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PRESIDENTIAL ADDRESS

AN INQUIRY INTO THE MEANING OF CLINICAL INVESTIGATION

BY EUGENE B. FERRIS, JR.

Having approached emeritus status in the Society and being Editor of its journal for the past four years, it has not been possible to avoid considerable thought about the meaning of Clinical Investigation.

Frankly speaking, this inquiry has not led to sufficient insight for me to circumscribe its perimeters or to identify its characteristics from those of other sciences. The query has, however, raised some thoughts which I would like to share with you, since we are a society for Clinical Investigation and should have such awareness of its meaning as is consistent with its rapid and inevitable change in character.

I naturally went back to the origin of this Society and its constitution. The objects of the Society, as set down in the constitution, were most desirable but yet were not defined sufficiently for my purpose—so I perused the address of the first President, Dr. Meltzer (1).

It appears that in the beginning the founders themselves may not have been too clear as to what Clinical Investigation would turn out to be, but rather desired to encourage American physicians to make any kinds of objective and orderly observation on patients.

The first President seemed most concerned with a gangrenous disease of clinical medicine which manifested itself through a suicidal process by which each time a scientific growth developed in the field, it was extruded, like a foreign body, to become a special science—leaving clinical medicine to maintain a certain peace of mind, freed from the fetters of maturation into a science. It was by such a process, according to Dr. Meltzer, that the medical sciences, such as anatomy, physiology, biochemistry, etc., broke away.

One stated purpose of the founders was to bring back to clinical medicine those scientific disciplines which it had previously cast out so that one might say that Clinical Investigation represented an endogamous type of marriage between the allied sciences and the art of medicine, for as Dr. Meltzer stated in 1908: "I am of the opinion that clinical medicine as it exists now is made up of two constituents. One part has all the elements of a pure science and ought to be coordinate to the other pure sciences of medicine, and the other part is the real practice of medicine, an applied science which has many elements of an art." It appears that he was partial to the scientific mate of the union for he warned the members, "The constitution does not keep you down exclusively to science but let me tell you; beware of practice. It is a bewitching graveyard in which many a brain has been buried alive, with no other compensation than a guilded tombstone."

I think it can be said that Clinical Investigation has developed far along the pathways envisioned by the founders of this Society. In fact from some quarters one hears that the content of our program and that of our Journal is investigation all right, but bears only a slight resemblance to anything clinical. Perhaps it may be that Clinical Investigation has become somewhat Frankensteinian to some adherents of practice and perhaps also to some adherents of the "pure" sciences of medicine in the light of the competition which Clinical Investigation offers to the latter. For it now brings to bear in its study of man not only the disciplines of the biological sciences but those of the natural sciences as well and is beginning to encompass the social sciences.

It is of interest that the constitution requires that Society members have the M.D. degree and this requirement may have some symbolic connotation in defining, in a narrow sense, the peculiar differentiation of clinical from other forms of investigation, namely, the investigation of man as opposed to other forms of matter. Man, in health and disease and all things that affect his health, would seem to be the perimeter of the field. In order to study man, the clinical investigator must have both the training and the legal prerogative to do so in a responsible manner.

By and large Clinical Investigation has tended to apply all forms of investigative techniques and disciplines derived from other sciences to the study of man. It has been slow in developing that part which sets it aside from other fields of investigation, namely, the differentiating characteristics of man himself.

As a matter of fact, man is anathema to a very widespread principle of science, namely, the need to control in order to study. Man has been resisting control and seeking freedom throughout the ages, so that it is no wonder that he—and this includes the observer and the observed—has resisted the application of accepted scientific approaches. This factor has been ignored by many clinical investigators and deliberately bypassed by many others through the use of orderly experimental designs and statistical approaches, and through the correct use of the placebo—methods which can, and sometimes do, get the answer in spite of the fact that man is man.

The ability to investigate the more intricate workings of man requires some recognition of the facts that man resists being controlled, that he perceives through other

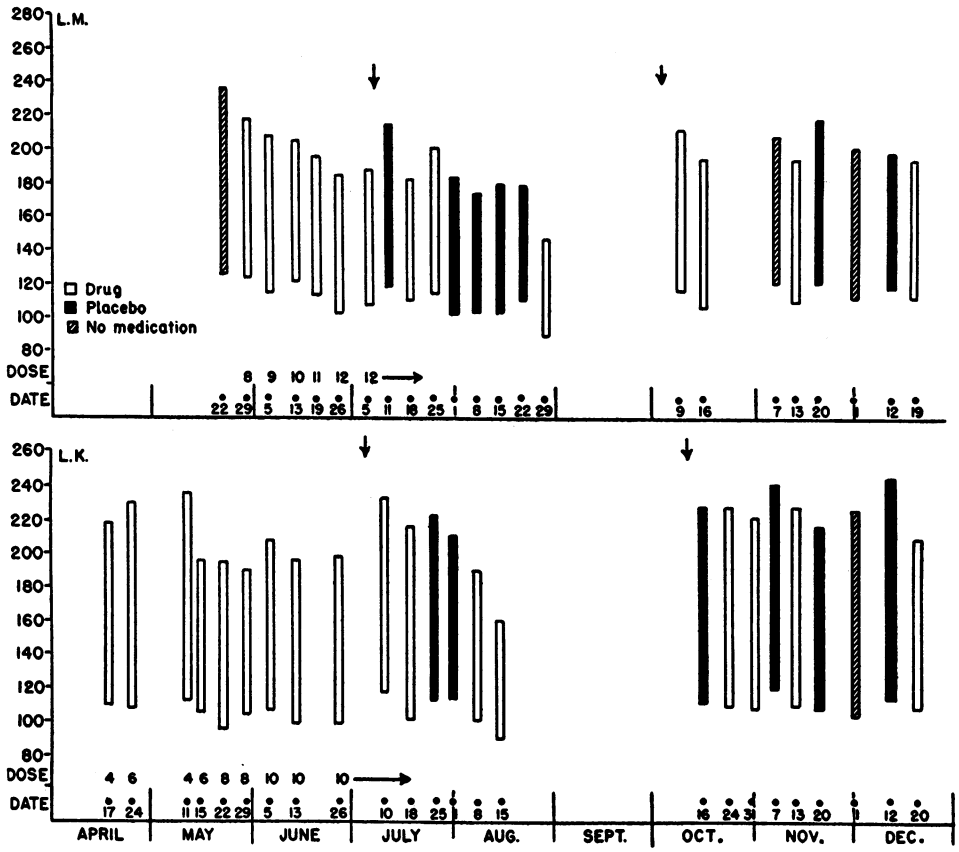

FIG. 1. THE EFFECT OF A DEPRESSOR DRUG ON THE STABILIZED RESTING BLOOD PRESSURE OF TWO PATIENTS HAVING ESSENTIAL HYPERTENSION

The dosage was built up to the indicated maximum and continued as drugs or placebo throughout the period of observation. Abscissa: BP in mm Hg; Ordinate: Dates as indicated. Bars represent systolic and diastolic limits of arterial pressure obtained by the auscultatory method.

than the five senses, that he communicates through the spoken and unspoken word, and that the investigator is just as human as the subject.

Thus we set up such conditions as the "basal state," "bed rest," etc., and may forget that people like bed rest or other forms of control to varying degrees. Some can relax without breakfast and others get angry at being denied it, so that controls may be more or less stressful depending on one's attitude toward them. It is known of course that feeling states, such as anxiety, can profoundly affect physiological homeostasis and that the placebo may be recognized by non-verbal communication.

The clinical investigator himself, through a need to control or dominate the patient, through his reaction to control or discipline of himself, and through his reaction to the success or failure of the patient to respond, may likewise affect the experimental result quite independent of his overt motives and desires or conscious integrity.

The following data from a group of studies to be subsequently reported serve as an example of the challenge to and special identity of Clinical Investigation as exemplified by the interaction of man, the investigator, with man, the investigated, and the intricacies which result therefrom.

Here are two women (Figure 1) in whom the depressor effects of a drug are being evaluated by one investigator-physician. As the dosage is raised to the point of optimum tolerance one notes a fall in blood pressure which is consistent with the hypothesis that it relates to the drug. It could also be related to the enthusiasm of the investigator and we know that, in this instance, there was such a feeling, so that it seemed appropriate to test the hypothesis further by the random substitution of blank pills for the drug. This procedure was acceptable to the investigator but it was more difficult for him to accept the further control that he, like the patient, should not know which was placebo and which was drug. In relation to the initiation of this control (July 9th), one notes an initial rise in blood pressure in the one case during blank administration and in the other case during the drug therapy, followed by a reduction in BP in both as the investigator accepted the new approach.

At this point, it is probable that as compared to the administration of blanks, the depressor effect of the drug

is evident but certainly not world shaking. It is apparent that further observations of the effect of drug and blank are necessary in order to establish the statistical significance of the rather uninspiring depressor effect of the drug.

During September several phenomena occurred in the investigator which had in common a lessening of enthusiasm in the experiment but which were not known to the patients: (*1*) there was a lessened enthusiasm for the therapeutic efficacy of the drug and a resistance to continuing the experiment, (*2*) the investigator volunteered in the armed services for future assignment, and (*3*) he married and took a two weeks' vacation.

The experiment was then continued as planned, and it is quite evident that the blood pressure thereafter returned to approximate pre-treatment levels in which the drug itself still exerted perhaps a slight depressor effect. It seems quite possible that the patients may have sensed and responded to a change in the experimental atmosphere despite continued administration of pills.

This phenomenon of the interaction of the observer and the observed and the variable response of both to rigidly conceived methods of control, represents one phase of investigation which, if not peculiar to, is certainly a delineating characteristic of Clinical Investigation.

Any dynamic system is altered to some extent by the impact of a direct measurement, but man, particularly, responds also to observational techniques in which neither physical contact nor direct transfer of energy occurs. This latter response is inherent in Clinical Investigation. Though it cannot be avoided, other responses can be isolated from it by a sophisticated experimental design which recognizes that such an interaction is continuous between the observer and the observed, and that to the subject the Clinical Investigator is always cast in the mold of a physician.

As the intricacies of our techniques require more attention to fewer subjects, this interaction may become a dominant factor in the response. It thus becomes imperative that controls be applied in the positive sense of observing the truth rather than the negative sense of domination.

Clinical Investigation, as elusive as it is, has, I suspect, surpassed the fondest expectation of the founders when they started this Society 44 years ago. When it systematizes man's total behavior and weaves it into the galaxy of other scientific disciplines which it has applied so successfully to the study of man, there may be little to distinguish the art from the science of medicine.

REFERENCE

1. Austin, J. H., A brief sketch of the history of the American Society for Clinical Investigation. J. Clin. Invest., 1949, **28**, 401.

ABSTRACTS

The Effect of Cortisone on Experimental Brucellosis. ROBERT ABERNATHY, Minneapolis, Minn. (Introduced by Wesley W. Spink).

The basis of these studies was an attempt to resolve the problem of therapy in chronic brucellosis. The granuloma is the fundamental tissue response in brucellosis, simulating that found in tuberculosis. Brucella localize intracellularly, where the organisms survive and probably multiply. There is evidence that intracellular brucella are protected against the lethal action of antibiotics. Since the lesion of tuberculosis is altered by cortisone and tubercle bacilli disseminated, it was of importance to ascertain if a similar sequence took place in brucellosis.

Mice, guinea pigs and rabbits were infected with *Brucella abortus, Brucella mellitensis* or *Brucella suis.* Appropriate groups of animals with acute and chronic disease were treated with varying doses of cortisone administered once daily for 5 to 14 days. Serologic, cultural and histopathologic studies were carried out. Cortisone did alter the clinical course and tissue reaction of the infected animals. There was an increased death rate and the lesions were more widely distributed following the use of cortisone. The hepatic granuloma was converted into a necrotic lesion by cortisone therapy, which was most pronounced in infections due to *Brucella suis.* The agglutinin response was not influenced by cortisone. A discussion of the results will be accompanied by photomicrographs of the lesions.

Interrelations of Serum Lipids in Patients Treated with Cortisone and Pituitary Adrenocorticotropic Hormone (ACTH). DAVID ADLERSBERG, STANLEY R. DRACHMAN, LOUIS E. SCHAEFER and RHODA DRITCH, New York, N. Y. (Introduced by Ernst P. Boas).

Previous studies revealed well defined changes in the serum lipid partition of patients treated with cortisone or ACTH. These patients received hormonal therapy for a variety of pathological conditions, including disseminated lupus erythematosus, polyarteritis, rheumatoid arthritis, asthma, leukemia, scleroderma. Serial determinations included total and esterified serum cholesterol (Sperry-Schoenheimer method), serum phospholipid (Sperry modification of the Fiske-Subbarow method), serum total and neutral fat (Bloor method). The changes in the serum lipids may be, in general, summarized as a rise in the level of total and esterified serum cholesterol, often resulting in sustained hypercholesteremia with prolonged therapy; a gradual elevation in serum phospholipid roughly paralleling that of total serum cholesterol; a diphasic effect of serum neutral fat characterized by a transient and often precipitous fall during the first 2–3 weeks of treatment and later a gradual elevation above pre-treatment levels. Turbidity of "fasting" sera was observed, often at normal levels of serum neutral fat, probably indicating changes in the relative concentrations of serum lipid fractions or a change in the ratio of free and conjugated lipids (lipoproteins).

A study of the interrelationship of the serum lipid components appeared to be profitable. It included the following three ratios: Free/total cholesterol; total cholesterol/phospholipid; free/total cholesterol: phospholipid. Although the ratios noted before, during and after hormonal therapy fell as a rule within the normal range, there was a consistent alteration noted. A definite lowering of the free/total serum cholesterol ratio was observed in almost every instance. There was a less marked rise in the total cholesterol/phospholipid ratio, largely due to a disproportion in the elevation of total serum cholesterol to that of serum phospholipid. The ratio of free/total cholesterol: phospholipid revealed a marked and consistent reduction. It is suggested that a change in the rate of esterification of cholesterol and phospholipid synthesis may account for the observed alterations of the interrelationship of serum lipids. The significance of these observations will be discussed.

A Quantitative Approach to Separation of Lipid Mixtures. EDWARD H. AHRENS, JR. and LYMAN C. CRAIG, New York, N. Y. (Introduced by Thomas M. Rivers).

Clinical investigations of lipid metabolism are hampered by lack of fundamental studies of chemical structure and physical properties of pure lipid compounds. Indeed, the incompleteness of basic data gives rise to many of the uncertainties of the methodology of the clinical laboratory. By their nature these methods can afford only approximate results: they are non-specific; calculations based on factors derived from studies of lipids in normal states may not be valid in abnormal states; certain lipid components can be estimated only by difference; the appearance of unusual compounds in disease may be completely overlooked. Primary difficulties in the organic chemistry laboratory have been produced by the strong association tendencies which hinder complete extraction of lipids and their subsequent separation into individual pure components. Attainment of single pure compounds is, however, essential to progress in this field. The present report will demonstrate that these forces can be overcome and lipid mixtures successfully separated by countercurrent distribution between immiscible solvents.

The countercurrent approach has made possible the quantitative separation of 5 grams of mixed lipids from one liter of human plasma into 3 main groups: phospholipids, cholesterol, and triglycerides plus cholesterol esters. The opportunity to characterize the composition of these

cleanly separated groups and to enumerate and define their components is now presented for the first time. Fractionation of the third group is currently in progress, and will be followed by quantitation of the hydrolyzed fatty acids by distribution in another solvent system, an effective procedure developed as part of the present study. The initial group separation also demonstrated the presence of less than 25 mgm. of free fatty acids in the original mixture.

Experience with a wide variety of lipids permits the conclusion that countercurrent distribution, which is simultaneously analytical and preparative, presents the possibility of all-inclusive analysis of extremely complex mixtures of lipids.

Pseudohypoprothrombinemia and Parahemophilia: Deficiency of Different Components Essential for Rapid Prothrombin Conversion. BENJAMIN ALEXANDER,* ROBERT GOLDSTEIN and GRETA LANDWEHR, Boston, Mass.

Recent recognition of non-prothrombin factors important in prothrombin conversion has permitted more precise delineation of the defect in certain patients who formerly would have been erroneously diagnosed as hypoprothrombinemia. Exhaustive study of these cases has disclosed hitherto unknown clotting components. The present observations indicate another plasma factor besides those already described. Its deficiency (or deranged function) causes retardation in the early activation of prothrombin despite normal prothrombin, Ac-globulin, Labile Factor and Factor V.

Two patients were studied with hemorrhagic diathesis and persistently elevated prothrombin times (70 and 60 seconds respectively) yet different coagulation defects. In the first, a 4 year old girl with hemorrhagic phenomena since birth, the clotting time, prothrombin consumption, plasma prothrombin, Ac-globulin, Labile Factor, and Factor V were normal, but serum prothrombin conversion accelerator (spca) elaboration was poor. Normal serum or purified spca rectified the abnormality in vitro and vivo. The disorder, hitherto unrecognized, is called "congenital hemorrhagic pseudohypoprothrombinemia."

In the other subject, a girl of 17 with bleeding episodes since 5, the abnormality was identical with Owren's case of parahemophilia. The following features distinguish this patient from the first: clotting time, elevated (42 min.); Factor V, Ac-globulin, Labile Factor activities were absent; prothrombin consumption poor; spca normal; prothrombin conversion retarded; corrected by normal plasma in vitro and vivo; serum and spca ineffective.

These contrasting features demonstrate convincingly the existence of two non-prothrombin plasma constituents important in thrombin evolution: (1) Factor V, which is unequivocally identical with Ac-globulin and Labile Factor; (2) the inert precursor of spca, which is activated during coagulation. Deficiency of either causes pathological bleeding and elevated prothrombin times simulating hypoprothrombinemia. Parahemophilia (Factor V deficiency) is benefited by *plasma*. Pseudohypoprothrombinemia (deficiency of spca precursor) is benefited by *serum*. The relative merits of the one- and two-stage prothrombin methods in distinguishing these disorders are apparent.

Toxoid Immunization in Clostridium Novyii Gas Gangrene. W. A. ALTEMEIER,* C. L. COITH, W. R. CULBERTSON, M. A. LOGAN and A. A. TYTELL, Cincinnati, Ohio.

In previously reported experiments conducted jointly by the Department of Surgery and the Department of Biological Chemistry of the University of Cincinnati, under the auspices of the National Research Council and the United States Army, a Clostridium welchii toxoid was produced which developed a high degree of immunity in animals against a severe form of experimental gas gangrene caused by a highly virulent strain of Clostridium welchii.

Subsequent and unreported studies, under the same auspices, have yielded an effective Clostridium novyii toxoid which gives excellent immunity against severe infections in animals produced by the injections of 10 to 100 minimum lethal doses of virulent Clostridium novyii into closed wounds containing crushed muscle and dirt. Control animals which had not previously received injections of toxoid all died within 72 hours of a fulminating and extensive gas gangrene after the same challenge. Blood titers obtained in humans with this toxoid will also be given.

The unsettled international condition and the prospect of mass casualties predisposing to the occurrence of gas gangrene in the civilian as well as the military populations, makes this subject of timely importance and interest.

The Loss and the Retention of Potassium Which Occur Immediately After Starting and Stopping Adrenocorticotropic Hormone, Respectively. FREDERIC C. BARTTER and PAUL FOURMAN, Boston, Mass. (Introduced by Fuller Albright).

The most constant metabolic changes which occur with adrenocorticotropic hormone (ACTH) administration in man are the transient K diuresis and retention which appear within 24 hours of starting and within 48 hours of stopping therapy, respectively. The present studies were undertaken to explore the mechanism underlying these changes.

