globulins and antibodies is related to plasma cells. We have initiated studies designed to test whether or not the phylogenesis of gamma globulins parallels that of the plasma cell.

Serum proteins from several marine invertebrates, elasmobranchs, and teleosts were analyzed by zone electrophoresis in starch gel (Smithies). Proteins migrating toward the cathode at pH 9.0 were considered to be gamma globulins. Microscopic examinations were conducted to determine whether or not plasma cells could be found in the blood or organs of the marine forms. Blood smears of invertebrates were fixed in osmic acid or formalin vapor. Blood of vertebrates was smeared on slides and dried. Spleens and other organs were sectioned, and the exposed surfaces lightly touched to glass slides. These touch preparations were dried and then treated with Wright-Giemsa stain. Some fresh preparations were examined with the phase contrast microscope.

The invertebrates studied included 14 species of crustacea, the horseshoe crab, scorpion, squid, and oyster. Within this group, no gamma globulin-like protein was identified and no cells suggestive of plasma cells were found in the blood and tissues. Among the eight species of elasmobranchs studied, all had gamma globulin-like bands in the electrophoretic pattern. Cells having morphologic characteristics of plasma cells were readily found in touch preparations of the spleens. Twelve species of bony fish were examined. In this group, no gamma globulin-like protein was found. After prolonged searching, it was possible to identify a small number of cells having some of the characteristics of mammalian plasma cells in the touch preparations from the spleens. In all species studied, there was remarkable specificity in the serum electrophoretic patterns and the pattern within a given species was consistent.

In the forms thus far examined, there appears to be a phylogenetic association between gamma globulin and plasma cells.

Selective Inhibition of Renal Concentrating Ability by Experimental Hypercalcemia. FRANKLIN H. EPSTEIN,* DAVID BECK, MANUEL J. RIVERA, HOWARD LEVITIN, and FRANK A. CARONE, New Haven, Conn.

Polyuria and polydipsia are well-known clinical accompaniments of hypercalcemic states. In order to investigate their genesis, 15 white male Sprague-Dawley rats weighing 300 to 400 grams were given 200,000 to 400,000 units of vitamin D_3 in oil subcutaneously daily for four days, resulting in a rise in serum calcium of 3 to 4 mg. per cent and spotty nephrocalcinosis, distributed chiefly in the medulla, but affecting the cortex as well. Maximum urinary solute concentration tested after dehydration and vasopressin fell from an average of 2,596 mOsm. per Kg. to 1,655 mOsm. per Kg. Blood urea nitrogen, urea clearance and phenolsulfonephthalein excretion were unchanged in most instances, although when larger amounts of vitamin D_3 were given, all renal functions were depressed.

The effect of hypercalcemia of relatively short duration on the ability of the kidneys to reabsorb water free of solute was investigated in dogs given 60 to 90 units per Kg. of parathyroid hormone subcutaneously in divided doses over a 24 hour period. Serum calcium rose 4 to 5 mg. per cent. $T_m{}^cH_2O$, measured during mannitol diuresis, dropped precipitously. Two dogs excreted urine hypotonic to plasma despite a constant infusion of large amounts of exogenous vasopressin. Glomerular filtration rate fell in some dogs but marked depression of $T_m{}^cH_2O$ was seen in those animals as well in which inulin clearance remained the same or rose following parathormone. Acute infusions of calcium salts, while producing comparable elevations of serum calcium, did not depress $T_m{}^cH_2O$ to the same degree as did parathormone.

These data suggest that hypercalcemia of sufficient duration specifically inhibits the ability of the renal tubules to concentrate urine and conserve water.

Chromatographic Characterization of the Serum Protein Changes in Pathologic Sera. JOHN L. FAHEY, PATRICIA F. McCOY, and ANN P. HORBETT, Bethesda, Md. (Introduced by Charles G. Zubrod).

Chromatographic fractionation of serum proteins into 16 or more components has been reported recently. Such fractionation, carried out on diethylaminoethyl-cellulose columns in the cold and utilizing a gradient elution system employing phosphate buffers, permits characterization of the serum proteins to an extent not previously feasible by electrophoresis or ultracentrifugation. The usual electrophoretic fractions are characteristically further subdivided into a number of separate components by this chromatographic procedure.

Modifications in the chromatographic procedure have made possible detailed examination of 1 and 3 ml. serum samples. Sera obtained from 36 patients with a variety of diseases have been examined chromatographically, electrophoretically and, in some instances, in the ultracentrifuge. In most of the disease states studied multiple serum chromatographic changes were seen and, in several, identifying serum chromatograms were obtained. Macroglobulinemic sera, although indistinguishable from multiple myeloma sera by electrophoresis, were usually easily distinguishable chromatographically. Macroglobulins are eluted later in the chromatogram than are myeloma proteins. The major gamma globulin peak was characteristically missing from the agammaglobulinemic sera. The ceruloplasmin-containing component was markedly diminished in Wilson's disease and significantly increased in several patients with Hodgkin's disease. Transferrin and albumin levels were decreased and alpha-1 and alpha-2 glycoproteins elevated in many of the sera from patients with infectious, neoplastic and collagen diseases. In selected abnormal sera the chromatographic distributions of protein-bound vitamin B_{12}, alkaline and acid phosphatases and other proteins of physiologic interest have been determined and compared with normal serum. Serum protein chromatography has been found to provide considerable information in addition to that obtainable by electrophoresis or ultracentrifugation.

Occurrence of a Sprue-Like Syndrome During Neomycin Therapy. WILLIAM W. FALOON, CURTIS J. FISHER, and KATHLEEN C. DUGGAN, Syracuse, N. Y. (Introduced by Eugene L. Lozner).

The occurrence of diarrhea during neomycin therapy in patients with cirrhosis has stimulated investigation of fecal constituents. In three patients receiving constant diets containing 76, 82 and 92 Gm. of fat daily, respectively, during successive six day periods of 1) control, 2) neomycin (12 Gm. per day orally), 3) neomycin plus ammonium chloride and citrate, 4) neomycin, 5) control, fecal excretion of the following was measured: total fat, free fatty acids, neutral fats, soaps, calcium, sodium, potassium and nitrogen.

Total fecal fat rose during neomycin administration in all three patients, the average percentage of daily intake in each period being: 1) 4 per cent, 2) 18 per cent, 3) 27 per cent, 4) 24 per cent, 5) 10 per cent. Soap excretion rose most markedly averaging eight times control. Free fatty acid excretion averaged six times control. Neutral fat excretion increased in only one period in one patient. Fecal calcium exceeded control excretion in all three of the neomycin periods in two patients but in only one period in the third. The average increase in fecal calcium was 200 mg. per day but the magnitude of increase did not correlate with the increase in fecal fat. Fecal potassium excretion rose by from 10 to 32 mEq. per day but fecal sodium was unchanged in two patients and increased by 5 to 15 mEq. per day in one patient. Fecal nitrogen increased consistently by from 1 to 1.9 Gm. per day. No deleterious clinical effects were noted and the changes reverted toward normal in all moieties with neomycin withdrawal.

The data indicate that oral neomycin produces a fecal excretory pattern similar to that commonly seen in sprue and which is accentuated by ammonium salts. This excretion results in significant fecal loss of calories and electrolytes in patients receiving neomycin. These observations may also suggest a technique for producing steatorrhea in the human.

The Febrile Response upon Injection of Bovine Albumin into Previously Sensitized Rabbits. RICHARD STUDLEY FARR, Pittsburgh, Pa. (Introduced by Wallace N. Jensen).

Fever is a manifestation of human allergy which can also be induced and measured in animals. An example is the fever thought by Stetson to be associated with the delayed type of hypersensitivity in tuberculous rabbits after an injection of nonpyrogenic products from tubercle bacilli. The present study was undertaken to determine whether rabbits could be sensitized to produce fever upon exposure to nonbacterial antigens.

Thirty-six rabbits were immunized with multiple intravenous injections of 10 to 25 mg. bovine serum albumin (BSA). Two to four weeks after the last injection, more than one-half of these rabbits responded with fever when challenged with 1 to 10 mg. BSA intravenously. The febrile response resembled that from bacterial endotoxin in that both exhibited: 1) a latent period between the injection and onset of fever; 2) a biphasic temperature curve lasting six hours; 3) development of tolerance after repeated daily injections; 4) restoration of a febrile response in tolerant rabbits by a tenfold increase in the dose injected; and 5) loss of tolerance following intravenous thorium dioxide.

The quantitative precipitin test and the ammonium sulfate method of measuring antigen-binding capacity revealed circulating antibody in all rabbits which responded with fever, while fever was never observed when antibody could not be detected. Conversely, however, fever did not always result when circulating antibody was present, and the correlation between fever response and total measurable antibody content was poor.

These experiments indicate, therefore, that hypersensitivity associated with fever is not restricted to the bacterial hypersensitivity of injection and provide further evidence that parenteral injections of nonbacterial antigens can induce fever similar to that resulting from infection.

The Pulmonary Collateral Blood Flow in Man. ALFRED P. FISHMAN,* GERARD M. TURINO, MARTIN BRANDFONBRENER, and AARON HIMMELSTEIN, New York, N.Y.

Anatomic observations have established that an extensive overgrowth of systemic arteries into the lung may accompany various types of heart and lung disease. The present study was designed to measure the rate of blood flow through such vessels, in particular the rate of collateral blood flow to the gas-exchanging surface of the lung ("effective"). For this purpose, the Fick principle was applied to three groups of subjects; in each group, the application was modified to conform with the anatomic abnormality. Thus, in the group of four subjects with congenital atresia of the pulmonary artery, the measurement of oxygen uptake and the oxygen content of arterial blood sufficed; in the group of two subjects with permanent occlusion of a major pulmonary artery, the combination of bronchospirometry, cardiac catheterization and arterial cannulation was employed; finally, in the group of six subjects with either acquired pulmonary disease or idiopathic clubbing of the digits, this combination was modified by the use of a balloon-tipped catheter to occlude transiently a pulmonary artery. Different rates of "effective" collateral blood flow were measured, ranging from zero in subjects with either carcinoma of the lung or recent occlusion of the left pulmonary artery, to approximately normal values for pulmonary blood flow in some subjects with congenital atresia of a pulmonary artery. Intermediate values, up to 1 liter per minute, were obtained in subjects with bronchiectasis, long-standing ligation of a pulmonary artery, and idiopathic clubbing of the digits.