The following points have been established: 1) The K changes are not due to contaminating antidiuretic hormone, since pitressin alone will not reproduce them; and they do not occur when the same preparations of ACTH are given to patients with unresponsive adrenals (adrenogenital syndrome). 2) The K loss is not due to catabolism, nor the K retention to anabolism of protoplasm, since the changes of K balance precede, and are relatively much greater than, those of N balance; furthermore, testosterone administered together with ACTH can prevent the changes of N but not those of K balance. 3) The K loss is not due to loss of normal intracellular fluid, since the loss precedes, and is relatively greater than, the loss of P. 4) The K retention is not a result of a non-

* Member.

specific, ACTH-induced K deficit alone, since it is not diminished by an increase in K intake before stopping ACTH. 5) The K changes are probably not secondary to reciprocal Na changes at the cellular level, since they are not modified by changes in the Na intake. 6) The urinary K loss precedes retention of Na and loss of P, electrical neutrality being achieved largely by a decrease of urinary acidity, an increase of urinary bicarbonate, or both. The urinary K retention precedes loss of Na, electrical neutrality being achieved largely by decreases of Cl and P.

Significant Remissions in Rheumatic Diseases with High Dosage Cortisone Therapy. THEODORE B. BAYLES, FRANCIS L. COLPOYS, PAUL FREMONT-SMITH, BRUCE C. FERGUSON and EMIL PAIGE, Boston, Mass. (Introduced by Eugene C. Eppinger).

Standard dosage ACTH and Cortisone therapy in rheumatic diseases has failed to produce remissions after cessation of therapy. Ninety per cent of 135 patients so treated by us have relapsed within ten days after treatment. The experience of others is similar. Nine of our patients developing marked physiologic effects on ACTH therapy have experienced prolonged remissions. Accordingly, we have treated 12 patients with high dosage Cortisone, including seven cases of rheumatoid arthritis, one of rheumatoid spondylitis, one of juvenile rheumatoid arthritis, two of disseminated lupus erythematosus, and one of smoldering rheumatic fever with carditis. Previous standard dosage of ACTH or Cortisone in four of these patients had failed to produce lasting improvement.

Five hundred milligrams of Cortisone were administered intramuscularly daily for 14–28 day periods to the eleven adults, the juvenile rheumatoid arthritis patient receiving 300 milligrams daily. Clinical and laboratory improvement was noted in all cases. Transient psychotic episodes in two patients and marked fluid retention in a third were the only serious effects noted. These did not persist.

The first five consecutive patients treated have been followed for 74–88 days after threapy and all are in good clinical remission. In all cases, no evidence of continuing hormonal activity by the usual clinical and laboratory criteria and no evidence of continuing adrenal suppression could be demonstrated during the period of clinical remission after therapy.

Four patients have been followed for 13–32 day periods following treatment and are in good clinical remission. Three patients have just concluded therapy.

The prolonged remission in the first five patients and the shorter but definite remission in the next four so treated is highly significant in view of the previous high relapse rate on standard dosage. High dosage therapy can be administered with safety and deserves further investigation.

Clinical Observations on the Effects of Adenylic Acid and Cozymase. WILLIAM B. BEAN,* Iowa City, Iowa.

Various adenine derivatives, including yeast- and muscle-adenylic acid and cozymase, have been injected intravenously into three classes of subjects: 1) convalescent hospitalized patients without cardiovascular disease, 2) patients who had experienced painful myocardial infarction in the past, and 3) patients with acute pellagrous glossitis. Control electrocardiograms were taken, using lead 2, and a continuous 2-minute tracing during and following injection with short runs 3, 5 and 10 minutes after injection was begun. All subjects experienced hyperpnea, a feeling of warmth throughout the body and occasionally abdominal pain and hyper-peristalsis. These usually disappeared within five minutes. Electrocardiographic changes were confined to T-waves and mechanism disturbance. Ninety-five per cent of patients had tachycardia after adenylic acid and about three-quarters showed T-wave changes. Similar changes occurred after cozymase was injected but they were not as common nor as extensive. Some patients who had had painful myocardial infarction in the past experienced sensations after adenylic acid resembling those they had with acute myocardial infarction.

Intravenous cozymase in doses of 100–300 mg. was found to produce a rapid improvement in pellagrous glossitis. This did not occur with yeast- or muscle-adenylic acid.

Studies on Acquired Resistance to the Shwartzman Phenomenon. IVAN L. BENNETT, JR. and LEIGHTON E. CLUFF, Durham, N. C. (Introduced by Julian M. Ruffin).

It has been amply demonstrated that with repeated injections of the products of Gram-negative bacteria animals and human subjects rapidly acquire a tolerance for many of the toxic effects of these materials (fever, leukopenia, hypotension). This tolerance is of short duration, is not passively transferred by serum, and is non-specific in that tolerance to the "endotoxin" of one species applies also to similar substances from heterologous organisms. Many of these bacterial products are highly active in the production of the Shwartzman phenomenon. If the Shwartzman reaction is elicited repeatedly in rabbits, there rapidly develops a state of non-reactivity.

The experiments reported here were designed to determine: 1) if the production of typical skin reactions was necessary for the development of non-reactivity, 2) if incomplete reactions produced by giving only repeated skin or intravenous injections caused resistance, and 3) if thorotrast injection eliminated tolerance.

The materials used for the reaction were meningococcus filtrate or Prodigiosus polysaccharide (Shear).

Rabbits made resistant to typhoid vaccine consistently develop a state of non-reactivity to the Shwartzman phenomenon which disappears in 3–5 weeks if daily injections are discontinued. This material, in the doses employed, does not elicit the Shwartzman reaction.

Groups of animals were treated simultaneously as follows: A) given repeated Shwartzman reactions at 2 day intervals, B) receiving intravenous injections only, C) receiving skin preparatory injections only.

Animals in A developed complete resistance after 2–4 reactions of diminishing intensity. At this time, B and C

animals uniformly showed complete tolerance to the reaction also. All animals exhibited a return of reactivity in 3–5 weeks.

Thorotrast injection promptly abolished tolerance in groups A, B, and C and also in animals treated with typhoid vaccine.

Studies on Adrenocortical Eosinopenia. WILLIAM R. BEST, ROBERT C. MUEHRCKE and ROBERT M. KARK,* Chicago, Ill.

We have described elsewhere the statistical errors and physiological variations in eosinophil counts of the circulating blood, and have demonstrated the importance of these factors in evaluating serial eosinophil counts. The present study is a clinical and statistical analysis of repeated four-hour eosinophil response tests to ACTH (Thorn test), epinephrine, ephedrine, and placebos in 135 healthy individuals and patients. The latter were ill with Addison's disease, malnutrition, cachexia, anorexia nervosa, proven pituitary or hypothalamic lesions, or other conditions. Several subjects were tested before and after splenectomy.

The majority show some eosinopenia following treatment with placebos (20% mean fall). A significantly greater eosinopenia (31% mean fall) is noted following ephedrine. The response to ephedrine is often much less than to ACTH in conditions bearing no known relationship to the pituitary-adrenal axis. On the other hand, significant eosinopenia was seen in patients with demonstrable lesions of the pituitary or hypothalamus.

The degree of response is independent of the initial eosinophil level. More consistency on repeated tests is noted in subjects with high initial counts and marked eosinopenic responses than in those with initial counts under 150/cmm. or with slight eosinopenic response.

From these studies we conclude: (1) Single tests with ACTH or ephedrine which fail to show significant eosinopenia do not establish the diagnosis of Addison's disease; (2) Marked eosinopenia indicates adequate adrenocortical function; (3) Repeated failure of response to epinephrine or ephedrine cannot be construed as indicating adrenal, pituitary, or hypothalamic disease; (4) Repeated failure of ACTH to produce eosinopenia strongly suggests nonfunctioning adrenals; (5) The level of response to epinephrine or ephedrine does not give accurate information regarding the integrity and functional activity of the pituitary-adrenal axis, and (6) The spleen does not play a part in the mechanism of adrenocortical eosinopenia.

Observations on Survival of Mice After Exposure to Lethal Doses of X Radiation. W. F. BETHARD and L. O. JACOBSON,* Chicago, Ill.

The discovery by Jacobson that spleen shielding in otherwise totally irradiated mice resulted in decreased mortality and more prompt hematopoietic recovery has been followed by a diligent search for the probable mechanism of action. Similar protection by cysteine, as shown by Patt, posed the question as to whether or not the two mechanisms were related. As a first step it seemed desirable to determine whether or not simultaneous use of the two methods would afford additive protection and for this an experiment was devised.

Approximate groups of mice (18 animals in each group) were subjected to 1100 r X radiation. Of the non-protected controls, all but one (95%) were dead within 28 days. Of those animals whose spleens were shielded with lead during irradiation, nine (50%) were dead in the 28 day period. A third group received 10 mg. of cysteine HCl intravenously 15 minutes before exposure to X radiation. Within the observation period 15 (83%) had succumbed. Animals of the fourth group received 10 mg. cysteine HCl as above. In addition, the spleen was lead-shielded during exposure to X radiation. Of these mice, only one out of eighteen (5%) died; and that death occurred on the first postoperative day.

Combined cysteine HCl administration and spleen protection yielded additive protection from X radiation over either method used singly. Further work is in progress to determine the LD50/28 days for mice given such protection.

The Influence of pH on Blood Coagulation. FREDERICK S. BIGELOW, Boston, Mass. (Introduced by Henry Jackson, Jr.).

Increase in alkalinity occurs in even briefly stored oxalated normal plasma, and has been found to cause errors in interpretation of certain coagulation tests. Within 2 hours during unstoppered storage at room temperature, the pH of 2 ml. samples rose to 8.4.

The thrombin-time of such stored plasma increased markedly. However, plasma samples, maintained at pH 7.4 by equilibration with mixtures of CO_2 and O_2 or N_2, showed unchanged thrombin-time for 6 hours at room temperature, and for 24 hours when frozen and prothrombin-free. Reduction of the pH from 8.4 to 7.4 shortened the thrombin-time to equal that of the equilibrated control. Thrombin-times were minimal in the pH range 6.25–8.60, at pH 7.30 in plasmas equilibrated with appropriate gas mixtures, and at pH 7.00 when adjusted by added 0.3N HCl or NaOH. The use of thrombin solutions buffered to pH 7.25 with imidazole failed effectively to control the pH of the reaction mixture.

Significant increases in recalcification-time of plasmas occurred within 1 hour of unstoppered storage. When kept at pH 7.4, there was but slight increase within the first 3 hours, but thereafter large increases occurred despite continued pH control. Systematic examination over pH range 6.25–8.8 revealed minimal recalcification times of 85–92 seconds between pH 6.2–7.5 with gas mixture equilibration and between 6.80–7.50 with HCl or NaOH adjustment.

Increase of the one-stage prothrombin-time occurred within 1 hour of unstoppered storage of mixtures of normal with prothrombin-free plasma. In equilibrated mixtures prothrombin-time was minimal at pH 7.35 and significantly lengthened at pH 8.00. Prothrombin-free plasma, after 72 hours in the frozen state at pH 7.40 did not retard coagulation when used as diluent in the one-stage procedure.

Myocardial Oxidative Metabolism and Initial Fiber Length of the Failing Human Heart. R. J. BING,* W. FALHOLT, R. HEIMBECKER and D. CARROLL, Baltimore, Md.

Oxidative myocardial metabolism and cardiac efficiency were investigated in 80 patients with and without cardiac failure, by means of coronary sinus catheterization and the nitrous oxide method. The diastolic volume of the right ventricle was determined by injecting Evans Blue into the right ventricle through a double lumen catheter and suctioning the dye and blood mixture from the pulmonary artery through an automatic dye recorder. From the slope of the dye dilution curve, the residual and diastolic blood volumes of the right ventricle could be estimated.

In patients with increased diastolic volume but without myocardial failure, as in peripheral A–V fistulas, the myocardial oxygen consumption per unit weight was elevated. In patients with myocardial failure, the myocardial oxygen usage was normal despite large increases in the initial myocardial fiber length. These findings indicate that (a) failure is accompanied by a defective conversion of oxidative energy into useful work and that (b) an increase in initial tension of the heart muscle fiber augments oxidative myocardial metabolism only in the absence of chronic failure.

Cardiac glycosides caused a decrease in the initial fiber length and a rise in the work of the failing human heart. They had no effect on myocardial oxygen consumption. This suggests that cardiac glycosides act by influencing the initial fiber length and by a more effective conversion of oxidative energy into useful work.

Effect of Portacaval Anastomosis on Hepatic Oxygen Extraction and Estimated Hepatic Blood Flow in Patients with Cirrhosis. S. E. BRADLEY,* A. I. MACPHERSON, A. GAMMELTOFT and A. H. BLAKEMORE, New York, N. Y.

Measurements of hepatic oxygen extraction were made in 12 patients with portal hypertension due to cirrhosis before and after portacaval anastomosis. In 10 of these individuals hepatic blood flow (EHBF) could be estimated by the Bromsulfalein (BSP) clearance method. The hepatic arterio-venous oxygen difference increased in all but two. This value ranged from 3.0 to 5.3 vol. per cent preoperatively and increased by 16 to 110 per cent during the post-operative period (14 days to 30 months), ranging from 4.5 to 8.4 vol. per cent. Of the two patients showing a fall in hepatic oxygen extraction, one suffered from hepatic disease of such severity that BSP extraction was less than 10 per cent and the second was studied only 7 days after operation. It is possible that disturbances of hepatic function attributable to anesthesia and operation may have altered the response in the latter. EHBF fell in every case (from 10 to 110 per cent, 940 to 1870 ml. per min. pre-operatively and 530 to 1500 ml. per min. post-operatively). BSP extraction increased markedly in nearly every instance. These changes may be attributed to decreased portal venous inflow following the fall in portal venous pressure; the less rapid perfusion permitting more effective extraction of oxygen and BSP. Apparently an appreciable fraction of the oxygen supply to the liver is provided by the portal venous blood even in the presence of cirrhosis. This phenomenon may afford a means of assessing the potency of the shunt postoperatively.

Blood Pressure Responses to ACTH and Cortisone in Normotensive and Hypertensive Subjects in the Resting State and During Autonomic Blockade with Tetraethylammonium Chloride. ALBERT A. BRUST, WILLIAM RANSOHOFF and MORTON F. REISER,* Cincinnati, Ohio.

Responses of the blood pressure and tetraethylammonium chloride (TEAC)"floor" to ACTH, cortisone, and desoxycorticosterone acetate have been studied. Appropriate metabolic changes were followed.

In six patients (3 hypertensive, 3 normotensive) who received ACTH, the "TEAC floor" rose above control levels within 24 hours after the drug was started and remained elevated throughout. Following discontinuance of the drug, "TEAC floors" fell below control levels. In three of the patients, changing the sodium intake produced additional shifts in the "TEAC floor" similar in direction and magnitude to the shifts induced by the same maneuver in the absence of adrenocortical stimulation.

Cortisone produced a slower but equally striking rise in "TEAC floor" in a normotensive and hypertensive patient, thus excluding pituitary pressor content of ACTH as the cause for the responses. With both drugs the usual depressor effects of TEAC were ultimately converted to a pressor rise, suggesting that autonomic blockade potentiates the vascular effects of ACTH and cortisone. DOCA did not produce a significant change in "TEAC floor" in a normotensive patient who responded dramatically to both ACTH and cortisone, ruling out DOCA-like action of cortisone in producing the observed vascular responses.

Resting blood pressures rose with both drugs, but the rise was less striking than that of the "TEAC floor." Blood pressure elevations were more prominent in normotensive than in hypertensive subjects.

The data suggest that: 1) Administration of ACTH or cortisone significantly alters the blood pressure and "TEAC floor." The responses are independent of sodium retention and indicate that the vascular effects of these drugs are mediated by a humoral mechanism which is potentiated by autonomic blockade. 2) The "TEAC floor" appears to be a sensitive indicator of adrenocortical activity as induced by ACTH and substituted for by cortisone. The responses precede eosinophil depression and other metabolic effects.

Elimination of Intravenous Carbon^{14} Bicarbonate by Man. DONALD L. BUCHANAN, Chicago, Ill. (Introduced by Austin M. Brues).

A body of data has been obtained concerning the excretion rates and tissue retention of inorganic carbon^{14} administered to rats and mice. The present study compares

the human excretion of injected $NaHC^{14}O_3$ to rates observed in smaller animals in order to provide a basis for the computation of permissible human doses of this isotopic compound. In this work 10 microcuries of material was injected intravenously into healthy human volunteers and all expired CO_2 was collected in fractions during the following 3 hours. With the subject at rest approximately 75 per cent of the radioactivity is exhaled as CO_2 during the first hour; 90 per cent is gone in 2 hours; and 95 per cent has been blown off at the end of the third hour.

Within the error of the methods used the rate of isotope excretion by different sized animals is proportional to the metabolic rate per unit mass of animal. When published data of others are included this relationship seems valid for animals as large as the cow (Kleiber) and as small as the mouse. On this basis the extrapolation to man of information obtained in long term experiments with rats and mice indicates that an intravenous injection of 20–40 millicuries of $NaHC^{14}O_3$ or the inhalation of a comparable amount of $C^{14}O_2$ would be required to give a 70 kg man a total radiation dose of 1 roentgen equivalent over his entire life span.

The Determination of Traces of Calcium and Magnesium in Plasma. EDWARD S. BUCKLEY, JR. and THERESA R. BORTOLOTTI, Boston, Mass. (Introduced by John G. Gibson, 2nd).

Currently available techniques are not sufficiently sensitive to assay calcium in plasma following its exposure to a cation exchange resin. No technique for the direct assay of plasma magnesium has been found satisfactory. Analytic techniques based on the principles outlined by Schwarzenbach have been developed. Murexide ammonium purpureate reacts with plasma calcium at pH values greater than 12.5 to give a red solution. Eriochromschwarz-T reacts with plasma magnesium at a pH of 10.8 forming a red color. Neither color obeys Beer's law and the murexide fades. If sufficient ethylene-diamine-tetra-acetate is added to either solution the metal-free color is obtained (purple for the murexide and green for the Eriochromschwarz). Ethylene-diamine-tetra-acetate reacts mole for mole with calcium and magnesium at these pH values, chelating all of the calcium before the magnesium. The calcium concentration can thus be computed from the murexide titration and the sum of the calcium and magnesium concentrations from the Eriochromschwarz titration. Potassium cyanide is included in the system to prevent interference from iron, zinc, and copper.

Using these techniques, plasma calcium concentrations ranging from 6.0 to 0.2 milli-equivalents per liter and magnesium concentrations from 2.5 to 0.10 milli-equivalents per liter have been measured.

Combined Antimicrobial Action Upon Penicillin Resistant Organisms In Vitro. PAUL A. BUNN * and LEONARD CANARILI, Syracuse, N. Y.

Two micro-organisms recovered from patients with subacute bacterial endocarditis have been tested *in vitro* with a variety of antibacterial substances, singly and in combination. Both organisms were resistant to penicillin, 3 and 5 units penicillin respectively, and effective concentrations of streptomycin against each were 10 units. As they were difficult of eradication *in vivo*, studies were designed to demonstrate an interfering, or a synergistic action among the combination of other agents.

The two organisms, alpha hemolytic streptococcus and group D enterococcus, were tested in various dilutions and combinations of penicillin, streptomycin, terramycin, aureomycin, and chloramphenicol. Each organism was exposed to concentrations of at least two agents equal to, higher than and less than the measured sensitivity levels. Observations upon growth of organisms were noted at periodic intervals for 72 hours.

True interference of action of one antimicrobial agent against another was not observed. Synergism of high order occurred only with use of penicillin and streptomycin, each at levels of measured sensitivity. Reduced activity was noted with streptomycin at effective and penicillin at less than effective concentrations. When both were added in sub-effective levels, antibacterial action was less than when adequate amounts of either alone were used. With all other agents in combination with penicillin, neither an additive nor a synergistic effect was noted. Adequate and higher than adequate concentrations of each, when combined with the effective amounts of penicillin, resulted in more growth than when penicillin was used alone. Growth curves after aureomycin, chloramphenicol or terramycin with penicillin were identical and corresponded with those seen after sub-effective amounts of penicillin or streptomycin used singly. Use of three agents together did not reveal differences from that observed with two. Presence of penicillin always enhanced activity of aureomycin, chloramphenicol and terramycin.

Studies on Amethopterin, Citrovorum Factor, and Crude X-Methyl Folic Acid in Leukemia. JOSEPH H. BURCHENAL * and E. M. KINGSLEY-PILLERS, New York, N. Y.

Amethopterin (4-amino-N^{10}-methyl pteroylglutamic acid) is chemotherapeutically active against several strains of mouse leukemia. From one of these sensitive parent strains of leukemia (AK4) a resistant mutant (AK4R) has been developed by successive passage through treated mice. This resistance is not due to a relatively increased content of folic acid (F.A.) or citrovorum factor (C.F.) in the AK4R cell, nor is it due to decreased adsorption or absorption of amethopterin by the resistant cell. AK4R spleen slices, incubated with amethopterin, show no increased ability to detoxify the drug. AK4R shows cross resistance to all 4-amino derivatives of F.A. tested, but not to 9-methyl folic, crude X-methyl folic, or 2,6 diaminopurine. The most likely explanations for this resistance would seem to be the development of an alternate metabolic pathway bypassing C.F. or a qualitative change in the enzyme for which both C.F. and amethopterin are

competing so that there is a decrease in the affinity for amethopterin.

Since these data suggest that the crude antagonist of folic acid might be effective in patients that have become resistant to amethopterin, and that the folic-citrovorum-amethopterin relationship may be altered in the development of resistance, clinical studies on the effects of the crude antagonist and on the effects of massive doses of amethopterin buffered by C.F. have been undertaken.

In one patient who, on 2.5 mg. of amethopterin daily for eight days, developed toxic mouth ulcerations and leukopenia, doses of 45–60 mg. daily for twenty-one days were tolerated when covered by simultaneous parenteral administration of 3 mg. of C.F. No more beneficial effects were noted on the leukemia than from 2.5 mg. of amethopterin alone. Titrations of the competitive citrovorum-amethopterin antagonism and the effects of crude antagonist in patients will be reported.

Studies on the Nature of the Adrenal Cortical Secretion in Patients with Malignancy. ROBERT B. BURTON, ALEJANDRO ZAFFARONI, ANTHONY J. IZZO and E. HENRY KEUTMANN, Rochester, N. Y. (Introduced by Lawrence E. Young).

The analysis of urine extracts by paper chromatography is so designed that each compound present having the chemical characteristics of the known corticoids can be isolated and its quantity estimated.

Study of the free urinary corticoids in patients with leukemia or lymphomas showed that with ACTH stimulation the steroid showing the greatest increase was Compound F. The excretion of this hormone rose ten to sixty times that seen in the pre-ACTH period. While Compound E was present in amounts equal to, or slightly greater than, Compound F in the pre-ACTH period, its concentration rose only three to ten times under therapy. The same response held true for a third steroid component whose identity will be discussed.

Untreated but well nourished patients with malignancy showed no significant difference from normal controls with regard to excretion of these major components. In cachectic patients with malignant disease the excretion of all components was moderately reduced.

In both normal subjects and in patients treated with ACTH a much larger quantity of each corticoid was excreted as glucuronide conjugate than as free corticoid.

Studies on the High Output Cardiac Failure of Occidental Beriberi. JAMES A. CAMPBELL, LOUIS A. SELVERSTONE and DANIEL L. DONOVAN, Chicago, Ill. (Introduced by S. Howard Armstrong, Jr.).

To study high output cardiac failure and to compare it with failure of the low output type, hemodynamic measurements were obtained on 2 patients in cardiac decompensation with biochemically proved beriberi. The patients were young alcoholics without underlying disease who entered with cardiac enlargement, pulmonary congestion, peripheral edema, glossitis, and peripheral neuritis. Avitaminosis was demonstrated by thiamine excretion and metabolic load tests.

Prior to therapy these patients showed elevated resting cardiac indices of 4.3 and 7.2 L/min/M^2, which remained fixed during mild exercise. During this stress the stroke volumes fell, and pulmonary artery pressures rose markedly. These responses parallel from a high initial level those seen in a series of patients with low output failure, and indicate cardiac incompetence.

Following effective therapy with clinical restoration of cardiac competence, the resting cardiac indices in these patients dropped to 2.5 and 2.7 L/min/M^2 respectively with concomitant decreases in resting stroke volumes and pulmonary artery pressures. However, during exercise they were then able to increase their cardiac outputs and stroke volumes appreciably, while pulmonary artery pressures rose only moderately. Their responses had become similar to those seen in normal subjects studied in this laboratory. In one patient, coronary sinus catheterization was also performed before and after therapy, and blood oxygen, glucose, lactic acid, and pyruvic acid levels were measured.

Our data again show that high cardiac outputs occur in beriberi heart failure and decrease toward normal with the patient's clinical improvement. During decompensation the high resting values appear to be maximal; recovery is characterized by renewed ability to increase cardiac output during stress. The analogy with the hemodynamics of low output failure is drawn.

Effects of Paritol on the Prothrombin Times, Anti-Thrombin Times, and Lee-White Clotting Times. DON W. CHAPMAN and ALAN A. ORY, Houston, Texas (Introduced by James A. Green).

Observations as to the effect of Paritol C on antithrombin and prothrombin activity and clotting times were made, in addition to antidote studies.

In 23 dogs, Paritol, administered intravenously in doses of 3, 5, and 7 mg/kg, produced a peak effect in 15 to 30 minutes with a prolongation of clotting times 2–3 times the normal value for 4–5 hours, and prothrombin time was notably prolonged. Protamine sulphate effects a prompt return of the clotting time to normal in a dose of 5 mg/kg. Toluidine blue was found to be unreliable.

Twenty-three patients, including 9 with acute myocardial infarctions, 4 with acute thrombophlebitis, 3 with arteriosclerotic heart disease, and 7 normals, have been treated with Paritol C administered intravenously for 24 to 72 hours, with an average of 50 hours without any demonstrable toxic effects or developments of clinical thrombosis or emboli.

Patients initially received a dose of 5 mg/kg of Paritol C intravenously and 3–5 mg/kg when the clotting time fell below 20 minutes. The clotting times were effectively prolonged to 10 hours with extremes of 6½ to 18 hours. This onset of action is within 10–15 minutes after its administration. Antithrombin activity was greatly increased immediately following the administration of Paritol C and fairly closely paralleled the clotting times. Di-

cumarol alone had no effect on the antithrombin activity in 14 patients. Serial prothrombin level determinations revealed an almost immediate prolongation in 8 of 12 cases tested with a maximum peak in 3–4 hours. In 2 the prothrombin levels dropped from 90 to 10 percent of normal without dicumarol.

When dicumarol and Paritol C are administered in combination, the antithrombin activity may enable one to differentiate their relative effect on the prothrombin time. When the prothrombin time is prolonged in the presence of increased antithrombin activity, Paritol C, in our opinion, may be partially responsible for its prolongation.

Because Paritol is an immediately acting anticoagulant of relatively low toxicity which has a more prolonged action than heparin, and is potentially economically more available, it may prove to be superior to heparin.

Effect of Sub-total Adrenalectomy on Renal Hemodynamics and Electrolyte Excretion in Human Hypertension. JOHN KAPP CLARK, ARCHER P. CROSLEY, JR. and HAROLD G. BARKER, Philadelphia, Pa. (Introduced by Francis C. Wood).

Measurements of renal hemodynamics and electrolyte excretion have been made before and after sub-total adrenalectomy with removal of approximately 90 per cent of adrenal tissue in 6 severely hypertensive patients. The change in glomerular filtration rate (inulin or endogenous creatinine clearance) was small and variable. In 5 of 6 patients effective renal blood flow (para-amino-hippurate (PAH) clearance and hematocrit) increased at a time when mean arterial blood pressure was decreased, thus indicating a reduction in overall renal vascular resistance. Tabular maximal excretory capacity (Tm PAH) was not consistently changed post-operatively. The characteristic pathological functional pattern of the hypertensive kidney consists of increased vascular resistance located chiefly in the efferent arteriole with resultant high filtration fraction and ischemia relative to the mass of perfused tissue. The data suggest that this pattern may tend to be reversed by sub-total adrenalectomy since the high pre-operative filtration fractions fell in all 6 patients and the ratio of effective renal plasma flow to Tm PAH increased in 3 out of 4 patients.

The rates of sodium, potassium and chloride excretion have been measured and correlated with the hemodynamic observations both pre- and post-operatively. The data show no systematic change in electrolyte excretion following surgery. This may indicate that enough adrenal tissue remained to prevent the electrolyte imbalance characteristic of Addison's disease, but the results are so variable, possibly due to changes in diet, rate of diuresis and state of hydration as well as to differences in the amount of gland still functioning that no conclusions can be drawn as to the effect of the operation on electrolyte excretion on the basis of these few cases.

Observations on the Metabolic and Anti-Inflammatory Effects of Desoxycorticosterone, Testosterone and Cortisone in a Patient with Rheumatoid Arthritis. WILLIAM S. CLARK, ELIZABETH L. MANNING and JANET E. APPLETON, Boston, Mass. (Introduced by Walter Bauer).

Mineral and nitrogen balances were studied for 250 days in a 43 year old male with typical rheumatoid arthritis to determine the metabolic and inflammatory effects of cortisone, desoxycorticosterone and testosterone (in doses of 150, 25 and 100 milligrams per day, respectively), singly, together and in binary combinations for periods of 8 days. Sodium, potassium, calcium, chloride, phosphorus and nitrogen were determined. The actual and derived data revealed no metabolic anomalies during control periods. The administration of ACTH resulted in the expected loss of potassium, nitrogen, phosphorus and calcium with sodium and chloride retention. All three steroids resulted in sodium and chloride retention, however administered. All treatment combinations involving testosterone resulted in potassium, nitrogen and phosphorus retention. Desoxycorticosterone and cortisone caused potassium loss which increased when the hormones were administered together. Although cortisone and desoxycorticosterone did not appreciably alter nitrogen and phosphorus excretion, cortisone counteracted the nitrogen and phosphorus-retaining effects of testosterone. Cortisone increased calcium excretion, whereas testosterone decreased it; however, the latter effect was counteracted by cortisone. Desoxycorticosterone increased urinary calcium when it was administered with cortisone or testosterone and cortisone. Desoxycorticosterone prevented the calcium-retaining effect of testosterone. The data add further evidence of the antagonistic effects of testosterone and cortisone on nitrogen, potassium and phosphorus balances. They reveal an effect of desoxycorticosterone on calcium excretion and no antagonism between desoxycorticosterone and cortisone in relation to sodium and chloride excretion. They further suggest that the ACTH calcium diuresis may indicate an electrolyte hormone effect. They also reveal that the anti-inflammatory action of cortisone is not altered by testosterone and/or desoxycorticosterone and that this effect cannot be related to protein and electrolyte metabolism as measured by mineral and nitrogen balances.

Enzymatically Hydrolyzable Formaldehydogenic Corticoids: Normal Values and Observations on Disease States. A. C. CORCORAN,* HARRIET P. DUSTAN and IRVINE H. PAGE, Cleveland, Ohio.

Treatment with beta-glucuronidase has been shown to increase yields of urinary corticoids. The present study deals with development and application of a standardized method for determination of total hydrolyzable formaldehydogenic corticoids (THFC) in normal subjects and in hypertensive disease; it includes observations in adrenal dysfunctions and on the effect of ACTH and cortisone.

Determinations in normal subjects yielded the following values in mg. per 24 hours: Males, mean 23.5 $\sigma \pm 16$; females, 16.6 $\sigma \pm 9.3$. Corticoid determined without enzymatic hydrolysis (FC) shows some correlation with

corticoid glucuronide; the ratio FC/THFC averages 18.3 $\sigma \pm 8.8$.

The mechanism of the wide variations in values is not apparent since such occurs in the same person. However, amounts of THFC are found to increase with urine volume (correlation coefficients, 0.53 in males and 0.71 in females). This is not due to effects of urine on activity of the enzyme. It is therefore attributable to some renal mechanism. This view is supported by the observation that total acid-hydrolyzable urinary glucuronide ("glucuronic acid") shows a similar association.

In essential hypertension we can demonstrate neither a sex difference in mean rates of excretion of THFC nor a correlation with urine volume. THFC values found in these patients were: mean 16 $\sigma \pm 8.9$ in males and 15.3 $\sigma \pm$ 8.5 in females. As previously surmized, the ratio FC/THFC is sometimes very low, because high levels of FC occur in hypertensive disease.

Cortisone (100 mg. daily) does not increase yields of THFC. Preliminary observations in systemic lupus erythematosus in acute relapse, under treatment with massive doses of cortisone or ACTH, indicate that yields of THFC increase as the process comes under control. Destruction of the steroid side chain probably decreases as the disease abates. These analyses may become rational guides to dosage schedules and may prevent hypercorticoidism as a complication of hormonal treatment.

In Vivo *Studies of the Role of the Kidney in Intermediary Metabolism in Man and Dog.* JAMES W. CRAIG, MAX MILLER,* WILLIAM R. DRUCKER, HIRAM WOODWARD, BERNARD L. BROFMAN and WALTER H. PRITCHARD, Cleveland, Ohio.

Renal excretory function has been emphasized in most studies of the normal and diseased kidney. However, there is evidence that this organ also plays a significant role in intermediary metabolism. The following studies of renal metabolic activities were therefore undertaken in man and intact anesthetized dogs.

The concentrations of glucose, pyruvic acid, citric acid, and malic acid were determined in urine and in samples of blood obtained simultaneously from the renal vein (by catheter) and femoral artery. Renal blood flow was measured by PAH extraction, and glomerular filtration rate by inulin (in man) and creatinine (in dog) clearances. Renal balances were calculated by the equation:

$$\text{Renal balance (mgm./min.)} = (A\text{–}V)\ RBF - U$$

where A and V = concentrations (mgm./100 cc.) in femoral artery and renal vein blood respectively.

RBF = Renal blood flow (cc./min.)
U = Urinary excretion (mgm./min.)

In the fasting resting state small positive balances of glucose and citric acid were found.

During glucose infusion the balances of glucose and pyruvic acid became increasingly positive.

During intravenous injection of sodium succinate the balances of pyruvic, citric, and malic acids became significantly negative. Glucose balance did not change. The urinary excretions of pyruvic, citric, and malic acids were increased strikingly. There was a diminution in the percentage of filtered pyruvic acid which was reabsorbed by the renal tubules and a decrease in the net amount of citric acid reabsorbed. Part of the increase in malic acid excretion appeared to be accounted for by renal tubular secretion.

If it is assumed that a positive renal balance of a substance signifies its utilization by the kidney and that a negative balance implies renal formation of the substance, the above results demonstrate that human and dog kidneys *in vivo* have metabolic activities in addition to the recognized excretory functions.

The Hemodynamic Actions of Protoveratrine in Essential Hypertension. CHARLES W. CRUMPTON, CARL K. FRIEDLAND, PETER T. KUO, TRUMAN G. SCHNABEL, JR., ELWOOD L. FOLTZ, ROBERT G. PAGE, JESUS ALANIS and JOSEPH H. HAFKENSCHIEL, Philadelphia, Pa. (Introduced by Calvin F. Kay).

Protoveratrine injected intramuscularly (6 micrograms per kilogram—18 patients) and intravenously (2 micrograms per kilogram—10 patients) decreased blood pressure markedly and produced a bradycardia of from 40 to 60, over a two hour period. Pressures remained low for four hours after intramuscular injection. Unpleasant symptoms were less severe than with "purified" veratrum viride extracts, but there was pain at the injection site. Intravenous dosage lowered blood pressure 30% for at least one hour, with minimal unpleasant symptoms and much less bradycardia. Oral dosage of 0.75 to 1.125 mgm., in 8 patients, induced a significant hypotension in 4, with unpleasant symptoms and complete heart block in 3 of these patients. No significant change in the circulation was observed in the other 4, who received 0.75 mgm.

Ballistocardiographic studies in 3 normal subjects and 2 hypertensive patients, before and after the intravenous dosage, showed an increase in the size of the wave form suggesting an increase in stroke volume, along with hypotension and bradycardia. However, direct recording of central aortic pulse pressure waves under similar conditions did not indicate a significant increase in stroke volume.

Measurement of coronary hemodynamics and oxygen metabolism in anesthetized dogs (2.5 to 3.0 micrograms per kilogram intravenously) suggested a slight decrease in coronary vascular resistance at the time of the hypotension. However, the effects on circulation did not indicate that protoveratrine has a therapeutically desirable action under these conditions.

Cerebral circulation studies in seven hypertensive patients (2 micrograms per kilogram intravenously) showed a significant reduction in cerebral vascular resistance one hour after injection. Cerebral blood flow, cerebral arteriovenous oxygen difference, and cerebral oxygen consumption remained constant.

The Metabolism of I^{131} Labelled Proteins in Rabbits: The Sensitivity of the Antigen Elimination Rate as an Indi-

cator of Antibody Response. GUSTAVE J. DAMMIN,* SAMUEL C. BUKANTZ,* FRANK J. DIXON and DAVID W. TALMAGE, Saint Louis, Mo.

Intravenously injected homologous globulin is eliminated from the blood in two distinct phases, a rapid dilution and a slow constant non-immune phase with a biologic half life (BHL) of 48 hours. The elimination of bovine gamma globulin (BGG) also presents initial dilution and non-immune phases, the latter continuing until the fourth day when a rapid elimination rate (BHL—16 hours) begins and continues until the 7–8th day when less than 0.1 per cent of the injected protein remains. Such tri-phasic elimination curves are obtained with total antigen doses of 1, 75 or 500 mgs. Circulating antibody appears inconstantly following the 1 mg. dose, but is detectable on the 7–8th day following larger doses, persisting until the 20th day. Following complete disappearance of antigen from the blood, there is no significant tissue radioactivity. About 80 per cent of the injected protein-bound I^{131} can be recovered in the urine as non-protein-bound I^{131}, neither antigen nor protein-bound activity appearing in the urine.

Because (1) the rate of the third phase of antigen elimination in the normal rabbit roughly parallels elimination in the sensitized rabbit, (2) the appearance of circulating antibody coincides with the disappearance of circulating antigen, and (3) rapid antigen elimination can be induced with passive antibody, this phase has been identified as an immune phase.

Rabbits injected with 1 mg. of BGG may develop no circulating antibody, but on re-injection exhibit a specific anamnestic response. X-irradiated (250 R) rabbits injected with BGG may show a short rapid third phase and develop no circulating antibody but on re-injection will exhibit a specific anamnestic response. Thus, with minimal antigenic stimulation or partial suppression of the immune response, no circulating antibody appears but the presence of a rapid antigen elimination phase constitutes immediate evidence of an immune response.

Adrenal Cortical Reserve in Severe Hyperthyroidism. WILLIAM H. DAUGHADAY and A. LEWIS FARR, St. Louis, Mo. (Introduced by C. M. MacBryde).

Abnormal adrenocortical function in hyperthyroidism has been suspected by many investigators. We have studied seven patients with severe hyperthyroidism with basal metabolic rates between plus 50 and plus 100 per cent. In each patient eosinophil leukocytes, urinary 17-ketosteroids and urinary formaldehydogenic steroids were measured before and after ACTH. Twenty-five mg. of ACTH were given, then 10 mg. every six hours for 48 hours. The average excretion of formaldehydogenic steroids was 1.9 mg./day (normal 0.5–1.5 mg.) before ACTH and 1.9 mg. and 2.2 mg. on the first and second days of ACTH treatment. The average excretion of 17-ketosteroids was 12.0 mg./day before ACTH and 12.7 and 12.7 mg./day on the 1st and 2nd day of ACTH. Normal rise of 17-ketosteroids on 2nd day of ACTH is 6 mg./day. The average fall in eosinophils four hours after 25 mg. ACTH was 46 per cent. Four patients had less than 40 per cent drop in eosinophils. Normal fall is 76 per cent. Repeated studies of adrenal function were made in 4 other patients with severe hyperthyroidism.

These results suggest: 1) that hyperthyroidism is a stimulus for increased adrenal activity and 2) that decreased adrenal reserve may be present in severe hyperthyroidism as measured by responses to ACTH of eosinophils, ketosteroids, and formaldehydogenic steroids.

Use of Mutants to Reveal a Vitamin (POB) Antagonizing the Chemotherapeutic Action of PABA. BERNARD D. DAVIS,* New York, N. Y.