Some Responses of the Pulmonary and Systemic Circulations of Man to Acute Alterations of the Alveolar Carbon Dioxide Tension. C. W. FRANK, A. JORDAN, F. KIEFHABER, and M. ZINN, New York, N. Y. (Introduced by Saul R. Korey).

Interest in a possible influence of the alveolar gas or arterial blood tension of carbon dioxide upon the pulmonary circulation stems from several theoretical and clinical considerations. The data to be presented concern the responses of 31 patients with rheumatic heart disease studied by means of right heart catheterization.

After a suitable control period during which the vascular pressures were recorded frequently and the cardiac output twice (Fick principle), an inspired gas containing 3 or 5 per cent carbon dioxide in air was administered and continued long enough to permit stabilization of the measurable parameters of ventilation and circulation (20 to 40 minutes), at which time the cardiac output measurement was repeated. The subsequent responses to another concentration of inspired carbon dioxide, a bout of voluntary hyperventilation breathing room air, and leg exercise (breathing air and then CO_2), were studied in various combinations.

In all 31 patients the inhalation of carbon dioxide resulted in an increase of the pulmonary artery mean pressure. The magnitude of this increase varied from 1 to 27 mm. Hg, with an average increase of 12 mm. Hg. Hyperventilation *per se* was shown not to be the cause of the rise. Changes in the cardiac output were inconstant, and did not correlate with the pressure rise. The "total resistance" to pulmonary outflow was increased above control in almost all subjects during the periods of respiratory acidosis.

Although the data so far available do not permit the clear identification of the mechanisms responsible for the increased hindrance to pulmonary arterial outflow, the response of several individual patients to suitably matched periods of exercise suggests that the "pulmonary vascular resistance" may have been increased by the respiratory acidosis.

The interplay of a variety of factors in the development of this pressor effect will be discussed.

The Effect of Smoking on Pulmonary Function in a Working Adult Population. WILLIAM FRANKLIN, Boston, Mass. (Introduced by Francis C. Lowell).

During a complete routine annual medical examination, 1,000 employees of a plant free of toxic or irritating dusts and fumes performed kymographic tracings of maximal forced expiration (E). They also answered a questionnaire dealing in detail with smoking habits and with respiratory symptoms and disease. In establishing standards for E it was found that measurement of the rate of flow between the point where 50 and 75 per cent of the vital capacity had been expired (E_{50-75}) was a reliable means of detecting early bronchial obstruction, and that it was less influenced by errors in performance than (E_{25-75}) or the volume expired in the first second.

In those between the ages of 40 and 65 years, unselected with respect to evidence of respiratory disease, the E_{50-75} was lower among those who smoked heavily than among those who smoked little or not at all. Among 105 male heavy smokers the mean E_{50-75} was $2,055 \pm 94$ ml. per second as compared with a mean of $2,520 \pm 102$ ml. per second for 103 males who smoked little or not at all. Among 36 female heavy smokers the mean was $1,575 \pm 130$ ml. per second as compared with a mean of $1,850 \pm 97$ ml. per second among 50 females who did not smoke. The p values for these differences were less than 0.01 for males and 0.05 for females.

These results indicate that heavy smoking can impair pulmonary function, that such effects are common among the population at large, that they frequently occur in the absence of any pulmonary abnormality of which the subject is aware, and that the abnormality does not represent a reaction peculiar to a few especially susceptible individuals. It is suggested that this abnormality is the forerunner of chronic obstructive pulmonary emphysema.

The Metabolism of Insulin by Human Placental Tissue. NORBERT FREINKEL * and CHARLES J. GOODNER, Boston, Mass.

In diabetics, requirements for insulin are markedly increased during pregnancy and acutely diminished in the immediate postpartum period. The possibility that placental degradation of insulin might contribute to this changing pattern prompted an inquiry into the metabolism of insulin by human placental tissue. Slices and homogenates of placenta were incubated with unlabeled and I^{131}-labeled crystalline beef insulin. In both cellular and cell-free preparations, proteolysis of the insulin could be demonstrated by the liberation of *a*) nonprotein nitrogen; *b*) free amino acids; and *c*) trichloracetic acid-soluble radioactive and ultraviolet-absorbing moieties. Loss of hypoglycemic potency was documented by bioassay. The placental system for the proteolytic degradation of insulin is thermolabile and may be inhibited by Zn^{++}, Cu^{++}, iodoacetate and *p*-chloromercuribenzoate. Activity of homogenates is enhanced in the presence of citrate buffers and reduced by dialysis. More than 80 per cent of the activity is localized within the soluble supernatant obtained by centrifugation at $100,000 \times G$. Analysis of the kinetics of insulin degradation revealed that the human placenta at term is at least as active as rat liver. No data are available for other human tissues.

The effects of extracellular factors upon this system were assessed. Insulin degradation by homogenates of normal placenta was studied during incubation with paired sets of maternal and cord sera obtained at delivery from normal and diabetic subjects. In seven normal pairs, insulin degradation was less in the presence of maternal than of cord serum. In four pairs from insulin-treated diabetics, both maternal and cord sera inhibited placental degradation of insulin. In the latter bloods, insulin-binding antibody was demonstrated by starch-block electrophoresis. Quantitative analyses of antibody titers were similar in each pair of sera, thus indicating free transplacental exchange of insulin-binding antibody.

The data support the suggestion that the human placenta may act as a major site of insulin degradation. Further studies are in progress to elucidate the possible role of this phenomenon in producing or modifying the abnormalities of the pregnant diabetic.

Effect of Acetazolamide (Diamox®) on Thyroid Function. J. L. GABRILOVE, New York, N. Y. (Introduced by L. J. Soffer).

The intravenous infusion of acetazolamide (Diamox®) into four euthyroid and two hyperthyroid subjects in a dosage of 125 mg. per kilo of body weight resulted in a decrease in the thyroidal uptake of radioactive iodine (I^{131}). Uptake was measured at 1, 2, 4, 6, and 24 hours following the oral administration of the tracer. The maximal depression obtained in the euthyroid group was 16, 27, 30, and 40 per cent of the control uptake and was generally noted in the 24 hour measurement. In the hyperthyroid group, the maximal decrease in uptake was observed in the six hour measurement and was 16 and 25 per cent of the control values. The effect on thyroid function is probably not secondary to the action of acetazolamide on the renal clearance of iodide since the administration of this agent to two patients with myxedema did not alter the urinary excretion of I^{131}. In these latter patients urine was collected at frequent intervals for a period of 50 hours.

Acetazolamide is a sulfonamide carbonic anhydrase inhibitor. In *in vitro* studies employing the colorimetric method of Wilbur and Anderson (J. biol. Chem. 1948 **176**, 147) carbonic anhydrase was demonstrated to be present in rat thyroid and the activity of the enzyme could be inhibited by adding acetazolamide to the test system. In other *in vitro* studies iodide was found to interfere with the action of purified carbonic anhydrase.

From these studies definitive proof cannot be adduced as to the importance of carbonic anhydrase in thyroid function. However, further investigations are in progress to test such a hypothesis.

Some Pathophysiological Aspects of Paroxysmal Nocturnal Hemoglobinuria. FRANK H. GARDNER,* EU-

GENE D. ROBIN, DAVID M. TRAVIS, DESMOND G. JULIAN, and CHARLES H. CRUMP, Boston, Mass.

The occurrence of enhanced hemoglobinuria during sleep has been described previously in paroxysmal nocturnal hemoglobinuria (PNH). It has been demonstrated *in vitro* that hemolysis may be increased by exposure of the abnormal erythrocyte to increased serum hydrogen ion concentration. It has been proposed that the respiratory acidosis of sleep is the major factor which accentuates hemolysis. The validity of this hypothesis has been studied in four patients with PNH.

Hourly measurements of plasma hemoglobin concentrations, arterial and venous blood pH, and breath to breath alveolar CO_2 tensions were determined during sleep and control intervals prior to and following the sleeping period. Two of the four patients showed a progressive rise in plasma hemoglobin during sleep. The time sequence of the increased hemoglobinuria did not correlate with respiratory acidosis. Two patients showed unvarying plasma hemoglobin levels during sleep, despite the development of respiratory acidosis. In one subject arterial and venous plasma hemoglobin levels were identical although there was a mean pH difference of 0.04 units. Two subjects inhaled 6 per cent CO_2 in air for 30 minutes. With this procedure a more profound respiratory acidosis developed than was observed during physiologic sleep. However, no significant further rise in plasma hemoglobin was obtained in the subsequent two hour period.

Nocturnal hemolysis in this disease is not related to the physiologic respiratory acidosis of sleep.

The Contrasting Ratios and Significance of the Tetrapyrryl (Bilirubinoid) and Dipyrryl ("Fuscin") Compounds of the Feces, in Certain Anemias. A. SIGRID GILBERTSEN, Minneapolis, Minn. (Introduced by C. J. Watson).

Earlier studies with P. T. Lowry, using N^{15} glycine, indicated that the fecal dipyrrylmethene or "fuscin" compounds are derived mainly from anabolic sequences on the pathway to heme rather than schism of tetrapyrrylbilirubinoids from destroyed hemoglobin.

The fecal "mesobilifuscin," partially purified by a modification of Siedel's method, has been compared in amount with urobilinogen in a group of normal subjects and in various anemias. The range of excretion of mesobilifuscin in normal human feces is 7 to 18 mg. per day and the urobilinogen/mesobilifuscin ratio (U/Mbf) is 8 to 14. In the presence of active hemolysis a disproportionate increase in fecal mesobilifuscin as compared with urobilinogen is often seen; hence the U/Mbf ratio is normal or low. This is also observed in cases of megaloblastic anemia. Remarkably low mesobilifuscin values are observed in cases of hyporegenerative or "refractory" anemia, even when the urobilinogen is normal or increased in amount, and in these the U/Mbf ratio is usually increased, often in marked degree. These results indicate that the magnitude of effective or attempted heme synthesis is more significant than hemoglobin destruction in determining the amount of mesobilifuscin in the excreta. Observations in certain other cases of anemia are also in accord with this concept.