In contrast to the antagonism of PABA to sulfonamides, the mechanism of antirickettsial chemotherapy by PABA is not understood. This problem was unexpectedly solved during studies on biosynthetic paths in *Escherichia coli.* Certain mutants blocked at an early stage in aromatic synthesis, which grow slowly on a mixture of tyrosine, phenylalanine, tryptophan, and PABA, were found to grow more rapidly on shikimic acid, a precursor of these metabolites. Since growth on the quadruple supplement was accelerated by culture filtrate of the parent wild type strain, we postulated a fifth aromatic metabolite to be derived from shikimic acid. This filtrate factor was identified as p-hydroxybenzoic acid (POB); it is fully active at a concentration (0.01 μg/ml) characteristic of vitamins. Its sulfone analogue (4,4'-dihydroxydiphenyl sulfone) was found to be a competitive inhibitor of *E. coli.* On the basis of structural considerations PABA was similarly tested; it slowed growth of *E. coli* at 150 μg/ml, and was reversed by POB in a 1/200 ratio. Preliminary experiments of J. C. Snyder have shown that POB also antagonizes rickettsiostasis by PABA in animals and chick embryos. Since POB is not present in significant amounts in liver extract, and one analogue of it (PABA) is known to have chemotherapeutic utility, the expectation has been realized that mutants could reveal metabolites peculiar to microorganisms, and hence suitable as models for the synthesis of chemotherapeutic analogues.

Measurement of Pulmonary Pressure. HOWARD G. DAYMAN, Buffalo, N. Y. (Introduced by David K. Miller).

Pulmonary pressure, as previously reported, is the difference between lung tension and pleural pressure, all expressed as cm. HOH pressure. The difficulty is measurement of lung tension. Rate of airflow and pleural pressure are continuously recorded by apparatus of original design in patients having a small pneumothorax space. At the instant of apnea between breaths of varying size, lung tension and pleural pressure are identical, and it is shown that $\frac{\text{Lung Tension}}{\text{Lung Volume}} = \text{K}\ (+4\%)$. From the airflow tracing, change in lung volume and thereby change in lung tension may be computed at $\frac{1}{10}$th second intervals during active respiration. This method of computing lung tension, not previously described, is the only means by which trends in pulmonary pressure may be

determined with any degree of accuracy. Knowing the rate of airflow, change in lung volume, pulmonary pressure, lung tension, and pleural pressure enables one to study the mechanical forces affecting ventilation. Resistance to airflow is of special interest (cm. HOH pressure necessary to produce 1000 cc/sec. airflow).

Limitations of method include: 1) Pulmonary pressure is certainly not uniform throughout the lung and the data thus express trends in pressure rather than absolute measurements. 2) Pneumothorax on one side disturbs pressure relations between the pleural cavities. Evidence is presented to indicate that such pressure difference is appreciable only in a position of extreme expiration. Tests were made, therefore, in the complemental air range so far as possible. 3) Computed resistance to airflow is inexplicably low at the inception (.1–.3 sec.) of both inspiration and expiration. Evidence indicates that this is due to sudden change in the size of dead space. 4) Lung tension/lung volume relationship is abnormal in advanced emphysema and lung tension can be computed only by plotting a curve expressing the relationship and applying that curve to a particular breath. The mechanics of ventilation in emphysema are so abnormal that such inaccuracy as may result from this method of computing lung tension is not serious.

The Behavior of Human Thyroid Tumors Transplanted to the Anterior Chamber of Guinea Pig Eyes. BROWN M. DOBYNS and BEATRICE LENNON, Boston, Mass. (Introduced by Oliver Cope).

The survival and growth of human tumors after transplantation to the anterior chamber of the guinea pig's eye has been regarded as a means of studying the behavior of tumors and diagnosing malignancy. Malignant lesions are said to grow, while benign lesions are absorbed. Twenty-three thyroidal tumors (many malignant or questionably malignant) as well as control tissues have been transplanted into 228 eyes of guinea pigs. Many malignant tumors have survived and grown. Some tumors, regarded as morphologically benign, have survived in the eye for over one year contrary to expectation. The survival but failure of multiplication of these tumor cells raises interesting speculation.

Some very undifferentiated thyroidal tumors which originally had no resemblance to thyroid have, after habitation in the eye, differentiated and acquired a resemblance to normal thyroid.

During a period of four years, one very undifferentiated thyroidal tumor was retransplanted through nine generations and 444 eyes during which time it gave rise to almost every histological pattern of carcinoma found in the human thyroid. These changes may be related to the variable character of some carcinomas growing in man.

Illustrations of the histological pictures serve to document the changes that have been found.

Acquired Hemolytic Anemia as the Presenting Syndrome of Lupus Erythematosus Disseminata. EDMUND L. DUBOIS, Los Angeles, Calif. (Introduced by Paul Starr).

The cause of the hematological abnormalities in disseminated lupus has been as great an enigma as the etiology of the disease; it is the purpose of this paper to present three patients with the previously unreported syndrome of acquired hemolytic anemia including positive Coombs test who subsequently developed the classic features of disseminated lupus. The second aim of the paper is to review the blood changes in this disease with the hope of showing that a causative mechanism is the secondary form of "hypersplenism" and to emphasize the importance of studies for Hargraves cells on all patients with evidence of any form of increased splenic activity.

The first and third patients had the classic pattern of acquired idiopathic hemolytic anemia. Soon after this they developed the other changes of disseminated lupus. As a result of these cases, routine "LE" studies were done on all patients with evidence of hypersplenism. In the course of this investigation, Case Two, who also presented herself with acquired hemolytic anemia, was diagnosed as lupus. Several days later bilateral pleural effusions appeared.

It is shown that there is no cross-reaction between the antibodies giving positive Coombs test and the LE factor.

The following changes may be due to increased splenic activity, namely, leukopenia, thrombocytopenia, and hemolytic anemia, provided that the bone marrow is hyperplastic. Leucopenia is well known in lupus and it is pointed out that the marrow is myelopoietically active.

Thrombocytopenic purpura has often been reported as an initial manifestation of disseminated lupus. Bone marrow studies show plentiful megakaryocytes not forming platelets.

The findings in these three patients and others suggest the hemolytic nature of the anemia in the early stages.

In view of the above it is postulated that hypersplenism of the secondary type plays a significant role in lupus.

Changes in Connective Tissue Reaction Induced by Cortisone—in vivo Observations using the Rabbit Ear Chamber Technique. R. H. EBERT,* Chicago, Ill.

Since earlier work in our laboratory using the rabbit ear chamber as an in vivo method of observation had provided insight into the dynamics of certain hypersensitive states, it was felt that a study of the effect of Cortisone on these reactions might provide basic information as to how this hormone modifies the inflammatory response. Tuberculous infection, the focal reaction, and serum sickness were chosen for study because considerable information about these reactions has been accumulated.

The inflammatory response was studied in 24 experiments using Cortisone (5–25 mg. daily) and 21 untreated controls. Cortisone produced certain consistent changes in the inflammatory response which were independent of the type of reaction studied.

1) In all reactions, vascular tone was better maintained in the Cortisone treated animal than in the untreated control. This effect seemed to be due primarily to a mainte-

nance of arteriolar tone. Even in the apparently normal chamber arteriolar tone frequently increased with Cortisone therapy and this effect was even more striking if non-specific inflammatory changes were present prior to treatment.

2) Cortisone appeared to maintain the integrity of vascular endothelium. In each of the reactions studied Cortisone treatment reduced the degree of damage to arteriolar and venule endothelium. Sticking of leukocytes to endothelium was diminished and swelling of endothelium was suppressed. Endothelium in treated animals tended to maintain its normal highly refractile appearance.

3) As a result of this increased integrity of endothelium there was a decrease in diapedesis of leukocytes and a reduction in exudate.

The modification of the inflammatory reaction by Cortisone in tuberculous infection, the focal reaction, and serum sickness was quantitative. If the stimulus was sufficiently intense there was no measurable reduction in the reaction.

Body Water, Water Distribution and Water Kinetics as Revealed by the Use of Deuterium Oxide. I. S. EDELMAN and F. D. MOORE,* Boston, Mass.

Following the rapid intravenous injection of D_2O, a characteristic arterial time-concentration curve is observed. Its early slopes are determined by cell permeability; its "equilibrium" value by total body water, and its late slope by water turnover ("biologic decay") rates. This paper deals with an analysis of these three phases of the heavy water curve. The pre-equilibrium portion of the arterial curve resolves into two rate components indicating two classes of rate—determining boundaries limiting the free diffusion of water in the living organism. In vitro dialysis of skeletal muscle containing D_2O, and arterial venous D_2O curves in the isolated hind limb of the cat, support the concept of two types of cell areas (one exchanging water with its interstitial fluid environment rapidly and the other slowly), accounting for the two rates of distribution noted. The zero-time volume of dilution, obtained by extrapolation of the curve and the application of the isotope dilution principle, is a measure of the extra-cellular water. The mean values so obtained were 15 per cent of the body weight in humans and 17 per cent of the body weight in dogs. Using this tri-cameral system for water exchange and from the arterial curves, we calculated the internal rates of water exchange and the distribution of body water. In toto, the extracellular water exchanges with cell water at the rate of 21 per cent/min. in humans and 25 per cent/min. in dogs.

The derivation of the above data, and the normal human total body water throughout the life span will be described.

Excessive Losses of Sodium, Chloride, and Water After Administration of Cortisone or ACTH, Suggesting Transient Adrenocortical Insufficiency. LEONARD P. ELIEL, OLOF H. PEARSON and CHARLES D. WEST, New York, N. Y. (Introduced by David A. Karnofsky).

In a series of patients with lymphoid tumors who had received cortisone or adrenocorticotrophic hormone (ACTH) for 3 or more weeks, marked asthenia, collapse, pyrexia, delirium, and death were observed in several instances after withdrawal of cortisone, while asthenia was usually noted after stopping ACTH. The sodium and chloride balances, and body sodium and water contents were determined in one of these patients, the balances alone in two others.

The first patient, after receiving ACTH (100 mg/day) for 30 days, lost 2.5 times more sodium and 5 times more chloride than were retained during hormone administration. An ensuing 36 day period of cortisone (100–200 mg/day) resulted in no significant salt retention, or loss on hormone withdrawal. Cortisone was subsequently given continuously for 7 months (64–171 mg/day), first intramuscularly and later orally. Hormone withdrawal then resulted on two occasions in extreme dehydration, pyrexia, delirium, a fall in serum sodium, chloride, and pH levels, and marked urinary losses of sodium and chloride. The body sodium content estimated by Na^{24} dilution during hormone deprivation showed a reduction to 34.0 meq/Kg from a value of 51.7 meq/Kg before hormone administration, while the total body water simultaneously estimated by antipyrine dilution showed a reduction of 11 liters. Exhibition of oral cortisone was followed by prompt clinical improvement and partial repair of the sodium and chloride deficit.

Large transient losses of sodium and chloride exceeding previous retentions by factors of 2 to 4.5 have been observed in two other patients after stopping administration of ACTH or cortisone.

The symptoms and excessive losses of sodium, chloride, and water observed in these patients following withdrawal of cortisone or ACTH, suggest adrenal cortical insufficiency which is usually transient but potentially fatal.

Observations on the Mechanism of the Hyperlipemia in the Nephrotic Syndrome. KENDALL EMERSON, JR.,* MARCEL ROCHE, STANLEY S. KAHN, HUGO W. MOSER and DALTON JENKINS, Boston, Mass.

In four adolescent and young adult patients with the nephrotic syndrome treated with ACTH for 7 to 14 days (80–100 mgs. daily in four divided doses) the following changes were observed. The initially high R.Q. fell transiently in the first 24 hours of treatment, then rose well above the initial level, frequently exceeding 1.00, reached a peak about the fifth day and tended to fall slowly thereafter even while treatment was continued. The serum level of phospholipids and either total or esterified cholesterol or both also rose during the first 5 days of ACTH treatment, falling slowly from then until withdrawal of ACTH and rapidly thereafter in conjunction with the diuresis which invariably occurred. The serum protein bound iodine rose from very low initial levels toward normal.

In a series of control patients similarly treated, including two normal subjects, two patients with hypothyroidism, one with Cushing's Syndrome and one with idiopathic

edema the R.Q. invariably fell during ACTH administration; no consistent variation occurred in the serum lipid levels and, except in the patients with hypothyroidism, the serum protein bound iodine level decreased.

It is concluded that the metabolic orientation of the nephrotic patient is in the direction of the conservation of fat. To this end his circulating thyroid hormone is decreased and adrenal stimulation calls forth a paradoxical response, namely increased combustion of carbohydrate and increased storage or synthesis of fat. It is suggested that this altered response represents a profound metabolic adaptation on the part of the liver, possibly analogous to the phenomenon of hibernation. Further studies are in progress to determine by means of P_{32} turnover rates whether the rise in phospholipids represents increased synthesis or decreased utilization.

The Hemostatic Defect in Thrombocytopenia as Studied by the Use of ACTH and Cortisone. WILLIAM W. FALOON, RICHARD W. GREENE AND EUGENE L. LOZNER,* Syracuse, N. Y.

Improvement in hemostasis and vascular resistance despite persistence of thrombocytopenia may follow splenectomy for purpura. Surgical procedures other than splenectomy may result in transient hemostatic improvement. Since these observations suggested the presence of humoral factors following stress, the effects of ACTH and Cortisone on the hemostatic defect in thrombocytopenia were investigated in four patients; two non-splenectomized adolescent girls, one man, 58 years old, and one woman, 66 years old. The man had been splenectomized one year previously without remission in thrombocytopenia or vascular fragility. The woman had been splenectomized six years previously with temporary remission of thrombocytopenia and subsequent relapse. The hemostatic studies included observations on vascular fragility (tourniquet test), platelet counts and the coagulation phenomena (prothrombin utilization and clot retraction) associated with changes in platelets.

During the administration of ACTH to three patients, all showed prompt improvement in vascular fragility. In the two girls, platelet counts and associated coagulation findings subsequently became normal. No imrovement occurred in the thrombocytopenia of the older man. In all three patients, discontinuance of ACTH was followed within a relatively short time by a return of the hemostatic defects.

One of the ACTH-treated girls subsequently received Cortisone orally, with maintenance of normal vascular resistance, but with almost complete relapse in thrombocytopenia and associated abnormalities.

The 66 year old woman has received only Cortisone. Capillary resistance approached normal without change in the platelet count or coagulation studies.

The data suggest that different adrenal hormones may be responsible for separate improvement in the vascular resistance and the platelet counts of thrombocytopenic patients. These studies may explain the dissociation between vascular fragility and thrombocytopenia which is observed clinically.

Renal Hemodynamics and Salt and Water Excretion During Induced Congestion of the Inferior Vena Cava of Man. SAUL J. FARBER, J. DEAVER ALEXANDER and LUDWIG W. EICHNA,* New York, N. Y.

Renal venous congestion has been postulated as a causative factor in the salt and water retention of congestive heart failure. In an attempt to evaluate the effect of venous congestion on renal hemodynamics and salt and water excretion in man, a method was developed whereby a balloon was inflated in the inferior vena cava, above and below the entrance of the renal veins. Inferior vena caval pressures of 160 mm. to 260 mm. saline were produced and maintained for 30 minutes. When the congestion included the renal veins, the excretion of water, sodium and chloride decreased markedly in 17 of 19 non-cardiac subjects. In the 7 subjects so studied, glomerular filtration rate and renal plasma flow decreased by approximately one-third during the first 20 minutes but began to recover during the last 10 minutes of congestion. In contrast, water and electrolyte excretions remained reduced or continued to fall throughout the period of congestion and returned to control values only after deflating the balloon. Neither the renal hemodynamic nor urinary changes were related to the degree of rise in venous pressure. Congestion of the inferior vena cava *below* the level of the renal veins induced rather similar effects; water and electrolyte excretion decreased in 6 of 9 subjects, and, in the 3 subjects studied, renal hemodynamics changed in the same manner as during inflation of the balloon above the renal veins.

Congestion of a sizable segment of the inferior vena caval drainage area appears to decrease the excretion of water and electrolytes and this reduction becomes practically invariable when renal congestion is present. Renal hemodynamic changes may contribute to the initial decrease in water and electrolyte excretion but are not necessary for the continuance of the effect. Factors other than renal venous congestion are involved in the antidiuretic effects observed.

The Role of the Pituitary Adrenocortical System in the Response to Anoxia. THOMAS F. FRAWLEY, MARCEL ROCHE, DALTON JENKINS and GEORGE W. THORN,* Boston, Mass.

In the present studies an estimate of the amount of endogenously liberated ACTH during a single exposure to anoxia has been made and the influence of ACTH and cortisone upon the altitude tolerance of normal males has been investigated. Anoxia was produced by breathing through a closed spirometer system containing atmospheric air with all expired CO_2 being absorbed by soda lime. The experiment was terminated when the subject became unable to respond to light signals. A comparison between the fall in circulating eosinophils caused by anoxia and that produced by the injection of a known amount of ACTH furnished an approximate estimate of the amount of ACTH secreted spontaneously by the human pituitary in response to the stress of anoxia. The per cent change in circulating eosinophils 4 hours after acute anoxia

ranged from 11 per cent to 62 per cent, with an average fall of 36 per cent. When compared to the fall obtained with ACTH in these same individuals, it appeared that under the circumstances of the test the anterior hypophysis secretes an amount of ACTH equivalent to slightly less than 25 mg. of injected ACTH.

ACTH in doses of 160 mg. daily over a five-day period produced an improvement in anoxia tolerance associated with an increased oxygen uptake. In five normal subjects the average initial altitude tolerance measured 28,800 feet, with a range of 23,200 to 32,700 feet. The average altitude tolerance during the administration of ACTH was 30,400 feet, with a range of 28,000 to 34,000 feet. Five hundred mg. of cortisone administered orally to two normal subjects over a 6-hour period preceding the anoxia test produced a change in altitude tolerance from 29,575 to 32,620 feet. Temporary pituitary-adrenocortical unresponsiveness to anoxia developed following ACTH and cortisone.

Reduction of Blood Pressure in the Normotensive and Hypertensive Animal Following Potassium Deprivation. MEYER FRIEDMAN,* S. CHARLES FREED and RAY H. ROSEMAN, San Francisco, Calif.

Recent studies indicate that reduction of blood pressure occurs in rats following restriction of potassium in their diet (0.01 per cent of total diet). Thus the average blood pressure of 33 *normotensive* rats fell from a control level of 103 mm. of Hg (Range: 88 to 128) to 87 mm. of Hg (Range: 66 to 116) after 7 weeks of potassium deprivation. Control rats had an initial blood pressure of 106 mm. of Hg (Range: 94 to 122) which rose to 116 mm. of Hg (Range: 102 to 130).

Similar potassium restriction had a much greater depressor effect on *hypertensive* rats. Thus the average pressure of 13 hypertensive rats fell from 165 mm. of Hg (Range: 140 to 200) to 123 mm. of Hg (Range: 100 to 150) after 7 weeks of potassium deprivation. The blood pressure of the control hypertensive rats rose from 153 mm. of Hg (Range: 140 to 165) to 171 mm. of Hg (Range: 140 to 198) during the same period.

This depressor effect of potassium restriction could be prevented by concomitant restriction of sodium (0.04 per cent of total diet). However, excessive sodium intake (0.70 per cent of diet) did not accentuate the depressor effect of potassium restriction.

Although foci of necrosis could be found in the myocardium of potassium deficient rats, no correlation could be established between severity of lesion and degree of induced hypotension. However, the pressor response of the potassium deficient rat to injections of epinephrine, renin, angiotonin and norepinephrine were respectively ½, ⅝, ½, and ⅛ those of the normal rat. These findings suggest that the depressor effect results from a loss of *peripheral* vascular responsiveness.

Metabolism of Pteroylglutamic Acid and Citrovorum Factor in Human Scurvy. GEORGE J. GABUZDA, JR., GERALD B. PHILLIPS, ROBERT F. SCHILLING and CHARLES S. DAVIDSON,* Boston, Mass.