Yet in some cases of anemia, especially those with rheumatoid disease, the amount of fecal mesobilifuscin has been small and the U/Mbf ratio high, despite an apparently normal or increased erythropoietic activity. This is as yet unexplained, but the possibility is considered that in these cases the immediate precursors of the "fuscin" compounds are more efficiently utilized.

The most consistent reduction of fecal mesobilifuscin and elevation of the U/Mbf ratio has been noted in cases of liver and biliary tract disease, probably due to diversion of mesobilifuscin to the urine, where marked increases are noted.

The Relationship of Serum Hexosamine Elevations to Immunological Reactions. ROBERT B. GILES, JR., MYRA MICHAEL, and JOE ED SMITH, Dallas, Texas. (Introduced by Elias Strauss).

Elevated serum hexosamine concentrations have been demonstrated in a group of diseases (collagen diseases, multiple myeloma, chronic infectious diseases) which are noteworthy for chronicity and tissue destruction, and, furthermore, which may be associated with immunologic disturbances. The elevated serum hexosamine concentrations have been attributed to: *a*) immunologic derangements; *b*) connective tissue destruction and/or proliferation of cells, particularly fibroblasts; *c*) destruction of leukocytes; and *d*) pyrogenic reactions. These experiments were undertaken to test these hypotheses and particularly to investigate further the role immunologic stimuli play in serum hexosamine alterations in the absence of tissue destruction.

Rabbits were sensitized to crystalline bovine plasma albumin (BPA), administered intravenously in 100 mg. doses at monthly intervals. Animals injected with saline served as controls. In order to dissociate serum hexosamine elevation from circulating antibody formation, on the one hand, and from leukopenia and hyperpyrexia, on the other, several animals were given 500 roentgens whole body radiation prior to antigenic stimulation, or were given *E. coli* endotoxin intravenously sufficient to cause hyperpyrexia and severe leukopenia. Serum hexosamine and precipitable antibodies to BPA were measured prior to and at frequent intervals after each antigenic stimulus.

These studies revealed: *a*) The initial injection of BPA did not elevate serum hexosamine concentration and elicited only minimal antibody response; *b*) Subsequent BPA injections caused serum hexosamine elevations four to six days following each antigenic stimulus, preceding or coinciding with a significant antibody rise; *c*) X-radiation prior to the challenging BPA injection suppressed both the antibody and hexosamine elevation; *d*) Endotoxin alone, sufficient to cause profound leukopenia and hyperpyrexia, followed by leukocytosis, was not associated with elevation of serum hexosamine.

These studies suggest that in the rabbit serum hexosamine elevations are related to immunologic responses occurring without apparent tissue destruction and do not appear to be related to severe leukopenia, leukocytosis or pyrogenic reactions.

The Site of Catabolism of Plasma Albumin. DAVID GITLIN,* JAMES R. KLINENBERG, and WALTER L. HUGHES, Upton, N. Y., and Boston, Mass.

While the organs or cells responsible for the synthesis of many plasma proteins have been established, the sites involved in the normal degradation of these proteins remains in question. Consequently, this study was undertaken to evaluate the relative importance of various organs in the catabolism of a major protein of plasma: albumin. Mouse and human serum albumins were labeled with I^{131} and given intravenously to mice. Behavior of both albumins was identical. The biological half-lives of albumin was determined by assay of radioactivity in the living whole mouse and by estimation of protein-bound radioactivity in pooled sera and in individual whole mice. The half-life of serum albumin was 0.7 days in normal mice; withdrawal of food increased the albumin half-life to 1.1 days. Sham operations did not significantly alter these values. Resection of 40 per cent of the liver markedly increased the albumin half-life to 1.7 days representing a 50 per cent decrease in the fractional rate of catabolism. Subsequently, albumin catabolism returned to normal as the liver regenerated. Removal of 15 per cent of the liver decreased albumin catabolism by 20 per cent. Bilateral nephrectomy or bilateral ureteral obstruction decreased albumin catabolism slightly, but resection of large and small intestine did not. Blockade of the reticuloendothelial system with thorotrast or India ink profoundly decreased albumin catabolism. The data indicate that the reticuloendothelial system is the major site of albumin catabolism and that the reticuloendothelial system of the liver plays the most prominent role.

Urinary Bacteriological Studies from Patients with Inlying Catheters. HARRY GLENCHUR, ROGER V. HAGLUND, WENDELL H. HALL,* and ROBERT H. WILCOX, Minneapolis, Minn.

Forty-three patients requiring an inlying catheter have been studied to determine the incidence of bacteriuria, the time required to acquire new species and their comparative resistance to antibiotics. Thirty-two patients acquired 55 strains of bacteria (over 5,000 per ml.); 17 of these patients had coincidental chemotherapy, as did 6 out of 11 acquiring no new strains. More resistant paracolons and fewer susceptible staphylococci were acquired with chemotherapy. New bacteria were found in one to three days. The acquired strains were resistant to the antibiotics used for prophylaxis.

Nine patients with protracted catheter drainage were given bladder lavages to determine the effect on their marked bactiuria. Lavage solutions included 2 per cent neomycin; crystalline penicillin G (1,000,000 units per L.) with streptomycin, chloramphenicol and neomycin (one gram each per L.); nitrofurazone (Furacin®) 0.012 per cent; acetate buffer (pH 4.4); and aqueous benzalkonium chloride 1:10,000. The solution was instilled in 50 ml. amounts b.i.d. through the catheter and left for 30 minutes. Quantitative urine cultures and antibiotic resistance tests were obtained weekly. The results were disappointing, as heavy bactiuria persisted. Sensitive bacterial species were eradicated, only to be replaced by more resistant bacteria or Candida species. The odor of the urine diminished.

Three patients without infection had a catheter inserted using neomycin unguent as the lubricant. Large numbers of Candida appeared within 7 to 14 days.

The findings indicate the potential danger of inlying urinary catheters.

Therapeutic Attempts with Cephalin Fraction of Bovine Brain in Thrombocytopenia and Classical Hemophilia. FRANCO GOBBI and MARIO STEFANINI,* Boston, Mass.

The protein-free fraction of bovine brain (Folch) was used *in vitro* and *in vivo* to correct the hemostatic defect of thrombocytopenia and hemophilia. *In vitro,* cephalin: *a*) shortened the clotting time and increased the prothrombin utilization of thrombocytopenic plasma at a lowest concentration of 1.7 mg. per cent; *b*) substituted for platelets in thromboplastin and thrombin generation tests; and *c*) at concentration of 50 mg. per cent corrected the prothrombin utilization in plasma of classical hemophilia; it was practically ineffective with PTC-deficient plasma. All five subfractions of cephalin exhibited platelet-like function, which was not, therefore, limited to phosphatidylethanolamine.

For use *in vivo,* cephalin was repeatedly washed with acetone and homogenized in saline. Aliquots were administered intravenously in 250 ml. saline into two patients with classical hemophilia and to seven with secondary thrombocytopenia. Results varied. Administration of 2.5 to 5 Gm. to thrombocytopenic patients induced: *a*) improvement of hemorrhagic manifestations; *b*) shortening of whole blood and of clotting time of recalcified plasma; *c*) normal utilization of prothrombin and labile factor (V) during coagulation; and *d*) improved generation of thrombin. Effects lasted 8 to 24 hours. Skin tests and repeated injections at short and long intervals failed to reveal sensitization or progressive reduction of therapeutic effects. An unexplained dissociation was seen after intravenous administration of 5 Gm. of cephalin to patients with classical hemophilia: *a*) the clotting time remained prolonged; *b*) the prothrombin utilization was greatly enhanced for at least eight hours, the effect being comparable to that of one unit of plasma.

It is concluded that: *a*) Cephalin at varying doses completely corrects the coagulation defect of thrombocytopenia and classical hemophilia *in vitro; b*) Cephalin of bovine origin is not antigenic and may be administered safely to humans. Significant, total or partial correction of the coagulation defect in classical hemophilia and

thrombocytopenia may thus be achieved *in vivo* with the use of a material of origin other than human.

Granulomatous Lesions—An Expression of the Hypersensitive State. MOSHE B. GOLDGRABER, Chicago, Ill. (Introduced by Joseph B. Kirsner).

The histologic study of chronic ulcerative colitis, based on surgical specimens and autopsy material, revealed granulomata with or without giant cells in a considerable number of cases. This finding, while a not uncommon feature of regional enteritis, has not been emphasized in ulcerative colitis. It has been suggested by Goddard that granulomatous inflammation is a "qualitative" characteristic of the hypersensitive state. The reproductibility of granulomas and giant cells, consequently, was investigated in sensitized and nonsensitized animals. Thirty guinea pigs were injected repeatedly into the subcutaneous tissue of the abdomen with crystalline egg albumin. After two weeks, 15 animals received four "eliciting" injections of the same antigen on four successive days; 15 received horse serum. Biopsies, taken on the third day after the last injection, demonstrated diffuse histiocytosis, proliferation of endothelial and perithelial cells in the test animals, at the site of the injections of egg albumin. Granulomas with giant cells were observed in 5 of 12 surviving animals given egg albumin, whereas only one of the control group developed diffuse histiocytosis and multinucleated cells. In additional experiments, granulomas with or without giant cells were noted in the colon of rabbits in serial biopsy studies of the Arthus and Shwartzman reactions 7 to 12 days after the induction of these reactions in the bowel wall. The granulomata in the subcutaneous and colonic sites appeared similar morphologically. It is suggested that granulomata with or without giant cells are an expression of the hypersensitive state.

Physiological Diagnosis of Disordered Coronary Circulation. RICHARD GORLIN,* NORMAN BRACHFELD, PIERRE BOPP, and COLIN MACLEOD, Boston Mass.

Objective physiological evaluation of the coronary circulation is needed in the diagnosis of coronary artery disease and evaluating therapy thereof. Such may be achieved by measuring coronary flow (CF) and vascular resistance (CVR) before and after induced vasodilatation.

Coronary venous catheterization was performed in 18 patients. Coronary flow per 100 Gm. left ventricular (LV) muscle was measured by the nitrous oxide technique at rest, and 5 to 12 minutes after 0.6 mg. sublingual nitroglycerin with simultaneous measurement of LV oxygen consumption, systemic arterial pressure, heart rate and indicator dilution output.