The urinary excretion of citrovorum factor (CF) was determined microbiologically following pteroylglutamic acid (PGA) administered orally to two men with scurvy before and during ascorbic acid therapy.

The patients were fed scorbutic diets. During the first three days urinary CF was 0.3 gamma, and 1.0 gamma daily, respectively. When 10 mg. of PGA were administered daily for six days to one patient, CF excretion became 8 to 16 gamma daily. During the next six days 1 gm. of ascorbic acid was given intramuscularly daily to this patient in addition to PGA. Urinary CF was 15 gamma the first day of ascorbic acid therapy, 30 gamma the second day, and ranged from 95 to 126 gamma daily for four additional days.

The second patient's urinary CF ranged from 9 to 13 gamma daily when 10 mg. of PGA were given daily for three days. One gm. of ascorbic acid was then given orally daily for seven days, in addition to PGA. Urinary CF was 17 gamma the first day of ascorbic acid therapy and increased to 110 gamma by the seventh day. PGA was then omitted, but ascorbic acid continued. Urinary CF returned promptly to initial levels. After six days PGA was given again in addition to ascorbic acid. On the first day urinary CF rose to 94 gamma, and averaged 133 gamma daily during three additional days.

In vitro measurements indicate that the quantities of PGA and of ascorbic acid excreted by these patients do not invalidate the microbiological assay of CF.

Conclusion: A small amount of CF was excreted in the scorbutic state and was increased by orally administered PGA. However, maximal CF excretion occurred only when both PGA and ascorbic acid were given. Thus, ascorbic acid facilitated conversion of PGA to citrovorum factor in man.

Ambient Air Recording Bronchospirometry and Differential Residual Volume Determination. EDWARD A. GAENSLER and DAVID W. CUGELL, Boston, Mass. (Introduced by Richard A. Bloomfield).

Conventional recording bronchospirometry requires the breathing of high oxygen mixtures. This places the diseased lung at a relative advantage and conclusions from its performance under these conditions do not necessarily apply to function under room air tensions. Differential lung volume determination is not possible because the initial concentrations of gases in the lungs are not known.

To obviate these difficulties a bronchospirometer fitted with twin air tight boxes and balloons was used which recorded respirations reflected by pressure changes within the boxes. One or both lungs could be supplied with ambient air or oxygen and the inspiratory gas composition could be changed instantaneously.

In normal volunteers oxygen and ambient air bronchospirometry resulted in the same relative oxygen uptake and ventilatory equivalent. Partially destroyed lungs showed up to twice the relative oxygen uptake with high oxygen tensions than with ambient air. The functional effectiveness of diseased lungs determined by high oxygen bronchospirometry is therefore overestimated.

Ambient air bronchospirometry was applied to the open circuit residual volume method of Darling, Cournand, and Richards. It solved one of the problems of this method by permitting visualization of the exact place in the respiratory cycle at which both lungs were turned into the oxygen circuit. The true functional residual volume was calculated after correction from the spirogram of the preparatory ambient air breathing period.

In normal volunteers the functional residual volume distribution corresponded closely to distribution of oxygen uptake, ventilation, and vital capacity. In a group of 40 patients with partially destroyed lungs the relative functional residual volume was often many times greater than other indices of function. This discrepancy was greatest in "imprisoned lungs" of fibrothorax and chronic empyema. Pulmonary mixing indices, often normal after combined oxygen breathing, showed marked mixing impairment by differential alveolar air sampling.

Observations on Insulin Labeled with Radio Iodine. JOSEPH W. GARDELLA and JOSEPH W. FERREBEE,* Cooperstown, N. Y.

Bovine insulin several times recrystallized from phosphate buffer has been labeled with radio iodine in amounts of 10 to 1000 microcuries of I^{131} per milligram of insulin, the total quantity of I^{127} and I^{131} organically bound being of the order of two atoms per molecule of insulin. Studies of insulin so labeled have revealed ranges within which the labeled insulin appears to have unimpaired biologic activity as measured by mouse convulsion test or by the acceleration of glycogen synthesis in isolated rat diaphragms. Physical chemical studies reveal no changes in salt precipitability of labeled insulin as compared with native insulin and no changes in adsorption on cellulose, indicating that the label has not greatly distorted the molecule. From these in vitro and in vivo observations it has been concluded that iodine labeled insulin may be used within limits to study the mechanism of insulin action and insulin metabolism. The pattern of localization of radioactivity in various organs following the injection of labeled insulin will be presented and the effect of previous administration of native insulin, glucose and other factors affecting carbohydrate metabolism will be reported. If time permits and appropriate AEC permission is granted, blood and urine curves of radioactivity following the injection of radio insulin into normal, diabetic and insulin-resistant individuals will be presented.

Cardiac Output in Acute Myocardial Infarction. ROBERT P. GILBERT, STEPHEN L. ALDRICH and LYNN ANDERSON, Chicago, Ill. (Introduced by Richard B. Capps).

The dye dilution technique for determining cardiac output, as described by Hamilton et al and as modified by Ebert et al, for dye collection offers a method which can be used at the bedside in sick patients.

So far three cases of acute myocardial infarction have been successfully studied by this technique. Arterial pressures have been recorded by a strain gauge manometer and venous pressures and circulation times determined by conventional methods. Two patients had low outputs and somewhat elevated pressures. One patient who had just recovered from a period of very low pressure was found to have a high output which suggests that his previous drop in pressure may have been associated more with a decreased total peripheral resistance. Particular attention will be paid to patients with low pressures in an effort to determine the relative importance of decreases in cardiac output and in total peripheral resistance.

Mechanism of Production of Lung Lesions in Virus Pneumonia. H. S. GINSBERG and F. L. HORSFALL, JR.,* New York, N. Y.

Lung lesions developing during experimental virus pneumonia induced with influenza virus or pneumonia virus of mice (PVM) increase at a rate much slower than the rate of increase in virus concentration. Moreover, high concentrations of Newcastle disease virus (NDV) cause extensive lung lesions in the mouse but no multiplication of this virus occurs. The gross and microscopic pathology of lung lesions caused by influenza, PVM, or NDV are indistinguishable. These findings indicate that in virus pneumonia factors other than the process of virus multiplication damage host cells.

The lung lesion factor is an integral component of the infective NDV particle itself: It is absorbed by and eluted from erythrocytes along with the active virus; it is not separable from the virus in high gravitational fields; and it is inactivated by either heat or ultraviolet irradiation at a rate closely similar to that of the infective virus.

Lung lesions caused by high concentrations of NDV develop at a rate similar to that found with influenza virus. Production of pneumonia by NDV is formally analogous to a virus infection: lung cell-virus combination is prevented by treatment of the living lung with receptor destroying enzyme (RDE), and in such treated lungs NDV does not cause lesions; NDV immune serum specifically prevents lung lesion production when given before or with virus but has no effect when given 30 minutes after; and prior multiplication of unadapted influenza virus in the lung completely inhibits lung lesion production with NDV.

These results support the following postulate: When a sufficiently high concentration of infectious virus is attained, either by multiplication or direct inoculation, cellular damage is caused. Lung lesions in virus pneumonia then are in large part attributable to the cytotoxic effects of the virus particle *per se.*

The Effect of Cortisone on Acute Bacterial Infections. ROBERT J. GLASER, JOHN W. BERRY, LENORE H. LOEB and W. BARRY WOOD, JR.,* St. Louis, Mo.

Cortisone and ACTH are known to depress a variety of inflammatory states, both in patients and experimental animals. Dramatic defervescence and subsidence of symptoms have resulted from their use in pneumococcal pneumonia, in spite of bacteriological evidence of continued infection. The experiments here reported have been designed to study the effect of Cortisone upon the course of

acute bacterial infections and particularly upon the cellular response of the host.

Streptococcal and pneumococcal pneumonia were produced in rats by intrabronchial inoculation. Half of the animals were treated with Cortisone beginning five days before infection, while the other half served as untreated controls. The mortality rate for each group was determined, and a systematic histologic study was made of the infected lungs. Similar studies were made in mice with streptococcal cervical adenitis produced by intranasal inoculation.

The survival rates in all experiments were less favorable among the animals treated with Cortisone. The host response to the infectious stimulus was strikingly altered. The lesions in the Cortisone treated animals contained excessive amounts of relatively acellular edema fluid in which there were many more bacteria than were ever seen in analogous lesions from control animals. The sparsity of cells in the edema fluid and the extraordinarily large bacterial population appeared to be related to a delay in the diapedesis of leucocytes. Cortisone *per se* was shown to exert no growth-stimulating effect upon the bacteria, nor did it influence significantly the phagocytic activities of the leucocytes which eventually appeared in the lesion.

Further studies are in progress relating to the mechanism whereby Cortisone depresses the process of cellular exudation, which in the case of acute bacterial infections constitutes one of the principal defenses of the host.

Dynamics of Eisenmenger's Complex. H. GOLDBERG, E. N. SILBER and A. GORDON, Chicago, Ill. (Introduced by L. N. Katz).

The patho-physiology underlying many of the manifestations of Eisenmenger's Complex has remained obscure. Chief among these is the delayed onset of cyanosis which is claimed to result from some congenital abnormality of the pulmonary epithelium or capillaries. A case of Eisenmenger's complex studied by cardiac catheterization is presented in which, in addition to the usual features, anomalous pulmonary venous drainage into the right auricle was demonstrated by entrance of the catheter into one of these veins. This afforded the opportunity to measure directly the total vascular resistance to blood flowing through the lungs and to determine whether or not a pulmonary factor for the production of cyanosis exists in this condition.

The pulmonary venous pressure and pulmonary blood flow were normal. It is concluded that the elevated pulmonary arterial pressure is due to an increased resistance resulting from a narrowing of the peripheral pulmonary vascular bed. This narrowing may be due to functional as well as organic changes. Although hypoxemia plays a role, it cannot account for all of the increased vascular resistance. Organic changes affecting the medial and intimal layers of the muscular arteries of the lung play a major role. The intimal changes are progressive and appear to be secondary to the pulmonary hypertension.

Blood obtained from the pulmonary vein was fully saturated. This indicates that the peripheral unsaturation is due solely to veno-arterial shunting of blood. This eliminates the presence of a pulmonary factor as a cause for the cyanosis in Eisenmenger's Complex.

The principles of surgical correction of this disease are discussed and may be summarized as follows:

1. A reduction of the pulmonary artery pressure.
2. Alleviation of the strain upon the right ventricle.
3. Return of the peripheral arterial saturation toward normal. Correction of the overriding aorta with elimination of the ventricular septal defect would accomplish these objectives.

Nor-epinephrine and Epinephrine in Human Urine (Addison's Disease, Essential Hypertension, Pheochromocytoma). MARCEL GOLDENBERG and MAURICE M. RAPPORT, New York, N. Y. (Introduced by Robert F. Loeb).

The inactivation mechanisms and the metabolic fate of epinephrine and nor-epinephrine are not established. Ingested epinephrine is partly excreted in the urine as a sulfate ester (Richter). Normal human urine contains a mixture of catechol-amines (epinephrine, nor-epinephrine and perhaps hydroxytyramine) (Holtz). This mixture is roughly composed of 85–90 per cent nor-epinephrine and approximates the composition of the sympathetic transmitter rather than that of the adrenal medullary hormone (70–90 per cent epinephrine). The normal daily excretion ranges from 15–45 micrograms nor-epinephrine in 24 hours (von Euler).

In an attempt to establish the source of these urinary catechol-amines, 7 cases of Addison's disease maintained on salt, Doca and cortisone were investigated. Of these, 5 were due to atrophy, in which case the adrenal medulla may have been preserved. Four of these showed a normal excretion, one a sub-normal value. Two cases of tuberculosis of the adrenal glands showing extensive calcification of both adrenals (in which it can be safely assumed that there is no remaining functioning medulla), showed an excretion of nor-epinephrine which was high normal in one case and far above normal in the other (maintained on cortisone). It therefore can be assumed that the adrenal medulla is not the principal source of the catechol-amines excreted in the urine. Since extra-adrenal chromaffin tissue is known to regress completely after fetal life except for small cell clusters in the sympathetic ganglia, the source must rather be the sympathetic or some other tissue. Three cases of extensive lumbo-dorsal sympathectomy showed the lowest observed sub-normal values. These findings are compatible with Cannon's "emergency" theory of adrenal medullary function which states that the resting secretion of the adrenal medulla is negligible.

The recent claim (Holtz) that an excessive excretion of nor-epinephrine occurs consistently in essential hypertension was not confirmed. Out of 14 cases only 2 showed an excretion above normal.

Finally, 2 cases of pheochromocytoma were investigated and found to excrete quantities of epinephrine and nor-epinephrine far in excess of the values obtained in normals and hypertensives (confirming Engel and von Euler).

In one of these cases the daily excretion amounted to 1600 micrograms of epinephrine and 400 micrograms of nor-epinephrine. Both cases showed the clinical characteristics of pheochromocytoma with persistent hypertension and repeatedly gave positive benzodioxane tests. Whether the urinary excretion is increased in the "non-humoral" phase of persistent hypertension due to pheochromocytoma, which poses the major diagnostic problem, is under investigation.

Studies in Diurnal Variation of Water and Electrolytes: Nocturnal Diuresis of Water and Sodium in Congestive Cardiac Failure, Cirrhosis of the Liver and Degenerative Glomerulonephritis. RALPH GOLDMAN, Los Angeles, Calif. (Introduced by Samuel H. Bassett).

The mechanism of sodium retention in edema-forming diseases has been the subject of much study and controversy. Two concepts dominate. The first theory is that sodium is reabsorbed at a fixed rate from the filtrate, and that the amount of sodium in the urine is related to the rate of glomerular filtration. The other theory is that the filtered sodium is subject to variable tubular reabsorption. Data obtained by others and ourselves indicate that in congestive cardiac failure there is often a nocturnal diuresis of sodium as well as water. This has been explained by a nocturnal increase in the glomerular filtration rate resulting from a decreased peripheral demand for blood during the resting state. Examination of the diurnal excretory cycle in thirteen patients with cirrhosis of the liver and ascites revealed that ten had a clear-cut nocturnal diuresis of water and sodium. A similar study of five patients with degenerative glomerulonephritis revealed that two of these patients also demonstrated a nocturnal sodium diuresis. The potassium excretion in two-thirds of these patients showed a daytime maximum, although the curves were moderately flattened. While it is possible to conceive of renal hemodynamics as the chief factor influencing nocturnal diuresis in congestive cardiac failure, there is no data to suggest that the nocturnal diuresis seen in cirrhosis of the liver and glomerulonephritis has the same cause. With the available data, it appears more probable that in these conditions, and perhaps in cardiac failure as well, the regulation of sodium reabsorption depends upon variations in tubular metabolism controlled by a humoral agent which can be affected by the functional state of the liver. Data will be presented.

The Effect of Mild Diabetes and Insulin on Heart Muscle Metabolism in Man. WALTER T. GOODALE, ROBERT E. OLSON and DONALD B. HACKEL, Boston, Mass. (Introduced by James P. O'Hare).

Studies on normal individuals by coronary venous catheterization have shown consistent patterns of extraction, and presumed utilization, of glucose, lactate and pyruvate by the myocardium. The coronary arteriovenous difference of each metabolite was related directly and linearly to its own arterial level, in both man and dogs. No significant myocardial glucose utilization occurred, however, below a mean arterial glucose level of 60 ± 10 mg. per cent, while lactate and pyruvate utilization was detectable down to arterial levels of 2.5 and 0.5 respectively. At average non-fasting arterial glucose, lactate and pyruvate levels of 103, 7.5 and 1.3, average coronary arteriovenous differences were 10.7, 2.7 and 0.4, and the myocardial RQ (MRQ), $0.86 - 0.93$. At *low* fasting arterial levels, glucose, lactate and pyruvate extractions were correspondingly small or negligible, and MRQ near 0.7.

Five patients with minimal asymptomatic diabetes showed negligible myocardial glucose extraction despite fasting glucose levels of $95 - 216$, with greatly reduced lactate and pyruvate utilization and an MRQ of $0.67 - 0.71$. Insulin restored lactate and pyruvate utilization to normal, and initially raised the diabetic MRQ to $1.1 - 1.2$, accompanied by very high coronary A–V glucose differences up to 29 mg. per cent as the arterial glucose level began to fall. Far more glucose was extracted than was needed theoretically as substrate for the simultaneous oxygen extraction, suggesting a partially anabolic non-oxidative utilization of glucose under the influence of insulin. With further fall in glucose level toward normal following insulin, the glucose, lactate and pyruvate utilizations and MRQ approached values seen in normal non-fasting individuals. These results suggest that insulin may promote phosphorylation of not only glucose but also other substrates such as pyruvate, and demonstrate an abnormal pattern of heart muscle metabolism in even minimal diabetes.

The Effects of Quiet Standing on Soluble Diuresis. A. V. N. GOODYER and D. W. SELDIN, New Haven, Conn. (Introduced by F. G. Blake).

The sharply decreased excretion of sodium during quiet standing is due at least in part to a potent stimulus for the increased tubular reabsorption of sodium and is usually not prevented by the administration of hypertonic saline.

It was of interest to investigate the extent to which this stimulus might interfere with the augmented excretion of sodium promoted by the administration of mannitol, or of sodium salts of acids, the anions of which must be rapidly excreted.

The experiments were performed in normal adults, most of whom had been deprived of water for 12 hours or more. After several preliminary periods in the supine position, each subject stood up and, 10–15 minutes later, an isotonic or hypertonic solution of mannitol, sodium bicarbonate or sodium phosphate was administered.

It was found that quiet standing limited greatly the usual augmentation of sodium excretion caused by mannitol diuresis. In several cases, the excretion of sodium actually decreased in the face of a concomitant mannitol diuresis. However, the usual relationship between total solute excretion and urine flow during water restriction was disturbed on only one occasion.

Quiet standing did not limit the usual increase in sodium excretion produced by the administration of sodium phosphate or sodium bicarbonate.

Measurements of GFR and RPF in some experiments

indicated that the changes in sodium excretion were primarily of tubular origin.

It is concluded that: 1) the rate of sodium excretion in mannitol diuresis is not strictly related to the quantity of mannitol in the tubular urine; it may be conditioned greatly by internal environmental factors affecting the renal tubular reabsorption of sodium; 2) the tubular rejection of sodium which attends the excretion of bicarbonate and phosphate takes precedence over the usual retention of sodium produced by quiet standing.

Circulatory Changes in Mitral Stenosis Following Valvuloplasty. RICHARD GORLIN, LEWIS DEXTER,* and DWIGHT E. HARKEN, Boston, Mass.

Eleven patients with mitral stenosis were studied before and 2 to 6 weeks after mitral valvuloplasty by the technique of cardiac catheterization. Cardiac outputs were measured by the direct Fick method. Pressures were recorded in the pulmonary "capillaries," pulmonary artery, and right heart with electromanometers. Mitral valve orifice areas were calculated from a hydraulic formula described previously. Mitral regurgitation was detected from the pulmonary "capillary" pressure pulse. Pulmonary resistances were calculated from standard formulae. The mitral valve orifice was significantly widened in 10 of 11 patients. In only one instance was mitral regurgitation produced at operation. In three others with regurgitation preoperatively, no increase occurred with valvuloplasty. Pulmonary "capillary" pressure decreased in 9, while cardiac output remained essentially unchanged in 9 and increased in 2. Pulmonary arteriolar resistance decreased in 5 of the 9 patients in whom it was elevated. Right ventricular diastolic filling pressure fell towards normal in 4 of 6 patients in whom it was elevated. Total pulmonary resistance, a measure of right ventricular load, decreased in 8. It is concluded that valvuloplasty effectively widened the mitral stenotic orifice without the production of regurgitation. Although the widened area was only 20–50 per cent of the normal of 4–6 cm.^{2}, this was sufficient to produce definite hemodynamic improvement because the reserve capacity of the mitral valve is approximately 80 per cent. If stenosis was severe, prior mitral regurgitation did not militate against successful valvuloplasty. Following operation, pulmonary congestion and pulmonary arteriolar resistance tended to decrease and right ventricular function to improve. These physiologic alterations have been attended by dramatic improvement in the clinical state of most of these patients. Long-term follow-up is desirable, although few such studies have been carried out to date.

The Specificity of the Relation Between Attitudes and Diseases. WILLIAM J. GRACE and DAVID T. GRAHAM, New York, N. Y. (Introduced by David P. Barr).

One hundred twenty-seven patients with one or more of 12 different bodily disturbances were interviewed to determine whether a specific attitude was associated with each symptom. When the life situation to which the patient was reacting had been clearly defined, he was asked to give a clear and ambiguous statement as to, a) how he saw himself in the situation, and b) what he wished to do about it at the time of the occurrence of the symptoms. These two components define the *attitude.* Conventional names of emotions, e.g. "anger" were not accepted. Only direct statements of patients, without reference to dream or associative material, were used in the study.

It was found that there was a specific attitude associated with each of the folowing symptoms: 1) Urticaria occurred in patients who felt they were being mistreated and were helpless. 2) Eczema occurred when the individual felt he was being interfered with or prevented from doing something and could do nothing about it. 3) Cold moist hands were noted in individuals who wanted to undertake some kind of activity. 4) Vasomotor rhinitis and asthma occurred in those who did not want to have anything to do with their problems. 5) Diarrhea occurred in persons who were wishing to get rid of their problems. 6) Constipation occurred in those who were grimly determined to go on in the face of difficulties. 7) Nausea and vomiting occurred when an individual was thinking of something which he wished had never happened. 8) Duodenal ulcer occurred in those who were seeking revenge. 9) Migraine headache occurred in an individual after he had been making an intense effort. 10) Arterial hypertension occurred in individuals who felt that they had to be prepared to meet all possible threats. 11) Low back pain occurred in those who wished to carry out some action involving the whole body, such as running away.

Many of these symptoms seem to be biologically appropriate to the attitude they accompany.

Evidence for an Adrenal Phase of Gastric Secretion. SEYMOUR J. GRAY, ROBERT W. REIFENSTEIN, HOWARD M. SPIRO and JOHN A. BENSON, JR., Boston, Mass. (Introduced by Marshall N. Fulton).

ACTH administered to experimental subjects in doses of 80 to 120 mgm. daily for 10–21 days produced an immediate and marked increase in the 24-hour urinary excretion of uropepsin and a significant rise in gastric juice pepsin within 7–14 days. A concomitant increase in gastric acidity was also observed on fasting serial aspirations and in the 12-hour nocturnal secretion. Both the concentration and total amount of hydrochloric acid, pepsin, and uropepsin were increased. An identical rise in uropepsin excretion was observed following the administration of cortisone orally or intramuscularly.

The administration of ACTH to a patient with a gastric ulcer resulted in a severe exacerbation of epigastric pain associated with marked epigastric tenderness, definite evidence of peritoneal irritation, localized protective muscle spasm, and guaiac-positive stools. The 24-hour excretion of uropepsin increased from 4900 to 9500 units during the 10 days of ACTH administration and was accompanied by a significant rise in gastric pepsin and acid secretion.

The increase in uropepsin, gastric pepsin, and gastric acidity (expressed as total mgm. of free HCl) approximated 100 to 400 per cent following ACTH administration.

A similar rise in the uropepsin excretion was observed following repeated injections of 0.6 mgm. epinephrine at 6-hour intervals for 4 days. This was associated with a significant fall in the level of circulating eosinophiles indicating a definite uropepsin response to adrenal stimulation.

The relation of this adrenal phase of gastric secretion to stress and the pathogenesis of peptic ulcer will be discussed.

Studies of Prevention of Some of the Toxic Effects of Prolonged Cortisone and ACTH Therapy. L. GREENMAN, T. S. DANOWSKI,* R. TARAIL, J. H. PETERS, F. WEIGAND and F. MATEER, Pittsburgh, Pa.

Electrolyte and nitrogen excretion in stools during a whole milk regimen administered to hospitalized patients has been compared with the output during a sodium-free milk diet. Carbohydrate and electrolyte-free milk protein were added to provide adequate calories and nitrogen. On the markedly restricted intake of sodium a) the daily excretion of potassium, b) the potassium in 100 grams of formed stool and c) the stool output of potassium in relation to nitrogen all fell significantly ("p" for the "t" test was less than 0.05 upon comparison of the above findings with those observed during maintenance on a whole milk formula). The sodium and the nitrogen output were not detectably altered while a statistically significant, though from the point of view of magnitude small, drop in chloride output was observed in the per 100 grams of stool and per gram of nitrogen categories. The presence of larger amounts of potassium in the low sodium milk together with the more complete absorption of potassium from this source provides the organism with 20 milliequivalents of potassium per day in excess of that obtainable from a comparable whole milk formula. The attributes of such low sodium milk formulae have been employed for the complete prevention of edema, maintenance of positive balances of potassium and of nitrogen, and deferral, minimization or prevention of hypokalemia, hypochloremia and alkalosis in a large series of patients receiving cortisone or ACTH for as long as 68 days.

The Results of Feeding Desiccated Thyroid to Thyrotoxic Subjects. MONTE A. GREER, Boston, Mass. (Introduced by Raymond D. Adams).

It has been observed that exogenous thyroid hormone in amounts of one to three grains daily will markedly suppress endogenous thyroid activity of normal human subjects, as measured by the accumulation of I^{131}. The BMR and PBI (serum protein-bound iodine) remain constant during such procedures and it has been assumed that this decrease in thyroid function has been brought about through suppression of pituitary thyrotrophin secretion.

It was therefore considered of interest to determine the effect of feeding thyroid hormone to thyrotoxic subjects. Following control determinations in sixteen untreated thyrotoxic patients, daily doses of desiccated thyroid were given, gradually increasing to twelve grains daily, increments being made at approximately weekly intervals. Thorough clinical evaluation, including pulse, weight, and I^{131} uptake, was made at each visit in every patient. In suitable patients the BMR and serum protein-bound iodine were also determined. It was found that a gradual suppression of the accumulation of I^{131} by the thyroid was produced, but that in no instance was this complete, never being less than 18 per cent/24 hours even when the patient was taking twelve grains of thyroid daily. The pulse, weight, BMR, and PBI, however, remained constant during the entire period of observation.

In one patient the 24-hour uptake of I^{131} was reduced from 84 per cent to 33 per cent by the ingestion of nine grains of thyroid daily. Six weeks following the cessation of therapy the I^{131} uptake was still at this low level and the patient was found to be accumulating I^{127} in the thyroid at a rate equivalent to only 0.4 grains of thyroid per day.

Since the I^{131} uptakes of 48 euthyroid subjects and 18 with simple goiter were all found to be suppressed below 15 per cent/24 hours by the administration of twelve grains or less of thyroid daily, it is suggested that the administration of twelve grains of thyroid daily for a period of two weeks may help to differentiate equivocal cases of hyperthyroidism.

Lack of Analgesic Effect of Cortisone and ACTH. ALBERT W. GROKOEST, DEGUISE VAILLANCOURT, ROBERT GOTTSEGEN and CHARLES RAGAN,* New York. N. Y.

It has been suggested that the beneficial effects of cortisone and ACTH on diseases such as rheumatoid arthritis are in part due to an analgesic effect of these hormones. A systematic evaluation of cutaneous, dental and visceral pain has been undertaken in patients with rheumatoid arthritis, before and during the administration of cortisone and ACTH. Cutaneous pain was tested by intradermal injections of saline and distilled water; tooth sensitivity by electrical stimulation and visceral pain by distention of a balloon placed in the duodenum. The pain threshold and intensity were similar before and during the administration of the hormones and no true analgesia could be detected. It is proposed that the beneficial effects of hormone administration in rheumatoid arthritis and similar diseases evolve from a suppression of the inflammatory reaction in the host and not from a central or peripheral "analgesic" effect which cannot be demonstrated.

A Study of the Hemodynamic and Pathological Changes Induced by Bilateral Nephrectomy and Ligation of the Ureters of Dogs Maintained for Prolonged Periods by Means of Intermittent Peritoneal Lavage. ARTHUR GROLLMAN, LOUIS B. TURNER and E. E. MUIRHEAD, Dallas, Texas (Introduced by Carl A. Moyer).

The elucidation of the role of the kidney, apart from its excretory function, in the animal economy has hitherto been rendered difficult because of the impossibility of maintaining the nephrectomized animal alive for extended periods. A procedure for intermittent peritoneal lavage has been devised which has permitted maintaining dogs alive in good condition for periods of months fol-

lowing bilateral nephrectomy, ligation of the ureters, or implantation of the ureters into the gut or vena cava. Following nephrectomy, the blood pressure gradually reaches hypertensive levels in the course of 1 to 2 weeks and remains so during the remaining life of the animal. The cardiac output, blood volume and venous pressure remain essentially unaltered, but there is an increase in peripheral resistance similar to that observed in hypertensive disease. Examination of the tissues of such animals reveals the acute lesions similar to those observed in the human dying of malignant hypertension. In animals maintained for a month or longer, the chronic lesions characteristic of those observed in the arterioles, myocardium and smooth muscle of the so-called benign form of hypertension in the human are observed. Animals retaining intact renal tissue (as when the ureters are ligated) and treated similarly develop neither hypertension nor these lesions. The results of these studies have an obvious bearing on the pathogenesis of hypertensive cardiovascular disease and indicate the role of the kidney in the maintenance of the normotensive state.

The method of intermittent peritoneal lavage offers a means of investigating other problems associated with renal function. It has been possible, for example, to differentiate the role of azotemia, the rise in blood pressure, etc. in the causation of the acute arteriolar necrosis observed in so-called malignant hypertension. The method as elaborated by the present study has also been applied successfully to the human.

Determination of Cardiac Output in Man by the Fick Principle without Gas Analysis. J. GROSSMAN and R. E. WESTON, New York, N. Y. (Introduced by Louis Leiter).

The usual method for measuring cardiac output by the Fick principle requires blood and air gas analyses with special laboratory equipment and procedures. However, cardiac output could be determined by cardiac catheterization without gas analysis by using any substance which is either added to or removed from the blood at an accurately measurable rate, if it were possible to produce an adequate difference between arterial and mixed venous blood concentrations of that substance. Unfortunately, the mixed venous (right ventricular) and arterial concentrations of substances added to or removed from only the greater circulation are identical. With a double lumen catheter, however, a measurable arterial-mixed venous concentration difference can be produced by injecting the test substance through the distal lumen, placed in the pulmonary artery. Mixed venous blood samples, then, can be obtained from the proximal lumen which opens into the right ventricle.

A number of potential test substances may be employed in this method. In these preliminary studies, sodium para-aminohippurate (PAH) was selected because of its virtually complete renal extraction, the large renal blood flow, the consequent high arterio-venous difference, and the ease of chemical determination of PAH in blood and urine.

It will be demonstrated that cardiac output may be calculated either as

$$\text{C.O.} = \frac{UV_{PAH}}{\frac{A_{PAH}V_{PAH}}{1 - \text{Hct.}}} \quad \text{or} \quad \text{C.O.} = \frac{C_{PAH}}{\frac{1 - \frac{V_{PAH}}{A_{PAH}}}{1 - \text{Hct.}}}$$

where A_{PAH} and V_{PAH} are arterial and mixed venous plasma concentrations of PAH, respectively, UV_{PAH} and C_{PAH} are the urinary excretion rate and renal clearance of PAH, respectively, and Hct. is the hematocrit.

Data on cardiac outputs concurrently measured in patients by the oxygen and PAH methods will be presented and discussed.

Central Nervous System Sequelae as Shown by Post-Recovery Electroencephalograms in Rickettsial Spotted Fever. GEORGE T. HARRELL,* RICHARD L. MASLAND and MARVIN J. ROSENBLUM, Winston-Salem, N. C.

Symptoms of encephalitis have been observed for many years in patients with rickettsial diseases. In patients dying during the acute phase of the disease pathologic sections often show either the acute microinfarct with arterial thrombosis and necrosis or the more chronic granulomatous "typhus nodule." Little information has been previously available on the residual damage to the central nervous system resulting from these infections.

Fifty-eight patients with rickettsial infections have been successfully treated in our clinic. Forty-three of these patients have been followed for periods varying from two months to eight years after the acute infection. At the time of re-evaluation a neurological examination and an electroencephalogram have been performed. The age of the patient and the type of treatment, whether supportive or specific, has been correlated with residual objective changes.

Even though these patients may be asymptomatic at the time of follow-up, abnormal electrical activity of focal, generalized or borderline character may be detected in electroencephalograms. Neurological findings are variable; in most patients the findings are normal, but in others bodily asymmetry, reflex changes, often involving the pyramidal system, or impairment of fine motion are noted. Four children have developed a convulsive disorder. The remaining children may be more easily disturbed emotionally, even though the electroencephalograms are normal. Some of the adults complain of impaired general health without specific localization of symptoms.

Many other types of infection may be accompanied by minor neurologic abnormalities during the acute febrile phase. Other disease processes, whether or not accompanied by high fever, show vascular changes pathologically which resemble in some aspects those of rickettsial diseases. Diffuse brain damage as a sequela of acute infection in diseases where the etiologic agent does not specifically parasitize nerve cells may be a more general phenomenon than is now recognized.

Demonstration of a Thrombocytopenic Factor in the Blood of Patients with Idiopathic Thrombocytopenic Purpura. WILLIAM J. HARRINGTON, J. WILLIAM HOLLINGSWORTH, VIRGINIA MINNICH and CARL V. MOORE,* St. Louis, Mo.

The existence of a circulating thrombocytopenic factor has been demonstrated in six patients with idiopathic thrombocytopenic purpura. Three had developed thrombocytopenia acutely, one had chronic thrombocytopenia for at least three years and in two the disease had recurred after splenectomy.

The thrombocytopenic factor was demonstrated by transfusing 250 to 500 cc. of citrated whole blood or 100–300 cc. of citrated plasma from these patients into normal subjects or into patients with incurable disease. When the larger amounts of whole blood or plasma were given intravenously, the platelet level fell in the recipients within 30 minutes and frequently was less than 10,000 per cu. mm. at the end of one to two hours. In the more severe reactions purpura resulted. Thrombocytopenia persisted for four to six days, during which time megakaryocytes showed characteristics of immaturity.

ACTH, given to one recipient, did not prevent the effects from occurring. That the spleen is not required for the action of the thrombocytopenic factor, was demonstrated by the fall which occurred in one recipient whose spleen had been removed at the time of gastric resection for carcinoma of the stomach. However, blood obtained from one of the patients both three and ten days after splenectomy, at which times her platelet counts were 1,000,000 per cu. mm., caused a moderate platelet decrease in a normal subject.

The thrombocytopenic factor was stable for at least eight to ten days at −5°, 5°, and 26°C. It has been found in the plasma of all patients with idiopathic thrombocytopenic purpura studied to date and has not been demonstrated in the blood of control subjects.

Attempts are currently being made to determine whether platelet lysis or agglutination is responsible for the platelet disappearance.

The Enzymatic Nature of the Factor in Normal Serum Which Hemolyses the Erythrocytes of Paroxysmal Nocturnal Hemoglobinuria. J. W. HARRIS, W. S. JORDAN, L. PILLEMER and J. F. DESFORGES, Boston, Mass. (Introduced by Thomas Hale Ham).

The erythrocytes in paroxysmal nocturnal hemoglobinuria are hemolysed by a thermolabile factor in normal serum most active at pH 6.5. This activity (PNH hemolysis) has been ascribed to both complement and ac-globulin.

Serum dialysed against isotonic sodium chloride, C.P., retained both complement and ac-globulin activity; PNH hemolysis was lost but was restored by adding magnesium. Complement activity was slightly augmented by either magnesium or calcium; ac-globulin activity was unchanged or diminished by magnesium.

Serum treated with barium sulfate and then dialysed showed PNH hemolysis on addition of magnesium; ac-globulin activity was diminished varying with the amount of barium sulfate used. A trace of PNH hemolysis present in a purified plasma ac-globulin preparation was not significantly altered when ac-globulin activity was (*1*) increased on conversion to serum ac-globulin; (*2*) varied by adjusting its concentration to less than, equal to, or greater than that in normal human serum.

Serum fractions prepared by ammonium sulfate or alcohol precipitation lacked PNH hemolysis. Removal of any component of complement was accompanied by loss of PNH hemolysis. After addition of magnesium, full PNH hemolysis was found in the supernatant obtained by dialysis of serum against distilled water or 0.02 M salt at pH 7.3; the precipitate formed on dialysis may contain an inhibitor. PNH hemolysis is inhibited by heated human serum.

Incubation of normal and PNH stroma with dialysed serum liberated nitrogen from only the PNH stroma and only in the presence of magnesium at pH 6.5, suggesting the selective action of a proteinase on the abnormal PNH stroma.

The serum factor which hemolyses PNH erythrocytes thus resembles a metal requiring enzyme which (*1*) differs from hemolytic human complement by its dependence on magnesium and inhibition by calcium; (*2*) is not related to ac-globulin activity.

Disturbances of Concentrations of Intracellular and Extracellular Electrolytes in Patients with Tuberculous Meningitis. HAROLD E. HARRISON,* LAURENCE FINBERG and EVELYN FLEISHMAN, Baltimore, Md.

Hyponatremia and hypochloremia are consistently found in patients with tuberculous meningitis. The sodium and chloride deficit cannot be explained by inadequate intake of sodium chloride nor by losses in vomitus, and the concentrations of these ions in the urine may be high despite the reduced levels in the serum. Similar findings have been reported in occasional patients with pulmonary tuberculosis.

Analyses of the red blood cells of subjects with tuberculous meningitis reveal an increase in red cell sodium with no significant change in cell potassium. The total concentration of univalent cations in red cell water is increased despite the decrease of total univalent cations in serum water. When remission of tuberculous meningitis results from streptomycin therapy the red cell and serum electrolytes return to normal and the normal ratio between cell and serum cations is reestablished.

Analyses of biopsy samples of skeletal muscle from patients with tuberculous meningitis show a reduction of muscle potassium and an increase of muscle sodium. Similar potassium deficiency and sodium excess are found in skeletal muscle obtained postmortem from subjects dying of tuberculous meningitis.

It is suggested that tuberculous infection causes an accumulation of sodium in cells by interference with the cellular mechanisms necessary for active removal of sodium from cells. Some of the intracellular cation, presumably the retained sodium, must be combined in an

undissociated form since intracellular fluid is in osmotic equilibrium with extracellular fluid. Accumulation of intracellular sodium is associated with a loss of cell potassium in skeletal muscle but not in red blood cells. Analogous changes in intracellular electrolyte of renal tubule cells might explain the disturbances of renal tubular reabsorption of sodium and chloride.

Studies on "Heparin Tolerance": An Analysis of Factors Influencing the Anticoagulant Activity of Heparin. ROBERT C. HARTMANN, C. LOCKARD CONLEY and JULIUS R. KREVANS, Baltimore, Md. (Introduced by A. McGehee Harvey).

A correlation between increased resistance to the anticoagulant activity of heparin and tendency to thrombosis has been reported by several investigators. However, the factors which influence the results of "heparin tolerance" tests have not been previously studied. A systematic investigation of factors concerned with "heparin tolerance" was carried out. It was shown that the concentration of heparin required to prolong the coagulation time of blood was directly related to the platelet count. It was also demonstrated that platelet-free plasma from normal human subjects as well as patients with thrombotic or hemorrhagic diseases did not affect the anticoagulant activity of heparin. Others have suggested that hypercoagulability of the blood and increased heparin resistance might be associated with liberation of thromboplastin into the blood, as, for example, following surgical operations. Therefore, experiments were carried out to determine the effect of intravenous infusion of thromboplastin on "heparin tolerance."

Normal dogs, anesthetized with nembutal, were given intravenous infusions of homologous brain thromboplastin. The rate of infusion and thromboplastic activity of the injected material were varied over wide ranges. *In vivo* and *in vitro* "heparin tolerance" tests were performed repeatedly before, during, and after the infusion of thromboplastin. In no instance was an increased resistance to heparin noted. Infusion of small amounts of thromboplastin resulted in an exaggerated response to heparin and larger amounts delayed coagulation of blood. These effects occurred before there was a measurable reduction in the fibrinogen level of the plasma.