Resting coronary flow (66 ml. per 100 Gm. per minute) and oxygen consumption (8.3 ml.) increased an average of 63 per cent (110 and 13.6 ml., respectively) with a 42 per cent fall in CVR after nitroglycerin in 10 noncardiacs. LV work remained unchanged and efficiency decreased. In eight patients with angina pectoris, due either to coronary artery disease or aortic valvular disease, coronary flow and oxygen consumption (normal at rest) fell or was unchanged, while CVR remained fixed ($\pm$ 10 per cent). LV work and efficiency fell precipitously in these eight.

Failure of coronary flow to increase with nitroglycerin in this second group is believed to indicate prior maximal vasodilatation (in relation to either obstructed coronary arteries or increased oxygen work demands), and as such may serve as a physiologic test to detect coronary insufficiency.

This study suggests that nitroglycerin may not relieve ischemic pain by increasing coronary flow, but may do so by sharply decreasing cardiac work (even at the expense of efficiency) as suggested in 1867 by Brunton.

Micropuncture Study of the Osmolality of Renal Tubular Fluid in Potassium-Depleted Rats. CARL W. GOTTSCHALK,* MARGARET MYLLE, ROBERT W. WINTERS, and LOUIS G. WELT,* Chapel Hill, N. C.

Potassium-depleted rats are unable to elaborate as concentrated a urine as normal rats, and an anatomical lesion has been described in their collecting ducts. Previous micropuncture experiments in normal rats have demonstrated that the tubular fluid is made hypo-osmotic in the loop of Henle by extraction of solute, probably NaCl, in excess of water. In the presence of antidiuretic hormone, tubular fluid again becomes isosmotic by the end of the distal convolution and the hyperosmotic concentration occurs as it flows through the collecting ducts. The following experiments were designed to localize the site of the concentrating defect in potassium-depleted rats.

Tubular fluid was collected by micropuncture from 18 distal convolutions in 4 anesthetized, hydropenic, potassium-depleted rats. The osmolality of the tubular fluid and simultaneous urine and plasma was determined microcryoscopically. The site of micropuncture was established by subsequent microdissection. The osmolality of the distal fluid was similar to that of the normal group, despite the fact that the urine was less concentrated. The defect is thus localized to the terminal hyperosmotic concentrating process. It is not known whether the defect is the result of decreased permeability of the collecting ducts to water due to their anatomical lesions, or to some other factor. The transfer of water across the collecting duct epithelium may be due to its diffusion into the interstitium of the medulla made hyperosmotic by the reabsorption of NaCl in excess of water in the loops of Henle. The latter mechanism appeared normal in these rats since their early distal fluid was as hypo-osmotic as in controls, and they excreted as dilute a urine as controls when water-loaded.

Cardiovascular Dysfunction During Rickettsial Toxemia. SHELDON E. GREISMAN and CHARLES L. WISSEMAN, JR.,* Baltimore, Md.

Hypotension characteristic of typhus fevers is attributed generally to peripheral vascular damage and, more controversially, to myocarditis. Despite the usual temporal coincidence between vascular dysfunction and vascular

histological lesions, fulminant cases may succumb before classical tissue reactions develop, suggesting the possibility that vascular toxins may also participate. Determination of man's susceptibility to known rickettsial toxins has not been feasible. Yet, the remarkable vascular effects induced in susceptible animals by typhus toxins strongly attract consideration. This report contributes new information about vascular effects of murine typhus toxin.

Intravenous injection of murine typhus toxin into white rats in amounts lethal in about 150 minutes induces a characteristic, reproducible pattern of changes in the mescoappendiceal vascular bed. After about 30 minutes, large arterioles, small arterioles and precapillaries manifest constriction that gradually intensifies until death. Simultaneously, capillary blood flow diminishes progressively, finally leaving the capillary bed markedly ischemic. Hemoconcentration is generally present terminally. Nevertheless, sensitivity of terminal arterioles and precapillaries to topical epinephrine remains constant throughout. Systemic arterial blood pressure in *unanesthetized* animals remains essentially unchanged until near death but it falls sharply in *anesthetized* rats at about the time that arteriolar constriction and slowed blood flow first appear and before hemoconcentration occurs.

These observations suggest: *a*) Increased vascular permeability, previously established, develops in a remarkably specific manner that leaves unaltered vascular epinephrine reactivity and capacity to participate in reflex compensatory mechanisms. *b*) The VEM mechanism usually operative in acute blood loss does not function. *c*) Cardiac output, at least in anesthetized animals, diminishes appreciably early in the toxemia prior to, and initially independent of, reduction in total blood volume from hemoconcentration. These distinctive vascular changes differ significantly from those we have observed after acute hemorrhage and after lethal amounts of typhoid endotoxin.

In Vivo Demonstration of Splenic "Conditioning" in Hereditary Spherocytosis—Alterations in Osmotic and Mechanical Fragility Related to Erythrocyte Aging and Splenic Sequestration. ROBERT C. GRIGGS, RUSSELL WEISMAN, JR., and JOHN W. HARRIS,* Cleveland, Ohio.

The increased erythropoiesis of hereditary spherocytosis made it possible in two patients to label new red cells *in vivo* with Fe^{59}, and produce an identifiable group of tracer cells of known age.

When first delivered to the peripheral blood, the group of labeled cells was less fragile to osmotic or mechanical forces than the general population. The cell fragilities progressively increased over a period of 10 to 17 days, a proportion of these cells becoming even more abnormal than the general cell population. Counting radioactivity over body surfaces indicated progressive erythrocyte sequestration in the spleen. The cells showing the most marked increases in osmotic fragility disappeared from the peripheral circulation within a few days.

At splenectomy blood recovered from the spleen was labeled with chromium^{51} and returned to the patient. This blood obtained from the spleen contained a large proportion of cells with a marked increase in osmotic fragility; these disappeared from the peripheral circulation within 48 hours. The remaining erythrocytes survived normally.

These *in vivo* studies indicate that the spleen "conditioned" the susceptible erythrocyte over a period of several days resulting in progressive increases in osmotic and mechanical fragility relative to cell age. If returned to the peripheral circulation, such a cell probably survives less than 48 hours. In the splenectomized patient cells can no longer be sequestered and subjected to "conditioning." Accordingly, the survival time is normal even though the erythrocytes continue to show the defects typical of hereditary spherocytosis.

Effects of Calcium on Urine Concentrating Ability. JACOB GROSSMAN, MARTIN F. MINES, ARTHUR G. GOLDMAN, and MORRIS WOLFMAN, New York, N. Y. (Introduced by Louis Leiter).

Several diseases of diverse natures producing long-standing hypercalcemia and/or hypercalciuria often result in renal insufficiency manifested by impaired urine concentrating ability and polyuria. This observation led to the investigation of the acute effects of intravenous calcium adminstration on urine composition and concentration. Experiments were performed as follows: Isotonic lactated Ringer's solution containing inulin, PAH and vasopressin (Pitressin®) (0.5 to 2.0 mU per Kg. per hour) was infused at 1.0 to 1.5 ml. per minute throughout the procedure. When a constant flow of hypertonic urine was obtained, a second infusion containing isotonic calcium chloride or gluconate (0.75 to 1.5 mEq. per Kg. per 120 minutes) was begun. Serum and urine osmolarities and electrolyte concentrations and renal hemodynamics were measured.

Calcium administration invariably produced a gradual, progressive increase in urine flow to 6 to 17 ml. per minute despite the continued vasopressin. Moreover, during control studies without vasopressin in the same patients, similar maximal urine flows were achieved suggesting that calcium either inactivates circulating vasopressin (ADH) or renders the renal tubules insensitive. The diuresis was associated with a rise in renal hemodynamics and natriuresis. While these changes resemble those of osmotic diuresis, the fall in urinary osmolarity to hypotonic levels (positive free water clearance) indicates dilution in excess of solute demands.

The observed diuresis is not dependent simply upon total serum calcium concentration since the urine flow falls shortly whereas the serum calcium level decreases very gradually. The similar impairment of urine concentrating ability of potassium deficiency and calcium excess suggests that their known antagonism, perhaps due to effects on membrane permeability, extends to the kidney and is based upon their relative rather than absolute concentrations. However, in one study, the simultaneous administration of KCl (15 mEq. per hour) and calcium

gluconate (27 mEq. per hour) during vasopressin administration did not prevent the usual diuresis.

Erythropoietin in Fractionated and Unfractionated Plasma. CLIFFORD W. GURNEY, Chicago, Ill.

Erythropoietin, an erythrocyte stimulating hormone, has been demonstrated in normal plasma and its concentration is increased in the plasma of some anemic animals and human beings. In bioassay procedures usually employed, most of the protein has been removed from the plasma by heat-denaturation and precipitation. We have studied the loss of activity resulting from fractionation of plasma and have subjected whole as well as fractionated human plasma to bioassay for erythropoietin.

Male Sprague Dawley rats in which erythropoiesis has been depressed by hypophysectomy, acute starvation, or transfusion-induced polycythemia were used for bioassay, employing the 16 hour incorporation of Fe^{59} by erythrocytes following two injections of plasma or heat-denatured plasma extract. In all three assay preparations used, heat-denaturation was responsible for a striking loss of erythrocyte stimulating activity of anemic rat plasma. Therefore the erythrocyte stimulating property of unfractionated normal and anemic human plasma was compared to that of extracts of the same specimens prepared by boiling for 10 minutes. The average Fe^{59} incorporation produced by whole plasma from nine normal donors, when tested in 42 starved rats, was 5.7 per cent (standard error equals 0.39 per cent). This average value was reduced to 4.3 per cent (standard error equals 0.30 per cent) when the plasma was fractionated. In 12 of 24 anemic patients, whole plasma specimens increased the Fe^{59} uptake to levels in excess of 10 per cent. In every instance, loss of activity was produced by fractionation, and only three plasma specimens were clearly positive after extraction.

Although it is generally held that erythropoietin is heat stable, these results indicate noteworthy amounts of activity are lost by fractionation, and some negative results in the literature may be explained on this basis.

Erythrocytic Acetylcholinesterase Defect in Paroxysmal Nocturnal Hemoglobinuria. ROBERT C. HARTMANN, JOSEPH V. AUDITORE, and WILLIAM C. HOLLAND, Nashville, Tenn. (Introduced by John B. Youmans).

While much attention has been given to the study of the various plasma factors in the paroxysmal nocturnal hemoglobinuria (PNH) hemolytic system, relatively little investigation has been directed toward the PNH erythrocyte. It is believed that the stroma of the PNH red cell is defective although the nature of the abnormality is not settled.

Previous work in this labortary indicates that there is a close correlation between permeability and the activity of the acetylcholinesterase system in normal red cells. Therefore, the activity of this enzyme system in PNH cells was investigated. In four patients with PNH the red cell acetylcholinesterase activity was 25 to 80 per cent lower than in normal red cells.

Reticulocyte-rich erythrocyte portions (top layers of packed cells) showed much higher acetylcholinesterase activity than reticulocyte-poor portions (bottom layer of packed cells) in both normal and PNH subjects. The increase in enzyme activity was approximately proportional to the reticulocyte concentrations. Reticulocyte-rich portions of PNH cells contained higher concentrations (5 to 10 per cent) of reticulocytes than in the normal. Nevertheless, PNH reticulocyte-rich portions showed considerably less enzyme activity than normal reticulocyte-rich or -poor portions. It appears that this basic enzyme defect is present in both young as well as mature PNH cells. PNH reticulocyte-rich and -poor portions of red cells were equally susceptible to hemolysis in the Ham-Crosby test. The *serum* cholinesterase activity was also low in PNH.

On the Diagnosis of Functional Coarctation. HANS H. HECHT,* DALI J. PATEL, and RAMON L. LANGE, Salt Lake City, Utah.

In four subjects with coarctation of the aorta, two arterial catheters (1 mm. ext. diameter) were passed simultaneously through a brachial and femoral artery. This simple and harmless technique permitted exact localization of the coarcted segment *a*) from qualitative and quantitative pressure differences proximal and distal to the lesion, *b*) from wedging of one or both catheters into the coarctation or passing through the narrowed channel, *c*) localizing the site of the defect by measuring the length of the catheters from their insertion to the wedge position. The pressure gradients as well as the slopes of these central pulse curves were determined. Slopes measured 670 to 955 mm. Hg per second above, and around 140 mm. Hg per second below, the coarctation (normal range, 538 ± 86). The differences remain significant with changes in flow. Anatomical coarctation may occur without change in mean pressure or pulse pressure. Occasionally constriction may be shown by X-ray studies but without variation of either slope or pressure values. This suggests that the slope value is the most critical index of functional coarctation. Animal experiments carried out by causing mechanical aortic constriction in dogs, as well as hydraulic and electrical analogues, demonstrate that the anatomic constriction alone can account for many if not for all of the pressure phenomena of coarctation, and that the earliest and most consistent finding is change in slope of the pressure pulse curve below the lesion.

Direct Measurement of Cholesterol Absorption via the Thoracic Duct in Man. LEON HELLMAN,* E. L. FRAZELL, and R. S. ROSENFELD, New York, N. Y.

The intestinal absorption of cholesterol has been directly studied in man by cannulation of the left thoracic duct and complete collection of lymph following oral administration of cholesterol-4-C^{14} and acetate-2-H^3. This procedure permitted simultaneous observations concerning biochemical alterations and kinetic behavior of dietary cholesterol during the absorptive process as well as the

contribution to lymph of endogenously synthesized cholesterol.

From 4 to 27 per cent of the cholesterol-4-C^{14} was absorbed into lymph collected up to three days. Cholesterol absorption is a slow process, reaching a maximum in six hours, with significant quantities still being absorbed during the second day. Despite complete diversion of the lymph outflow of the left thoracic duct, a small quantity of C^{14} cholesterol still appeared in plasma. From this it was calculated that the fraction of the tracer dose absorbed through all other routes was less than one-fifth the amount which was absorbed through the left thoracic duct. In contrast, tritium-labeled biosynthetic cholesterol appeared in plasma in quantities and at rates usually observed in intact subjects indicating that acetate is absorbed through the portal system. The specific activities of lymph free and ester biosynthetic cholesterol were lower than simultaneous plasma samples. This is due to the expected dilution of plasma cholesterol, which serves as the source of the biosynthetic lymph cholesterol, by newly absorbed dietary cholesterol lacking the tritium label.

A minimum of 50 per cent of the C^{14} lymph cholesterol was in the ester form indicating that dietary cholesterol is esterified in the intestine during absorption. The plasma of *intact normo*cholesteremic subjects contains labeled free, but *no* labeled ester cholesterol during the first few hours following an oral tracer dose of cholesterol. However, labeled plasma ester cholesterol *is* present in identical studies in intact *hyper*cholesteremic subjects. It is therefore apparent that the ester cholesterol of lymph must be efficiently cleared from the circulation of *normo*cholesteremic subjects immediately following absorption and that either the esterification or clearance mechanism is defective in *hyper*cholesteremic subjects.

Effect of Ether Anesthesia on Human Skeletal Muscle Metabolism. DOROTHY H. HENNEMAN and LEROY D. VANDAM, Boston, Mass. (Introduced by Francis D. Moore).

Phosphocreatine, total creatine, and lactic acid concentrations have been measured in triplicate on nontraumatized paired biopsies of rectus abdominis muscle taken from women during 20 to 140 minutes of ether anesthesia for ovarian biopsy, hysterectomy, or cholecystectomy. The data were correlated with changes in blood pH and serum inorganic phosphorus. Twenty minutes after induction concentrations of phosphocreatine were 14 to 23 micromoles and total creatine 25 to 37 micromoles per gram of wet tissue; lactic acid was 0.12 to 0.84 micromoles per gram of wet tissue. During two hours of anesthesia, phosphocreatine fell to 8.5 to 10.8 micromoles per gram and total creatine to 11 to 20 micromoles per gram. Lactic acid concentrations showed no significant changes. Biopsies from anesthetized dogs without surgery showed comparable changes. Serum inorganic phosphorus increased as expected without any significant change in pH.

Ether anesthesia appears, therefore, to inhibit the synthesis of phosphocreatine without an associated increase in lactic acid. This may reflect a decrease in glycolysis. Studies during ether anesthesia in man have demonstrated abnormal elevations in blood glucose and serum inorganic phosphorus following administered glucose or epinephrine. In addition, there occurs resistance to the glucose and phosphorus-lowering effects of administered insulin. These abnormalities are not associated with abnormalities in lactic, pyruvic, or citric acid concentrations under the same conditions. Ether in some manner alters the transfer of glucose across the cell membrane. It is unknown whether this reflects a change in cellular permeability, insulin activity, or phosphorylating reactions associated with the transfer of glucose.

The Metabolic Defect Responsible for Uric Acid Renal Stone Formation. PHILIP H. HENNEMAN, STANLEY WALLACH, and ELEANOR F. DEMPSEY, Boston, Mass. (Introduced by A. P. Forbes).

Uric acid renal stones occur primarily in middle aged and elderly persons and especially in Jews and Italians. In a series of 22 patients with uric acid stones 5 had gout, 3 had chronic diarrhea and 14 were otherwise normal. In patients with the latter idiopathic variety of uric acid stones serum uric acid levels are nearly always normal and, more significantly, urinary uric acid excretion is not increased above normal (150 to 900 mg. per day) on unselected diets. Two constant biochemical peculiarities in patients with idiopathic uric acid stones are hypocalciuria and constantly very acid (pH 4.5 to 5.5) urine. Since normal human urine is supersaturated with uric acid at pH values less than 6.0 the cause of precipitation of uric acid in these patients is probably the constantly very acid urine.

Complete balance studies on neutral ash diets in two patients with idiopathic uric acid stones have shown persistently acid urines but have not shown excessive fecal base loss. In two uric acid stone patients the pCO_2 of urine collected under oil was not increased. Balance studies on neutral ash diets with measurement of all major urinary electrolytes in four idiopathic uric acid stone patients demonstrated an inverted ratio of ammonium to titratable acidity-minus-bicarbonate due to subnormal ammonia secretion. The administration of 140 mEq. of ammonium chloride daily for six days elicited less increase in urinary ammonium and a greater degree of systemic acidosis in two patients with idiopathic uric acid stones than it did in three normal subjects.

Thus it appears that most uric acid renal stones are the indirect consequence of an acquired isolated defect in renal tubular ammonia secretion.

Mechanism of Intrinsic Factor Action in the Isolated Rat Small Intestine. VICTOR HERBERT, New York, N. Y. (Introduced by Robert G. Bloch).

The effect of hog intrinsic factor concentrate (HIFC) on Co^{60}-labeled vitamin B_{12} uptake by sacs of everted rat small intestine was studied by methods previously applied

to a similar study with rat liver slices. Vitamin B_{12}-Co^{60} uptake was not enhanced when intestine sacs were incubated in a mixture of HIFC and vitamin B_{12}-Co^{60}. Up to fivefold enhancement was repeatedly observed when the sacs were incubated with HIFC, washed three times, and then incubated with vitamin B_{12}-Co^{60}. Using sequential incubation, it was demonstrated that maximum increase of mucosal vitamin B_{12}-Co^{60} uptake did not occur if calcium was replaced in the incubation medium by sodium. The uptake produced by HIFC was reversible to an appreciable degree by ethylenediaminetetraacetate. The similarity of these findings to those obtained with rat liver slices suggests the mechanism of HIFC action is identical in both systems.

That the increase in mucosal vitamin B_{12}-Co^{60} uptake after preincubation with HIFC is due to its intrinsic factor is suggested by the facts that: *a*) The increase is abolished by preheating the HIFC at 100° C. for 15 minutes. *b*) Performance of either incubation at pH 1.85 markedly reduced the effect of HIFC. *c*) 5, 6-Dimethylbenzimidazole, either alone or in combination with $CoCl_2$, does not affect vitamin B_{12}-Co^{60} binding in the system, as it does not affect vitamin B_{12} absorption *in vivo*.