The results provide no evidence that the intravenous injection of thromboplastin brought about increased resistance to heparin. No evidence was found to support the view that the "heparin tolerance" test is a measure of circulating thromboplastin.

The Capacity of Patients with Chronic Hepatic Disease to Produce Antibody, and the Effect of ACTH and Cortisone on This Function. W. PAUL HAVENS, JR.,* JAMES M. SHAFFER and CHARLES J. HOPKE, JR., Philadelphia, Pa.

Twenty-seven Schick-negative patients with chronic hepatitis or cirrhosis and twenty-nine Schick-negative patients with other diseases were inoculated with 50 Lf purified diphtheria toxoid, and the amount of circulating antitoxin was determined at frequent intervals during the following month. Thirty-one percent of the control subjects had maximum measured amounts of antitoxin greater than 80 units/ml. serum, with an average of 97 units/ml. Seventy-four percent of the patients with hepatic disease had greater than 80 units antitoxin/ml. serum, with an average maximum measured amount of 286 units/ml. There was no apparent relationship between the unusual capacity of patients with chronic hepatic disease to make antitoxin and the age of the patient, severity of disease, state of nutrition, amounts of serum albumin and globulin, and resistance to intercurrent infection.

Cortisone and ACTH were administered to eight patients for periods of 9–15 days, beginning the day of injection of toxoid. All patients produced antitoxin—some in large amounts. Simultaneous determinations of serum globulin and antitoxin indicated, however, that tremendous increases in antitoxic protein (3 gm./100 ml. serum) were not manifested by comparable increases in serum globulin. It is undetermined whether this suggests a preferential use of globulin for antibody or an equilibration of two opposing forces—(1) the catabolic action of ACTH or cortisone and (2) stimulation for production of antibody afforded by the toxoid.

Cardiovascular Adjustments in Subjects with Organic Heart Disease Before and After Conversion of Atrial Fibrillation to Normal Sinus Rhythm. HANS H. HECHT,* WILLIAM J. OSHER and ARTHUR J. SAMUELS, Salt Lake City, Utah.

Respiratory and cardiac function at rest and on exercise were measured by venous catheterization in twenty-four subjects with chronic atrial fibrillation and organic heart disease. All patients had been under the influence of digitalis and diuretics and seemed to derive no further benefit from hospital management. In fifteen, the examinations were repeated upon restoration of a normal sinus mechanism by quinidine. No adverse reaction occurred during or following a total of 154 output determinations on these patients, and no embolic phenomena developed upon conversion. The data were compared to those obtained under similar conditions from twelve normal subjects and from twelve patients with pulmonary heart disease in a comparable state of "compensation."

Three types of responses were noted, apparently irrespective of the clinical state prior to conversion. One group derived no apparent improvement in cardiac output from conversion either at rest or on exercise, another demonstrated significant improvement upon conversion, both at rest and more particularly on exercise. A third group revealed definite improvement on exercise only.

The average resting output of all subjects examined was below normal before conversion and changed little when normal sinus rhythm had been restored. The average cardiac output on exercise improved significantly upon conversion. Oxygen extraction failed to rise on exercise prior to conversion but rose considerably thereafter. Pulmonary ventilation after exercise rose strikingly in subjects with atrial fibrillation before but changed only

moderately after normal sinus rhythm had been established. The arterio-venous oxygen difference was high during the stage of fibrillation and on the average returned toward but did not reach normal values upon conversion. Significant pressure changes were only occasionally encountered.

These observations indicate that a coordinate contraction of the cardiac atria aids in cardiac filling during demand periods and that this reserve function is lost in atrial fibrillation.

Effect of Testosterone upon the Human Testis. CARL G. HELLER,* WARREN O. NELSON, WILLIAM O. MADDOCK, EDWIN C. JUNGCK, C. ALVIN PAULSEN and GLENN E. MORTIMORE, Portland, Oregon, and Iowa City, Iowa.

Twenty normal adult men were given intramuscular injections of testosterone propionate 25 mg. daily for 24 to 100 days, or were implanted with testosterone pellets. Testicular biopsies, urinary gonadotrophins and sperm counts were performed before, at end of and 6 to 33 months after cessation of therapy.

At conclusion of treatment gonadotrophins and sperm counts were zero. Testicular biopsies revealed germinal and Leydig cell damage indicated by 1) decreased size of seminiferous tubules, 2) severe sclerosis and hyalinization of the membranes of the tubules, 3) necrosis and desquamation of spermatogenic cells, 4) decrease in total numbers of germinal elements and 5) absence of Leydig cells.

Six months after cessation of treatment slight improvement in histological appearance of the testis was noted. By 17 months the testes were near perfect in microscopic detail, sperm count was as high or higher than initially and gonadotrophins were normal (having been elevated in the immediate post-treatment period). Improvement appeared to be sustained at 33 months in one patient in whom a fifth testicular biopsy was obtained.

The most remarkable change was the disappearance of the hyalinization and sclerosis of the membranes of the seminiferous tubules. Some of the testes were originally involved with sclerosis and poor spermatogenesis. This too disappeared. These observations are being applied to clinical infertility.

The Metabolic Fate of Radioactive Carbon Labelled Acetate and Glycine in Humans. LEON HELLMAN, WENDELL PEACOCK, MAXWELL EIDINOFF, ROBERT ROSENFELD and THOMAS GALLAGHER, New York, N. Y. (Introduced by Konrad Dobriner).

During a study of the metabolic effects of ionizing irradiation, 6 patients with limited life expectancy were given 200 microcuries of 2-C^{14} acetate or 2-C^{14} glycine with the approval of the Atomic Energy Commission. The amount of activity administered would deliver only 20 per cent of the daily tolerance dose of radiation if uniformly distributed and permanently retained in the body.

In the first day, approximately 60 per cent of the dose appeared in exhaled breath, 3 per cent in urine, and 2 per cent in stool. During the next two weeks, more than 90 per cent of the dose was accounted for through excretion. Two patients came to autopsy and analysis of tissues showed there was no significant localization of radioactivity and that the highest specific activity of C^{14} was comparable to that of exhaled CO_2.

There was enough activity incorporated in tissue constituents to allow measurement of some dynamic aspects of hemoglobin, protein, and steroid metabolism. The average life of serum protein was 14 ± 2 days and that of serum cholesterol 11 ± 2 days. The average life of urinary ketosteroids was comparable to serum cholesterol. Data concerning the rates of synthesis of these constituents will be presented.

These studies have demonstrated that C^{14} in the form and quantity used does not present a radiation hazard and is a valuable tracer for use in humans.

Hypoxia as a Respiratory Stimulant in Cardiac Dyspnea. J. B. HICKAM,* H. O. SIEKER and J. M. RYAN, Durham, N. C.

Arterial blood oxygen is not usually thought to be significant in regulating breathing during congestive heart failure, because there is little arterial unsaturation. In normal man similar unsaturation is only a small respiratory stimulant, and the administration of 100 per cent O_2 to normal man at sea level is reported to cause only a small immediate decrease in ventilation. However, 100 per cent O_2 during severe exercise normally diminishes breathing significantly. It was thought possible that a similar "oxygen sensitivity" might occur in congestive failure.

Ventilation volume of subjects was recorded while breathing different gas mixtures. Arterial blood samples were taken through an in-lying needle.

Twelve patients with varying amounts of cardiac insufficiency, but not dyspneic at rest had an average arterial O_2 saturation of 93 per cent. They showed an average decrease of 14 per cent in ventilation volume on breathing 100 per cent O_2. The decrease occurred within the first minute and ventilation quickly climbed toward the previous level as the arterial pCO_2 rose. Twelve normal subjects breathing 15 per cent O_2 had a similar response to 100 per cent O_2. It is concluded that the cardiac group was not significantly different from the normal, but that oxygen lack played an appreciable role in driving respiration in both groups.

In 4 subjects with congestive failure and orthopnea the immediate ventilation decrease on 100 per cent O_2 averaged 36 per cent. These subjects were not significantly more hypoxic than the other group and still showed a large ventilation decrease on 100 per cent O_2 when their arterial saturation was previously raised above the group average by 30 per cent O_2. Preliminary results with the same technique suggest that persons with mild congestive failure are more sensitive to hypoxia during light exercise than are normal subjects.

These early results suggest that arterial oxygen unsaturation is a significant respiratory stimulant in cardiac dyspnea.

An Experimental Study of Practical Diets to Reduce the Human Serum Cholesterol. EUGENE A. HILDRETH, SHERMAN M. MELLINKOFF, G. WALKER BLAIR and DOROTHY M. HILDRETH, Philadelphia, Pa. (Introduced by Isaac Starr).

The aim of this investigation was to discover whether the serum cholesterol in human subjects could be lowered by reducing the cholesterol of the diet alone; or must the total fat of the diet also be reduced? Since vegetable fats contain no cholesterol, we investigated the effects of the ingestion of vegetable fat on the serum cholesterol concentration.

Three of the authors served as subjects, remaining on special restricted diets for periods up to one year. One of the subjects had a family history of coronary artery disease and was found to maintain a constant hypercholesterolemia. The second subject had normal serum cholesterol levels but a family history of coronary artery disease at an early age. The last subject had a normal serum cholesterol concentration and a negative family history for coronary artery disease.

Each subject was studied, first on his customary diet, which was essentially a normal fat, normal cholesterol diet; second, when both fat and cholesterol were restricted; and third, when, by a diet containing vegetable fats, cholesterol was restricted but total fat was normal in amount. The protein and caloric content of the diet were maintained constant throughout the study; the subjects lost no weight. Serum cholesterol concentrations were estimated twice weekly.

The diet low in fat produced a significant fall in the serum cholesterol concentration in all three subjects and that of the hypercholesterolemic subject fell to normal levels. In all three subjects the subsequent addition of vegetable fat containing no cholesterol to this diet was associated with a rise in the serum cholesterol concentration to the control levels. Therefore, in these subjects, the restriction of total fat and not of cholesterol alone was the decisive factor in lowering the serum cholesterol concentration.

Variations in Blood Glucose in Diabetes Mellitus. LAWRENCE E. HINKLE, JR. AND STEWART WOLF,* New York, N. Y.

Significant variations in urine excretion and blood ketones have been demonstrated in normal and diabetic humans under the stress of daily life. To investigate the relation of fasting blood glucose to these changes, serial measurements of blood glucose, blood ketones, and rates of water, chloride, and glucose excretion were made on 22 non-diabetic persons and on 27 diabetics in various states of compensation, in a post-absorptive state 24 hours after their last insulin injection. Stress was provided by an interview arousing significant emotional conflicts. The observations were controlled by a second "neutral" experiment.

In control experiments there was comparatively little variation in blood sugar. During stress situations accompanied by feelings of anxiety, hostility, and depression, a fall in blood glucose of 10 to 35 mg per cent was often observed in non-diabetic subjects. Among diabetics large and rapid changes in blood sugar occurred during stress; falls as great as 80 mg per cent in 60 minutes and as rapid as 59 mg per cent in 11 minutes were observed, and rises as rapid as 41 mg per cent in 15 minutes. The direction of the change appeared to be related to the subject's attitude toward the situation as well as to his previous metabolic state. There was neither a fixed relation between the concentration of glucose and ketones in the blood, nor a positive correlation between blood glucose and the rate of excretion of water, chlorides, and glucose in the urine, except in that glycosuria occurred only when blood glucose was relatively elevated.

The findings indicate that random samples of fasting blood glucose are not a reliable guide to the compensation of diabetes and do not correlate directly with other indicators of diabetic metabolism. Moreover, it is apparent that a careful study of the patient with his experiences and attitudes is of first grade importance in the management of diabetes mellitus.

The Life Span of Transfused Human Blood Platelets. ERWIN O. HIRSCH and FRANK H. GARDNER, Boston, Mass. (Introduced by Clifford L. Derick).

Human blood platelets were introduced into thrombocytopenic patients by transfusion of platelet-rich blood from polycythemic donors using syringes and needles coated with silicone. By this method platelet counts were increased by an average of 90,000 per cu. mm. Such platelet transfusions were followed by cessation of spontaneous bleeding and return of the bleeding time, prothrombin conversion, clot retraction and vascular fragility towards normal, indicating that a primary vascular defect is not the cause of bleeding in the thrombocytopenias examined. Platelet survival was determined by noting the return of the platelet count to pretransfusion levels.

Repeated transfusions in one case of aplastic anemia and one of acute leukemia indicated platelet survival of four to six days. In three patients with chronic (idiopathic) thrombocytopenia who had normal numbers of megakaryocytes and no splenic enlargement platelet survival was four to eight days, as determined on repeated occasions. In these patients with normal platelet survival even widespread hemorrhages and in one case splenectomy did not appear to affect the lifespan of the transfused platelets. Hemorrhage thus does not involve significant utilization of platelets and their life span is not significantly increased by removal of a normal spleen. Moreover it appears that platelet destruction is not the cause of the low platelet count in the chronic thrombocytopenic states characterized by normal numbers of megakaryocytes and non-palpable spleens.

In three cases of secondary thrombocytopenia associated with normal megakaryocytes and splenomegaly and in two cases of acute (idiopathic) thrombocytopenia without splenomegaly platelet survival was 24 hours or less in-

dicating that in these diseases rapid platelet destruction may at least contribute to the thrombocytopenia.

These observations illustrate two types of mechanisms for thrombocytopenia. In our experience platelet transfusions were beneficial in both acute and chronic thrombocytopenias, controlling acute hemorrhagic episodes and in preparing patients for surgery.

Characterization of Leucocyte Zinc Protein. FREDERIC L. HOCH and BERT L. VALLEE, Boston, Mass. (Introduced by Mark D. Altschule).

The behaviour of a soluble zinc protein from human leucocytes with stepwise ammonium sulfate fractionation and activity measurements has been used to differentiate it from carbonic anhydrase due to erythrocytes present with the leucocytes. The amount of carbonic anhydrase present *calculated* on the basis of hemoglobin measurements (Keilin and Mann) agrees well with that recovered by ammonium sulfate precipitation at 85–90 per cent saturation as determined by zinc measurements, confirming its differentiation from leucocyte zinc protein.

The *soluble* leucocyte proteins have been further investigated for behaviour under varying conditions of pH and ionic strength. Zinc protein fractions, twice precipitated between 50 and 60 per cent ammonium sulfate saturation, were soluble at pH 7.2. Lowering of the ionic strength by dialysis against distilled H_2O, 4°C, or against a buffer of pH 6.0, ionic strength 0.01, 4°C precipitated a fraction containing 0.2 per cent zinc. Identical results were obtained at pH 6.0, ionic strength 0.1, alcohol 10 per cent, −6°C. This fraction was tested for solubility over the range pH 3.0 to 9.4, ionic strength 0.1 to 1.0, 4°C, its maximum being at pH 5.0, ionic strength 0.5. Dissociation of zinc occurs at pH 4.0, ionic strength $\geqq$ 0.5, and at pH 3.0, ionic strength 0.1. Ultracentrifugation studies of these soluble fractions are in progress, as are emission spectrographic analyses for cation distribution in all leucocyte protein fractions.

The leucocyte fractions *insoluble* with the extraction procedures contain 20 per cent both of the original total zinc and protein. Hot and cold trichloroacetic acid precipitations indicate that nucleic acids comprise 9 per cent of the total dry weight of this residue. Such substances, with an absorption maximum of 260 to 265 mμ, were soluble above pH 6.2, when the residue was tested for solubility from pH 4.2 to 9.2. There was no correlation between nucleic acid and zinc solubilities.

Effect of Pitressin Induced Water Retention on Sodium Excretion. B. C. HOLLAND, Durham, N. C. (Introduced by Eugene A. Stead, Jr.).

Patients with congestive heart failure retain salt and water. Anti-diuretic materials are found in urine of patients with congestive heart failure. This study was designed to determine if water retention from pitressin would cause sodium retention. Pitressin has been shown to have the same effect on salt and water excretion as endogenously produced post-pituitary antidiuretic hormone.

Observations with and without pitressin were made on 12 subjects. Three days before the experiment, these subjects received a 2500 calorie diet containing 8 grams of sodium chloride. On the day of the experiment they drank 200 cc of 0.2 per cent sodium chloride solution every hour for 24 hours. Urines were collected hourly. Blood samples were taken at the beginning and end of each experiment.

After a three day balance period, this experiment was repeated. Subjects received in addition 1 milliunit/kg pitressin intravenously every hour. In 4 subjects the pitressin experiments were done first.

In control experiments subjects did not gain weight; there was no edema; blood sodium remained the same; they excreted 50–70 per cent of their sodium intake in 24 hours.

In pitressin experiments subjects gained 2 to 4 kg. in weight. All became puffy in the face and had traces of ankle edema. All had extreme oliguria. Two had falls of blood sodium levels from 140 to 130. Yet despite water retention and electrolyte dilution, sodium excretion was unchanged. Analysis of data after 16 hours of water retention showed no significant retention of sodium in the last 8 hours.

The following interpretations seem possible: 1) Pitressin exerts sodium diuretic action which exactly counterbalances sodium retaining mechanisms of electrolyte dilution. 2) Fall in sodium concentration without decrease in total body sodium is not strong stimulus for sodium retention. 3) Fall in sodium concentrations is a stimulus for sodium retention but 24 hours is not long enough to set in motion sodium retaining mechanisms.

Comparative Effects of Compound F (17-Hydroxycorticosterone) and Cortisone Injected Locally into the Rheumatoid Arthritic Joint. JOSEPH L. HOLLANDER, ERNEST M. BROWN, JR., RALPH A. JESSAR and CHARLES Y. BROWN, Philadelphia, Pa. (Introduced by Francis D. W. Lukens).

Injection of 25 to 50 mg. of cortisone directly into the synovial cavity of one inflamed knee joint in each of 11 patients with rheumatoid arthritis failed to cause any beneficial effects as measured by symptoms and physical signs, by lowering the cell count of the synovial fluid, or particularly by a significant reduction (i.e. more than 0.4°C.) in the intra-articular temperature. In contrast, the administration of 25 mg. of Compound F of Kendall into a single diseased joint in each of 7 patients with rheumatoid arthritis has resulted in marked local improvement in symptoms and signs, a fall in the cell count of the synovial fluid, and a significant fall (0.6° to 1.9°C.) in intra-articular temperature within 24 hours in every instance. Contralateral diseased joints were used as controls and showed no significant change, nor were there other signs of systemic effect.

The intra-articular temperature was measured by an electronic potentiometer with the filamentous copper-constantan thermocouple inserted into the knee joint space through an aspirating needle. As previously reported, the maximum daily variation of knee joint temperature in

rheumatoid arthritis is ± 0.2°C. under standard conditions of rest and room teemperature. The joint temperature returned to pre-treatment level within 48 hours in most instances. Some clinical improvement has persisted for as long as 10 days after a single injection of 25 mg. of Compound F into the joint. It would appear that the demonstration of a direct action and beneficial effect of Compound F, in apparent contradistinction to Compound E, upon the tissues of the rheumatoid arthritic joint, further emphasizes the physiological importance of Compound F.

Effects of Protoveratrine on the Circulation in Hypertension. S. W. Hoobler, R. W. Corley and T. G. Kabza, Ann Arbor, Mich. (Introduced by C. C. Sturgis).

Protoveratrine, a purified alkaloid of Veratrum album, was demonstrated by Meilman and Krayer to reduce the blood pressure and pulse rate when injected into hypertensive patients, without production of nausea or vomiting. These observations have been confirmed and hemodynamic studies to be reported indicate that the drug reduced peripheral resistance to blood flow in the forearm an average of 30 per cent in 6 cases, and in the foot by 32 per cent in 13 cases. After lumbar sympathectomy protoveratrine did not produce dilatation in the foot, suggesting that its peripheral vasodilator action is mediated over sympathetic pathways. Renal blood flow was unchanged or slightly increased, and glomerular filtration rate (creatinine or mannitol clearance) showed no change or slight decreases. Effects on cardiac output and total peripheral resistance were variable, as determined in seven hypertensive patients by the direct Fick technique. In two patients with marked reductions in blood pressure, cardiac output increased and total peripheral resistance declined. In the remainder no change or a slight decrease in these indices occurred.