It is postulated that intrinsic factor has the ability on one hand to bind vitamin B_{12} and on the other to attach to liver or intestine receptors in the presence of calcium. Possession of these two properties simultaneously may be unique to intrinsic factor.

Pathways of Ribose Synthesis in Normal and Pentosuric Human Subjects. HOWARD H. HIATT, Boston, Mass. (Introduced by H. L. Blumgart).

Ribose synthesis from hexose via the pentose phosphate pathway has been observed in several mammalian systems. Recently, an additional mechanism for ribose biosynthesis has been postulated as a result of the demonstration in animal tissues of the following reactions: D-glucuronic acid → L-gulonic acid → L-xylulose → xylitol → D-xylulose → D-xylulose 5-phosphate → D-ribulose 5-phosphate → D-ribose 5-phosphate. A block in this pathway would explain the excretion of L-xylulose by persons with the genetic disorder, essential pentosuria.

We have studied ribose biosynthesis in man with a trapping technique suggested by Tabor and Hayaishi's observation that imidazoleacetic acid (IAA) riboside appears in the urine following IAA administration. Patients given 1,500 micromoles of IAA and glucose-2-C^{14} excreted as much as 570 micromoles of riboside ribose containing 0.05 per cent of administered C^{14}. Fifty per cent of the C^{14} in the ribose molecule appeared in carbon 1, and 39 per cent in carbon 2, consistent with hexose conversion to ribose via the pentose phosphate pathway. Administration of IAA and D-glucuronolactone-U-C^{14} to a subject with normal carbohydrate metabolism was followed by the excretion of ribose containing 0.06 per cent of the administered C^{14}. In striking contrast, a pentosuric subject given IAA and glucuronolactone-C^{14} excreted a comparable quantity of ribose, which was virtually free of C^{14}, and a large amount of L-xylulose-C^{14}.

From our observations we conclude that human subjects with normal carbohydrate metabolism may synthesize ribose by at least three mechanisms: *1*) the oxidative loss of carbon 1 of hexose phosphate; *2*) the nonoxidative series of reactions catalyzed by transketolase and transaldolase; and *3*) conversion of glucuronic acid to ribose via L-xylulose. Our data provide strong support for the postulated block in the third pathway in essential pentosuria. Evidence from human studies and from experiments with rats given IAA and glucose-3,4-C^{14} will be presented to indicate that the first two mechanisms are quantitatively most significant in ribose biosynthesis.

The Mode of Action of Chlorothiazide and Mercurial Diuretics as Antihypertensive Agents. WILLIAM HOLLANDER and ARAM V. CHOBANIAN, Boston, Mass. (Introduced by Robert Wilkins).

The antihypertensive action of chlorothiazide and mercurial diuretics was studied in 20 hypertensive and 20 normotensive subjects. Oral and intravenous chlorothiazide or parenteral mercurial diuretics significantly reduced the blood pressure in 14 of 20 hypertensive subjects but did not alter blood pressure in the normotensive controls. Changes in weight, sodium excretion, and creatinine clearance were comparable in both groups. Body sodium (Na^{24} space) and extracellular fluid volume ($S^{35}O_4$ space) were not significantly altered in 12 hypertensive subjects after prolonged oral chlorothiazide therapy, although significant reductions in body potassium (K^{42} space) occurred.

Sixteen metabolic balance studies revealed: *1*) The antihypertensive effect of chlorothiazide or mercurial diuretics was greater than that of a 9 mEq. sodium diet alone. *2*) Net sodium losses as small as 50 mEq. were found during the antihypertensive effect of chlorothiazide or of mercurial diuretics, but were usually (as were net potassium losses) as large as 150 to 250 mEq. *3*) Upon simultaneously withdrawing the diuretics and reducing the dietary intake of sodium to 9 mEq. daily, the antihypertensive effect and cumulative negative sodium balance persisted until sodium intake was increased, but cumulative potassium balance reverted to pretreatment levels. *4*) Increase in dietary sodium intake to as much as 225 mEq. daily did not block the antihypertensive action of chlorothiazide. *5*) Addition of 9-α-fluorohydrocortisone to the chlorothiazide regimen restored sodium balance to control levels without blocking the antihypertensive effects. *6*) Addition of the steroidal antagonist SC-8109 (Searle) [19-nor analog of 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl), propionic acid γ-lactone] produced further loses in body sodium and increased the antihypertensive effect of chlorothiazide.

In conclusion, chlorothiazide and mercurial diuretics appear to have a selective antihypertensive action which is not necessarily dependent on, but which may be potentiated by a reduction in body sodium. Maintenance of a reduced body sodium may prolong the antihypertensive effects of these diuretics.

Cross Resistance to Cerebral Typhoid Infection and Influenza Virus Neurotoxicity. EDWARD W. HOOK and ROBERT R. WAGNER,* Baltimore, Md.

Salmonella typhosa inoculated intracerebrally in mice produces an acute purulent meningo-encephalitis and death in 1 to 10 days. Large doses of non-neurotropic influenza virus injected intracerebrally also cause an inflammatory reaction of the brain and meninges, characteristic tonic convulsions and death in 48 to 72 hours. Salmonellae multiply rapidly to high titer in brain tissue, whereas proliferation of influenza virus at this site is extremely limited. Despite marked differences in pathogenicity and the nature of the infecting organisms, resistance to both infectious agents can be induced in strikingly similar fashion. Animals injected intracerebrally with typhoid vaccine 24 hours previously resist challenge with either *S. typhosa* or influenza virus to about the same degree. Injection of subtoxic doses of influenza virus also protects mice against virus neurotoxicity although pretreatment with virus has only a minimal effect on cerebral typhoid infection.

The most pronounced refractory state is produced by purified bacterial endotoxins; lipopolysaccharides prepared from *Escherichia coli* or *S. equiabortus* markedly inhibit bacterial multiplication in brain and protect mice against 400 LD_{50} of *S. typhosa*. The route of inoculation is also important. Ten micrograms of *E. coli* lipopolysaccharide injected intraperitoneally in mice produces only a slight increase in resistance to intracerebral challenge, whereas 0.01 microgram of lipopolysaccharide given intracerebrally affords effective protection against both cerebral typhoid infection and influenza virus neurotoxicity.

Resistance to either typhoid infection or virus toxicity develops in a few hours, is maximal at 24 to 48 hours, and gradually disappears in about a week. The similarities in time of onset, duration and degree of host resistance to two such different types of infection are additional evidence for a general phenomenon of acquired resistance unrelated to specific immunity.

Adequacy of Renal Oxygenation in Essential Hypertension. WILLIAM E. HUCKABEE, Boston, Mass. (Introduced by Chester S. Keefer).

The question whether anoxia is present, in the sense that rate of O_2 supply is insufficient to meet requirements, cannot be answered by measuring the blood flow to or even the mean P_{O_2} in a tissue. To determine whether renal anoxia in this sense is present in patients with essential hypertension, rates of anaerobic metabolism (AMR) of their kidneys were compared with those of normal subjects. From analyses of 24 hour urines for lactate, pyruvate (corrected for ketoglutarate) and creatinine, the urinary excretion of excess lactate, XL, was estimated (lactate unaccounted for by the normal $DPNH_2$ /DPN ratio). Both groups excreted widely varying quantities of total lactate (U/P ratio < 1.0) and pyruvate (U/P ratio > 1.0), but excretion of calculated XL (the result of anaerobiosis) was relatively constant in any one individual during two to three years. The mean rate of excretion of XL in 22 hypertensive patients without pyelonephritis was significantly greater than in normal subjects (109 mEq. per Gm. creatinine per day, $p < 0.01$). It was not affected by exercise, infusion of pyruvate or lactate, or chronic administration or restriction of salt, nor by reduction of blood pressure during reserpine therapy. However, AMR of the kidney could be increased by reducing blood pressure with ganglionic blocking drugs or, in dogs, by constricting the renal artery. Renal AMR was proportional to the mean starting blood pressure (untreated) ($r = 0.66$), but was independent of current blood pressure. It was concluded that the kidneys of these hypertensive patients were abnormally dependent upon anaerobic metabolism because of relative inadequacy of O_2 supply to the urine-producing cells (those capable of altering tubular filtrate composition with respect to lactate), since the only mechanism for altering lactate concentrations is the intracellular lactic dehydrogenase system, which also necessarily reflects the $DPNH_2$/DPN ratio of the cells.

Pre-Albumin, a New Thyroxine-Binding Protein of Human Plasma: Its Isolation and Physiologic Activity in Normal and Abnormal States. SIDNEY H. INGBAR,* Boston, Mass.

During electrophoresis in veronal buffer, physiologic concentrations of thyroxine migrate in association with moieties in the interalpha area, termed thyroxine-binding protein (TBP). In contrast, in a new electrophoretic system to be described, endogenously and exogenously labeled thyroxine migrate both with TBP and with protein moving approximately 20 per cent faster than albumin at pH 8.6 (pre-albumin). Comparative studies in the two systems indicate that failure to observe thyroxine-binding by pre-albumin in veronal is due either to trailing or pre-albumin or to protein interactions in this buffer. In the new system, physiologic concentrations of thyroxine distribute almost equally between TBP and pre-albumin. Additionally, in contradistinction to findings during conventional electrophoresis in veronal, total thyroxine bound to TBP reaches a plateau as the concentration of thyroxine is increased. Thus, absolute binding capacities of both TBP and pre-albumin can be assessed. In normal sera, the total thyroxine-binding capacity of TBP approximates 25 μg. per 100 ml. of serum, whereas that of pre-albumin is approximately 100 μg. per 100 ml.

Thyroxine-binding capacity of TBP is normal in patients with untreated Graves' disease and increased in pregnant women at term. However, in both groups, binding capacity of pre-albumin is markedly decreased. Findings in other abnormal states are being assessed.

Pre-albumin seems distinct from TBP for the following reasons: *a*) In pathologic sera, the absolute thyroxine-binding capacity varies independently of that of TBP. *b*) At low carrier concentrations, TBP binds triiodothyronine, but pre-albumin does not. *c*) TBP is present in Cohn Fractions IV-7 and IV-8 of human serum, whereas

pre-albumin is not. *d*) Treatment of proteins with neuraminidase alters the isoelectric point of TBP, but not of pre-albumin.

Pre-albumin of exceedingly high thyroxine-binding potency has been isolated in an electrophoretically and ultracentrifugally homogeneous state. Its sedimentation and electrophoretic characteristics, as well as its amino acid and carbohydrate contents, have been ascertained and will be described.

The Demonstration of Bilirubin Sulfate in Bile. KURT J. ISSELBACHER, Boston, Mass. (Introduced by Mandel E. Cohen).

It is now apparent that the metabolism of bilirubin by the liver results in the formation of a water-soluble derivative which is excreted into the bile and gives a "direct" Van der Bergh reaction. Previous investigators have identified this pigment as the glucuronide conjugate of bilirubin.

Since the hepatic conjugation of other alcoholic and phenolic compounds, such as corticosteroids and estrogens, involves the formation of sulfate as well as glucuronide derivatives, it seemed reasonable that an ethereal sulfate conjugate of bilirubin at its hydroxyl group might also occur. Such a derivative would be water soluble, but, in contrast to the alkali-labile glucuronide ester, the ethereal sulfate derivative of bilirubin would be relatively alkali-stable. Previous observations that 10 to 15 per cent of the "direct-reacting" pigment of human bile was alkali-stable gave additional support for this hypothesis.

Bile was collected from rats with bile fistulas and from the gall bladder of cats following the intraperitoneal injection of S^{35}-labeled inorganic sulfate. After treatment with *p*-diazobenzenesulfonic acid, the resulting azo-pigments were purified and separated from bile salts by extraction with heptane-butanol and by adsorption of the pigments on zinc hydroxide gel at pH 7 to 8. Paper chromatography of the purified azo-pigments in two solvent systems and subsequent autoradiography consistently revealed the presence of radioactivity in the "direct-reacting" azo-pigment band. The eluted labeled material was stable to alkali at 25° C. and 37° C. but was readily hydrolyzed in 1 N HCl at 100° C. with the liberation of inorganic sulfate. Labeled taurine was not identified as a product of this hydrolysis. The azo-derivative of synthetic bilirubin sulfate was prepared and shown to have the same chromatographic properties as the labeled azo-pigment in the bile.

While bilirubin glucuronide probably represents the major constituent of the "direct-reacting" bile pigment, the present studies indicate that other conjugates, such as bilirubin sulfate, also occur.

Comparison of the Effectiveness of Transfusions of Fresh and Lyophilized Platelets in Controlling Bleeding Due to Thrombocytopenia. DUDLEY P. JACKSON,* DALE K. SORENSEN, EUGENE P. CRONKITE,* and VICTOR P. BOND, Upton, N. Y., and Baltimore, Md.

Enumeration of red blood cells in thoracic duct lymph provides a semiquantitative measure of the degree of bleeding in animals rendered thrombocytopenic by whole body irradiation. This method was employed to evaluate the hemostatic effect of platelets lyophilized by the method of Klein and his associates.

Seven dogs were rendered thrombocytopenic by the administration of 500 to 550 r whole body irradiation. After thrombocytopenia developed, large numbers of red blood cells were observed in thoracic duct lymph. Lyophilized dog platelets were infused in amounts calculated to increase the recipient's platelet level by approximately 200,000 per cu. mm. However, following infusions, the recipient's circulating platelets did not increase. The number of red blood cells in the lymph did not decrease significantly in any of the seven dogs after administration of the lyophilized platelet preparations.

Four of the dogs subsequently received infusions of freshly prepared platelet suspensions. Circulating platelet levels increased, and there was a dramatic decrease in the number of red blood cells in the lymph. In a representative experiment, the mean output of red blood cells in the lymph before infusion was 373 million red blood cells per minute. Following infusion of lyophilized platelets, the mean output was 466 million per minute. Following infusion of a comparable number of fresh platelets, the mean output decreased by approximately 95 per cent from 466 million per minute to a level of 17 million per minute.

Thus, lyophilized platelets did not circulate in thrombocytopenic recipients and did not effect hemostasis. Infusions of fresh platelets increased circulating platelet levels and had a dramatic hemostatic effect.

Common Cold Neutralization by Human Immune Globulin. GEORGE GEE JACKSON,* HARRY F. DOWLING, and TRUMAN O. ANDERSON, Chicago, Ill.

It is generally believed that infection with the common cold does not produce immunity to reinfection. It is not even known whether such an infection elicits a specific antibody response in the host and if so whether the antibody is protective. In the present investigations the common cold was transmitted to volunteers by means of filtered infectious nasal secretions. Pooled human immune globulin was tested for its capacity to neutralize the infectivity of two secretions which produced an acute infectious coryza after appreciably different incubation periods. Human albumin and buffered salt solution also were tested for neutralizing activity. Among 65 volunteers who were challenged with an infectious secretion suspended in salt solutions, 52 per cent developed a common cold. When the secretion was suspended in albumin solution, 12.0 Gm. per 100 ml., 50 per cent of 38 volunteers had an experimental cold. The use of human immune globulin, 8.0 Gm. per 100 ml., reduced the number of experimental colds to 10 per cent of 59 volunteers. During the time of the experiments "spontaneous" colds were observed in 15 per cent of 61 volunteers who were challenged with a control solution. The data are highly

significant and showed that the globulin fraction of human serum contains potent, heat labile substances capable of neutralizing infectious agents of the common cold in nasal secretions. The implication is that this is antibody elicited by previous natural infections. Studies on the presence of serum globulin in nasal secretions will be presented. The findings suggest that the susceptibility of the nasal epithelium to infection with common cold agents may be independent of circulating antibody.

Sequestration of Reticulocytes and of Abnormal Red Cells by Filtration at Low Pressures. JAMES H. JANDL,* Boston, Mass.

Reticulocytes from animals of several species possessed properties similar to antibody-coated red cells; they were selectively agglutinated by Coombs sera and by agents which induce rouleaux-formation. After severe anoxia from bleeding or phenylhydrazine injection, autoagglutination of reticulocytes in native plasma was observed. In animals reticulocyte levels of spleen pulp blood exceeded those of peripheral blood, as observed by Berendes, despite the absence of splenic erythropoiesis. Sequestration of reticulocytes was particularly striking during reticulocytoses, and was not observed in other organs.

In order to understand why reticulocytes and sensitized red cells are trapped in the normal spleen, while agglutinated red cells are sequestered in the liver, conditions were examined affecting the passage of red cells through Millipore filters with a pore size of 5.5 microns. Sicklemic red cells, at low oxygen tensions, and spherocytes were readily separated from normal red cells. Similarly, reticulocytes and antibody-coated red cells suspended in plasma fortified with polyvinylpyrrolidone or fibrinogen were selectively trapped at low perfusion pressures; higher pressures (above 8 to 10 mm. Hg) caused the cell clumps to dissociate, permitting passage of these cells. Red cells directly agglutinated by "complete" antibodies penetrated the filter only under still higher perfusion pressures. The ability of red cell rouleaux or agglutinates to penetrate filters was a function of the force required to dissociate these aggregates.

Thus, 1) immature red cells resemble antibody-coated red cells in their agglutinability and filterability *in vitro* and in their susceptibility to splenic sequestration *in vivo;* 2) splenic sequestration of reticulocytes may explain certain apparent suppressive effects on erythropoiesis of "hypersplenism"; and 3) the sites of sequestration of immature, abnormal, or agglutinated red cells may be determined largely by the pore sizes and perfusion pressures of different vascular filter-beds.

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

The fibrinolytic effect of streptokinase, as first demonstrated by Tillett, has been firmly established. The present studies were undertaken to extend these observations and to demonstrate that intravascular clot lysis may be produced by the intravenous infusion of purified streptokinase (SK), in patients, under optimum biochemical conditions.

Blood clots 5 to 20 centimeters long were produced in peripheral veins of volunteers. Twenty-four or 48 hours later, SK was infused intravenously into a contralateral extremity. The presence of the clot was determined by inspection, palpation, and X-ray venograms taken before, during, and after the infusions.

Biochemical studies on each patient's plasma prior to the infusion made it possible to determine the SK required to neutralize the SK antibody and inhibitor, *in vivo*. Therefore, additional infused SK was free to be effective in an active fibrinolytic system. Further studies were performed during the infusion to maintain an appropriate balance of plasmin, plasminogen, plasmin-activator, SK and prothrombin.

In 11 instances, SK was infused in amounts calculated to obtain optimal clot lysis, *in vivo*. Eight clots, 24 hours old, lysed within 20 hours, and 3 clots, 48 hours old, lysed in 33 hours. No clot reformation occurred. Embolic complications have not been observed.

In addition, SK was deliberately infused in amounts smaller and larger than the calculated optimum. Clot lysis failed in seven instances when smaller than optimal amounts of SK were infused. Circulating plasmin was present in all seven without detectable free SK. Variable clot lysis occurred in six instances when larger than optimal amounts of SK were infused. Incomplete lysis, or clot reformation occurred in five of these, presumably due to a deficiency of circulating plasminogen. One clot lysed completely without reforming.

The results demonstrate that SK will produce intravascular clot lysis when infused intravenously into patients under defined biochemical conditions.

Genetic and Intrauterine Influences on Steroid Hormone Production in Man. ATTALLAH KAPPAS and T. F. GALLAGHER, New York, N. Y. (Introduced by Allan T. Kenyon).

Isolation, characterization and quantitative estimation of individual steroid metabolites during several experimental periods in identical triplets have demonstrated significant heritable and prenatal environmental influences on steroid hormone production. Urinary ketosteroids comprising amounts of androsterone, etiocholanolone, dehydroisoandrosterone, 11-hydroxyandrosterone, 11-hydroxyetiocholanolone and 11-ketoetiocholanolone form patterns that are reproducible and constant for any individual over long periods. These patterns differ quantitatively from subject to subject. Individual differences likewise appear during stimulation with adrenocorticotrophic hormone (ACTH).

The steroid patterns of adult, normal male dichorionic monozygotic triplets were examined in detail during control and ACTH periods. In two triplets these patterns were qualitatively and quantitatively virtually identical before, during and after ACTH administration. This concordance, in view of the highly individual nature of

these patterns, is impressive evidence of an important heritable influence on steroid hormone metabolism. The third triplet, however, produced significantly smaller amounts of these metabolites during all periods. This finding is interpreted as evidence for the existence of an important environmental influence by means of which steroid metabolism had been modified. The intrauterine focus of this influence is strongly suggested by the small birth weight and slower growth of this triplet compared with his brothers. Related physiological differences in monochorionic twins have been attributed to demonstrated vascular asymmetry and unequal distribution of the mutual fetal circulation to one partner of the pair. The dichorionic character of the triplet membranes makes it evident that a fetal situation comparable to that seen in such twins existed for two of the brothers. It can be presumed, therefore, that the triplet with these divergent steroid patterns was one of this fetal pair and that his differences in production and metabolism of steroids represent a physiologic aftermath of relatively deficient fetal circulation.

Asymptomatic Bacteriuria and Pyelonephritis of Pregnancy. E. H. KASS,* A. L. KAITZ, R. COTRAN, W. L. CLOUGH, and R. L. NICHOLS, Boston, Mass.

Relatively high rates of occurrence of asymptomatic bacteriuria have been found in various population groups. Among these, pregnant women were found to have more than 10^5 bacteria per ml. of urine in 11 per cent of specimens obtained at delivery. Further studies have shown the following:

1. Clean voided specimens may be used for the detection of bacteriuria by quantitation of the bacterial flora. The agreement with results obtained using catheterized specimens was greater than 95 per cent. The use of catheterized specimens for quantitative bacteriologic study of urine is rarely necessary.

2. Asymptomatic bacteriuria was found in 10 per cent of about 4,000 patients making *first* prenatal visits. Bacteriuria was not related to the duration of pregnancy and was found as early as the second month.

3. Untreated bacteriuria persisted throughout pregnancy and about 40 per cent of patients with bacteriuria at the first prenatal visit developed symptomatic pyelonephritis during the last trimester or in the immediate postpartum period.

4. When asymptomatic bacteriuria was suppressed in alternate patients by continuous antibacterial treatment, pyelonephritis did not occur.

5. Reliable differentiation of patients with and without bacteriuria on the basis of history, physical findings, or pyuria could not be made.

6. Significant residual urines were not found in the bladders of bacteriuric patients, using a method which eliminates catheterization.

7. The bacteriologic flora of the vaginal secretion in patients with bacteriuria was not significantly different from the flora of patients without bacteriuria; coliform organisms were rarely found in either case.

The results suggest that asymptomatic bacteriuria and pyelonephritis are pathogenetically related, and that detection and management of bacteriuria may eliminate pyelonephritis of pregnancy. The demonstration of bacteriuria early in pregnancy suggests that bacteriuria may persist in many young women between pregnancies or that a metabolic disturbance occurring early in pregnancy predisposes to the development of bacteriuria.

Anergy and the Response to Homografts in Hodgkin's Disease. W. D. KELLY, R. A. GOOD,* and R. L. VARCO, Minneapolis, Minn.

At present the best evidence indicates that homograft rejection in man is an immune response which in many details closely resembles the phenomenon termed delayed bacterial hypersensitivity. A recent report that Hodgkin's disease is associated with a high incidence of anergy to common bacterial and viral antigens applied as skin tests would appear to offer an opportunity to evaluate the role of delayed hypersensitivity in homograft rejection in man. The present report concerns such a study. At first, 22 patients with Hodgkin's disease and 207 patients not having lymphoma or terminal cancer were skin tested with the following antigens: 1) Schick control (diphtheria toxoid), 2) streptokinase-streptodornase, 3) mumps virus, 4) mumps control solution, 5) trichophytin audouini, 6) candida albicans, and 7) P.P.D., intermediate strength. The incidence of positive reactions was recorded by observation at 24 and 48 hours. The results show a high incidence of anergy in the Hodgkin's disease group in contrast to the control group.

Ten of the patients with Hodgkin's disease have had full-thickness skin homografts carried out. The grafts which were tattooed to aid in identification were followed by frequent observation for periods ranging from three to eight months. The results indicate a distinctly altered pattern of reaction varying from essentially complete acceptance of the graft to complete rejection. In every instance, except one, the rejection time was prolonged when compared to rejection times for skin homografts observed in immunologically normal persons. The predominant response observed is a slow, prolonged disintegration of the graft by repeated episodes of blistering, exudation with crust formation and partial sloughing.

These results confirm the high incidence of anergy in Hodgkin's disease and report a new finding of altered skin homograft rejection in these patients.

Stimulation of Amino Acid Penetration into Mammalian Skeletal Muscle by Insulin. DAVID M. KIPNIS and MATTHEW M. NOALL, Saint Louis, Mo. (Introduced by Robert E. Shank).

Insulin stimulates the incorporation of amino acids into proteins of skeletal muscle. To test whether this response is secondary to an accelerated rate of amino acid transport or to a direct stimulation of protein synthesis, a nonmetabolizable amino acid analogue, α-aminoisobutyric acid (AIB), has been used to study amino acid penetration in skeletal muscle.

Control studies indicated that AIB-1-C^{14} was neither incorporated into the protein or lipid of rat diaphragm or gastrocnemius nor oxidized to CO_2. Total tissue radioactivity was extracted with 0.008 M acetic acid and when chromatographed migrated in one spot corresponding with authentic AIB.

Rates of intracellular penetration were determined *in vitro* by incubating "intact" rat diaphragm preparations under conditions where the medium concentration of AIB (3.2×10^{-5} M) remained constant. AIB penetration was twice as rapid in diaphragms of rats fasted for 72 hours as in those of nonfasted animals. Insulin increases the rate of AIB penetration 300 to 500 per cent in fasted and nonfasted preparations in the presence or absence of glucose. Glucose (0.01 M) decreases the rate of AIB penetration 30 per cent in the presence or absence of insulin. In these experiments, AIB concentration gradients (intracellular content/extracellular content) of two to three were established. The rate of attainment of a steady-state distribution of AIB between plasma and muscle water and the final concentration gradients established in nephrectomized normal and diabetic animals were measured following the intravenous injection of 1 μM of AIB-1-C^{14}. The results of these *in vivo* studies were similar to those obtained with the *in vitro* technique.

These results indicate that current concepts of insulin action must be broadened to include the stimulation of both sugar and amino acid penetration. This suggests that impairment of amino acid penetration may contribute to the disturbed metabolism of diabetes mellitus.

The Effect of Parathyroid Extract (PTE) on the Renal Clearance of Diffusible Calcium. CHARLES R. KLEEMAN, ROBERT E. ROCKNEY, and MORTON H. MAXWELL, Los Angeles, Calif. (Introduced by Samuel H. Bassett).

In unpublished observations, the authors have noted that: 1) Hypoparathyroid subjects of recent origin, not on vitamin D, may have normal or elevated calcium excretion while *hypocalcemic,* and, after parathyroid extract administration, urinary calcium may fall while serum calcium rises. 2) Hyperparathyroids with hypercalcemia but normal renal function may have normal calcium excretion. 3) In normal subjects on high calcium diets, chronic PTE administration may cause hypercalcemia without further hypercalciuria. All the above suggest that parathyroid hormone may specifically decrease the renal *clearance* of calcium.

To clarify this point, paired studies of the renal clearance of diffusible calcium were determined in normal subjects during a four hour infusion of 1 Gm. of calcium ion ($CaCl_2$) with and without simultaneous administration of PTE. Serum diffusible calcium was obtained by the Lavietes anaerobic ultrafiltration technique.

The serum diffusible calcium averaged 59 per cent, and this magnitude was not affected by the PTE or calcium infusion. The pH of the serum and ultrafiltrate was constant (7.3 to 7.5) during the ultrafiltration. The basal clearance of diffusible calcium with PTE was 2.0 ml. per minute—without PTE, 4.5 ml. per minute. During the infusion the diffusible calcium clearance rose (mean peak, 12 ml. per minute), but, for any given filtered load of calcium, the clearance was always *less with* PTE ($C_{Diff.\,Ca.}$ PTE $\div$ $C_{Diff.\,Ca.} = 0.5$ to 0.6).

PTE inhibited the serum phosphorus rise associated with calcium loading by increasing the renal clearance of phosphorus. The injection of PTE into the renal artery of one dog caused a decrease in the calcium clearance on the homolateral side.

Studies are in progress to compare beef PTE with human PTE prepared in this laboratory.

It is concluded that PTE specifically *decreases* the renal clearance of calcium in humans, confirming the rat data of Talmage [Proc. Soc. exp. Biol. (N. Y.) 1955, 88, 600.]. PTE (hormone?) maintains serum calcium by *three* mechanisms: a) it "liberates" bone calcium, b) *increases* renal phosphorus clearance, and c) *decreases* renal calcium clearance.

Immediate Wheal Hypersensitivity. I. The Diphtheria System as a Model in Studying the Nature of Reagins. WILLIAM J. KUHNS, Pittsburgh, Pa. (Introduced by Frank J. Dixon).

Although production of allergic reagins seems to be related to the genesis of immediate wheal allergies, little is known about their nature except that they are ordinarily classified as antibodies. The ability of reagins to sensitize skin characteristically refers to their persistence in skin tissues or mucous membrances, so that allergic antigens introduced later cause immediate wheal reactions. Passive transfer methods for detecting reagins are based upon these properties. However, for technical reasons, it was previously impossible to determine exact times of survival of these components in skin. Similarly, it has been difficult to exclude the possibility that skin persisting antibody in addition to other serum components collectively comprise reagin and are required for wheal production. If one antibody apart from other serum components can be calibrated in human skin, it may be possible, using appropriate skin testing methods, to determine whether: 1) only antibody is able to exhibit the biological properties of reagin, and 2) residence of antibody in skin differs appreciably from persistence in circulating blood.

Antibody which is also antitoxin may be measured using sensitive toxin neutralization techniques. Thus, rabbit skin tests were employed by us to accurately measure diphtheria antitoxin in immune sera from human subjects. It was found that when calibrated sera were passively transformed into Schick positive subjects, some antitoxins present in amounts equivalent to the Schick test dose could convert local skin areas to the Schick negative state for seven days or more. Toxic reactions caused by Schick reagent at control sites were used for comparison with test areas. Variations in this property, and associations with other immunological characteristics of antitoxin will be discussed.