Atropine abolished drug induced bradycardia and usually produced some elevations in blood pressure, but not to pre-injection levels.

0.750–1.5 milligrams orally produces a gradual lowering of blood pressure and pulse rate over a 4–6 hour period and may relieve headache, hypertensive encephalopathy, and cardiac dyspnea with reduction in heart size and venous pressure. The oral use of the drug is occasionally associated with nausea and vomiting. It has been administered 1–3 times daily over periods of 2–8 weeks in five severe ambulatory hypertensive patients who have kept daily records of their blood pressure. Evidence thus accumulated indicates that a significant reduction has been achieved and maintained over substantial portions of the day without serious side effects.

The Role of Infection in the Radiation Syndrome. Joe W. Howland, F. W. Furth and M. Coulter, Rochester, N. Y. (Introduced by Herbert Morgan).

Study of the role of infection and infectious factors in large numbers of dogs and rats exposed to lethal dosage (LD/85–90) of whole body 250 KV x-radiation has been carried out. Bacteriological study indicated that 16 per cent of 120 dogs and 22 per cent of 250 rats shows positive cultures for oganisms of the type normally found in the gastro-intestinal canal. Similar organisms are found in all organs cultured at autopsy. The effect of oral administration to the rate of antibiotics including aureomycin, streptomycin, chloromycetin, polymyxin and others indicates that all to some extent inhibit the diarrhea, lessen weight loss and enhance early recovery in the rat. At this dosage level of x-radiation, mortality is not improved in high degree. In the dogs oral aureomycin and terramycin in daily dosage of 50–100 mg./Kg. for 28 days are superior to others tested in that the latent period is extended for 10–14 days longer than the parallel controls, 50–70 per cent of which will die before the first treated animal becomes ill. Improvement in mortality (120 days) in consecutive experiments is shown in a reduction from LD/85–90 to an approximate LD/35–40. Most of the deaths in treated dogs are related to the hemorrhagic diathesis or the presence of or development of resistant organisms to the antibiotic used in this experiment. Alterations in intestinal flora resulting from prolonged administration of antibiotics are similar to those reported for man. The development of significant cross resistance in gram negative bacteria treated with a single antibiotic to other antibiotics is shown. In these experiments no therapy other than administration of antibiotics has been used.

Ferrokinetics in Normal Subjects and in Patients Having Various Hematopoietic Disorders. Rex L. Huff, Paul J. Elmlinger, Joseph F. Garcia, John Oda and Marion Cockrell, Berkeley, Calif. (Introduced by John H. Lawrence).

With the advent of high efficiency scintillation counters, it is possible to study the movement of Iron-59 in the body even when less than tolerance doses are given. Approximately 30 patients with abnormalities in blood formation and 7 normal male adults have been studied by a combined blood sampling and in vivo counting technique. The depletion from the plasma of the instantaneously injected Iron-59 Globulin IV-7 by bone marrow, liver, spleen, or by combination of the three tissues can be readily observed. The formation of red cells in abnormal sites such as spleen and liver is apparent with continued study.

Storage of greater than normal quantities of tracer in the liver has been observed in certain cases.

Excessive rates of destruction of red cells were interpreted as occurring because of higher splenic body surface counting rates at a time when newly tagged cells were leaving the bone marrow to enter the circulation. In such patients corroborative evidence of excessive cell destruction was obtained.

Patients with polycythemia vera may or may not have splenic erythroid metaplasia while chronic myelogenous leukemia patients invariably show erythroid metaplasia of the spleen and liver as evidenced by the Iron-59 kinetics.

A large spleen seen with chronic lymphatic leukemia does not exhibit the ability to form red cells but may show excessive destruction of cells.

Hypoplasia or aplasia of the erythroid elements of the bone marrow is evident with failure of the tracer to accumulate and discharge from the normal marrow locations. The tracer evidence of hypoplasia and aplasia has been corroborated by marrow biopsy.

Beta Glucuronidase Production by Beta Hemolytic Streptococci. RALPH F. JACOX,* Rochester, N. Y.

Recent investigations of streptococcal enzyme systems by other workers have revealed that several enzymes are elaborated which depolymerize hyaluronic acid and nucleic acids. Another streptococcal enzyme concerned with breakdown of glucuronides has been found in this laboratory. This enzyme, a beta glucuronidase, is elaborated by 4 of 29 subtypes of group A streptococci studied, namely, types 2, 11, 13 and 25 and by several of group C streptococcal strains. Adaptive enzyme production is not stimulated by addition of a glucuronide substrate in distinction to the findings in the case of glucuronidase synthesis by *E. coli* and *Staphylococcus albus;* nor is stimulation of glucuronidase formation found when enzyme positive strains are grown in a glucuronide medium. Streptococcal glucuronidase is chiefly an intracellular enzyme in distinction to the extracellular characteristics of *E. coli* glucuronidase. A point of similarity between glucuronidase of streptococci and *E. coli* is a similar pH optimum of 6.5 for maximum enzyme activity.

Assay of 60 strains of streptococci isolated from children with uncomplicated acute tonsillitis reveals that none produced glucuronidase. Fourteen of 30 strains isolated from streptococcal carriers in a boys' institution of 300 inmates were positive for glucuronidase. It is of interest that prior to this investigation ten cases of acute rheumatic fever had occurred among this group of 300.

Preliminary antibody studies against streptococcal glucuronidase in sera of patients with rheumatic fever have failed to reveal any inhibition of enzyme activity for group A or C streptococci.

These observations on streptococcal glucuronidase appear to be of chief interest because of the possible biochemical implications of infections with glucuronidase positive strains of streptococci since enzymes of this type may play a role in hyaluronic acid degradation or with any metabolite conjugated in a beta glucosidic linkage.

Lack of Correlation Between Immunologic and Electrophoretic Estimation of Gamma Globulin in Human Serum. B. V. JAGER* and E. L. SMITH, Salt Lake City, Utah.

With rabbit antisera to human gamma globulin and with the quantitative precipitin technique, the gamma globulin content of human serum can be estimated immunologically. In a previous study we indicated that the values for gamma globulin obtained immunologically are appreciably greater than the values for gamma globulin estimated by the electrophoresis technique.

To investigate the problem further, a number of rabbit antisera to human gamma globulin was studied. These antisera react to a considerable degree with human serum from which the gamma globulin has been removed completely by a salting-out procedure. When the gamma globulin-free human serum was tested against the antiserum with the agar diffusion technique of Oudin, it was found that at least two antigenic substances present in this fractionated human serum react with the antiserum to human gamma globulin. It would appear that this cross-reacting antigenic substance is responsible for the excessively high immunologic values for gamma globulin as contrasted with electrophoretic estimations of this component.

By means of quantitative procedures it was found that with a single antiserum to gamma globulin, the discrepancies between immunologic and electrophoretic estimations varied considerably with the individual human serum being examined for gamma globulin content.

With different antisera to gamma globulin, immunologic estimations of gamma globulin on a single sample of human serum gave widely different values.

Partial absorption of gamma globulin antiserum with gamma globulin-free normal human serum did not eliminate the cross-reacting properties of the antiserum and failed to yield an antiserum which was specific for gamma globulin.

These studies reveal some of the difficulties that arise in estimating the gamma globulin content of human serum with an immunologic technique.

The Blood Pressure of Patients with Hypertension After Subtotal Adrenalectomy: Relationship to the Amount of Residual Adrenal Tissue and Substitution Therapy. WILLIAM A. JEFFERS,* HAROLD A. ZINTEL, JOSEPH H. HAFKENSCHIEL, FRANCIS C. DOHAN* and NELLY J. KEFFER, Philadelphia, Pa.

Eighty-five to 95 per cent of their adrenal tissue has been removed from 17 patients having severe essential hypertension. Only two of the surviving 14 patients have failed to show a considerable fall in blood pressure after observation for from two weeks to 12 months. Three have died, from 7 to 30 days after operation, each from causes other than adrenal insufficiency.

This report is an attempt to correlate levels of blood pressure post-operatively with the size of the adrenal remnant, residual cortical function (urinary corticoids and ACTH-eosinophile test), and the type and amount of substitution therapy administered.

Preliminary observations suggest that a prolonged hypotensive effect may be accompanied by diminished urinary excretion of corticoids, without concomitant abnormalities of serum electrolytes. It is our current impression that sufficient adrenal tissue should be removed to render these patients susceptible to transient periods of adrenal insufficiency in order to produce an optimal effect upon blood pressure. Conversely, the majority of patients have as yet not required the prolonged use of substitution therapy following subtotal adrenalectomy.

Chemical Abnormalities in the Erythrocytes in Sickle Cell Anemia, Their Relationship to Sulfhydryl Metabolism

and the Effects of ACTH. EDWARD H. KASS, SIDNEY H. INGBAR, JOHN W. HARRIS and ALLYN B. LEY, Boston, Mass. (Introduced by Maxwell Finland).

Sulfhydryl (SH) inhibitors inhibit sickling *in vitro*, suggesting that SH activity is related to the sickling phenomenon. The reduced glutathione (GSH) content of the erythrocytes of patients with sickle cell anemia was significantly greater than that of normal erythrocytes. Prolonged saline dialysis removed the GSH from the erythrocytes and rendered them incapable of sickling. Purified sickle cell hemoglobin contained 3 moles of SH/mole Hgb., by amperometric determination, whereas normal Hgb. contained 2 moles SH/mole Hgb. Variations in the apparent SH content of hemoglobin may be obtained by altering the technique of measurement.

Because ACTH may alter GSH content of erythrocytes and change the SH : N ratio of proteins, ACTH was administered to two patients with sickle cell anemia. In one patient, typical crises occurred twice following administration of ACTH. DOCA had no effect. The hematologic data and GSH content of the erythrocytes showed no significant changes. In the second patient a mild crisis was precipitated by ACTH but when the hormone was continued, the erythrocyte count rose gradually from 2.6 to 4.8 million rbc/cu.mm. The osmotic and mechanical fragilities of oxygenated blood samples, which were initially increased, became normal. Reticulocyte counts decreased fourfold. The number of permanently sickled cells in the blood fell from about 35 per cent to less than 5 per cent, and the fecal urobilinogen decreased fivefold. The erythrocyte GSH, initially 0.13 mcg. per million rbc, decreased to 0.074 mcg. When ACTH was discontinued, the hematologic findings reverted rapidly to their original status, and the GSH rose to .115 mcg.

The data suggest that the genetically determined abnormality in sickle cell anemia is related to sulfhydryl activity within the erythrocyte and that this abnormality may be altered by the action of ACTH.

Cardiac Output in Auricular Fibrillation with Observations on the Effects of Conversion to Normal Sinus Rhythm. ROSS C. KORY and GEORGE R. MENEELY, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Circulatory dynamics were studied by the technique of cardiac catheterization in eighteen patients with auricular fibrillations. Cardiac output was determined by the direct Fick principle with patients at rest and after standard exercise. Prior to catheterization all clinical resources except quinidine were used to place these patients in the best possible state of compensation with well-controlled ventricular rates. At the time of catheterization venous pressures were elevated in only two patients, but circulation times were prolonged in all eighteen.

In all of these patients the resting cardiac output was low (mean cardiac index: 1.89 liters/min/m^2 of body surface). Not only was the resting output low but the response to a standard bout of exercise was considerably below normal.

Eight of these patients were restudied after conversion to normal sinus rhythm with quinidine. Six of these had organic heart disease, cardiomegaly, and previous congestive failure. In these six patients there was a striking increase (average 43%) in both resting and exercise outputs after conversion to sinus rhythm. The two remaining patients had functional or idiopathic auricular fibrillation with normal heart size and no history of congestive failure; in these no significant change in cardiac output followed conversion.

It is suggested that auricular fibrillation in organic heart disease despite full compensation and a controlled ventricular rate is accompanied by a low cardiac output not only at rest but after exercise and that conversion to sinus rhythm is of real value in improving cardiac function.

Normal and Abnormal Swallowing Mechanisms Studied by the Measurement of Intraluminal Esophagus Pressures. PHILIP KRAMER, HERBERT MINKEL, HEDDY FRANK and FRANZ J. INGELFINGER,* Boston, Mass.

Normal and abnormal mechanisms of deglutition have been studied by measuring intraluminal pressures at various points between the nasopharynx and cardia. Pressures were recorded by means of a Sanborn electromanometer connected to an open-tipped, water-filled venous catheter positioned under fluoroscopic control. Such pressure records adduce information not obtainable by fluoroscopic or balloon-kymographic methods.

In normal subjects characteristic phasic complexes were noted after "dry" and "wet" swallows. The range of phasic pressures observed (20 to 50 mm. Hg) exceeded those heretofore believed to exist in the pharynx and the esophagus. The pressure waves, as measured by simultaneous recording from three esophageal points, progressed at a rate of 2.5 cm. per second. Negative pressures of -25 mm. Hg, which according to modern texts of physiology are integral to swallowing, were never observed. During rapidly repeated swallowing, the pressure waves of esophageal peristalsis were clearly inhibited until after the last swallow.

Two patients unable to swallow following bulbar poliomyelitis failed to develop adequate intrapharyngeal pressures on attempts to swallow, but qualitatively and quantitatively normal intraesophageal pressures could be initiated by the unsuccessful attempts at deglutition. It appears that the pharynx, although too feeble to propel liquid into the esophagus, may nevertheless initiate reflex esophageal motility normally.

In patients with cardiospasm, intrapharyngeal pressures were normal, but the normal esophageal pressure patterns were replaced by an irregularly progressive elevation of intraluminal pressure. This elevation presumably reflected the accumulation of swallowed liquids in an esophagus with impaired propulsive motility. The characteristic spasmogenic effect of Mecholyl in cardiospasm, previously studied in our laboratory by balloon-kymograph methods, is also demonstrable by measurement of intraesophageal pressures.

Extreme Hypergammaglobulinemia in Young Women with Liver Disease of Unknown Etiology. H. G. KUNKEL,* E. H. AHRENS, JR., W. J. EISENMENGER, A. M. BONGIOVANNI and R. J. SLATER, New York, N. Y.

Elevation of the gamma globulin fraction of serum in patients with liver disease is almost invariably present, but is rarely sufficient in degree to cause elevation in the total protein concentration of the serum. However, in a 5-year period, during which the sera of 1300 patients with liver disease were analyzed for protein fractions, 12 patients showed total proteins ranging from 9–13 gm. per cent. Electrophoretic studies showed that this rise was due entirely to gamma globulin increase.

Eleven of these 12 patients were females, and of these the maximum age was 32. The onset of disease was insidious, and the course was prolonged and either stationary or downhill, and frequently marked by the unusual features of periods of high fever, arthralgia, and arthritis. Pathologic study of the liver in 9 patients showed a remarkable degree of plasma cell infiltration, reaching 30–40 per cent of the nucleated interstitial cells. Plasmacytosis was not found in sternal marrow biopsies; in the liver it diminished during the course of disease. The etiology of the syndrome remains unknown.

The abnormal gamma globulin of 6 of these patients was found to be immunologically similar to normal gamma globulin or a fraction thereof, and could be measured quantitatively with rabbit antiserum prepared against normal gamma globulin.

Physiological Observations on a Case of Beriberi "Heart Disease" with a Note on the Acute Effects of Thiamine. W. J. LAHEY, D. B. ARST, M. SILVER, C. R. KLEEMAN and P. KUNKEL, New Haven, Conn. (Introduced by Arthur J. Geiger).

A patient with Beriberi and anasarca was studied on four occasions by cardiac catheterization. Measurements before thiamine revealed a cardiac output of sixteen liters per minute, slight elevation of right heart and pulmonary artery pressures, low glomerular filtration rate, and marked oliguria. Two injections of thiamine, one hour apart, were then given. The changes observed were an increase in the A–V O_2 difference, an increase in oxygen consumption, a fall in cardiac output, slight transient rises in pulmonary and brachial artery pressures and increases in glomerular filtration rate (GFR) and sodium clearance (C_{Na}). A single determination showed the renal plasma flow (RPF) to be one-half normal as contrasted with a cardiac output of three times normal during the same period. During the next two weeks the patient lost forty pounds. The venous pressure returned to normal. Restudy revealed no change in central pressures despite marked reduction in cardiac output. The GFR was normal. There were no significant changes after thiamine. A partial third study two weeks later showed no change in the mean auricular pressure. The fourth study, after the patient had been home for one week, revealed re-appearance of a high cardiac output and diminished A–V O_2 difference. The right heart pressures were unchanged. The peripheral venous pressure was normal. The RPF and GFR were near normal. It is concluded that the clinical picture of congestive failure may appear and be resolved in Beriberi in the absence of marked changes in cardiac function as indicated by right heart and pulmonary artery pressures. The peripheral venous hypertension seen may be due largely to transmission of arterial pressure through the dilated arteriolar bed. Abnormal distribution of blood flow with selective depression of flow to the kidneys and resultant disturbance of salt and water balance would appear to be of primary importance.

An Antidiuretic Mechanism Not Regulated by Extracellular Fluid Tonicity. ALEXANDER LEAF and AUDLEY R. MAMBY, Boston, Mass. (Introduced by J. H. Means).

Verney has demonstrated that an increase in solute concentration of plasma stimulates an increased output of antidiuretic hormone with reduction in urine flow while a dilution of the plasma inhibits posterior pituitary activity allowing a copious diuresis.

We have confirmed this sequence of events in normal humans and dogs. With water restriction there occurs an increase in total serum solute concentration and a very hypertonic urine. On administration of water, dilution of the extracellular fluid occurs and a hypotonic urine is formed. Assay of the serum shows antidiuretic activity with water restriction and none during water diuresis. This cybernetic mechanism is ideally designed to preserve constant tonicity of the body fluids.

Clinically we encounter patients who for long periods have low serum sodium and chloride concentrations. Such patients also have a reduction in total solute concentration of their sera. Why then do they not have a copious water diuresis and return their solute concentration to normal?

This has been investigated in uncontrolled Addison's disease and in patients with congestive heart failure who have low serum sodium concentrations. In both groups there is observed a dilute extracellular fluid, but in spite of this, a markedly hypertonic urine, an inability to have a water diuresis after water ingestion, and a high titre of antidiuretic activity in the serum.

It was thought that this abnormal antidiuresis might be related to depletion of extracellular electrolytes. By depleting a normal dog of extracellular fluid electrolyte it was possible to convert from the normal diuretic mechanism to this abnormal pattern.

Thus it appears that in the face of extracellular fluid electrolyte depletion the animal will sacrifice tonicity of the extracellular fluids, retain water and attempt to restore volume. The implications of these findings to regulation of extracellular fluid volume and edema formation are discussed.

Some Hemodynamic Aspects of the Peripheral Vessels in Hypertensive Vascular Disease. RICHARD E. LEE, New York, N. Y. (Introduced by Ephraim Shorr).

Certain features of the bulbar conjunctival capillary bed have been studied in 87 patients with hypertensive vascular disease. In comparison with normal subjects and patients with non-hypertensive illnesses, the terminal arterioles of hypertensive patients were found to be narrowed, with a slight, but significant reduction in velocity of arteriolar, capillary, and venular blood flow, as determined by micrometric methods. In addition, spontaneous vasomotor activity in arterioles, and in pre-capillaries, was Grade 3–4 in 10 of 12 hypertensives studied, as opposed to Grade 1 in 3 of 12 controls. Sixty two per cent of the hypertensive cases showed Grade 3–4 elongation, looping, and tortuosity of the true capillaries, while 9 per cent had Grade 1–2 such abnormalities. Tortuosity and coiling of capillaries, of Grade 1, were found in 8 per cent of controls.

There were no abnormalities observed in corpuscular flow (i.e. clumping of rbcs., or other cellular elements) that could be considered specific, either of hypertension or of any of the diseases studied. In addition, vasoconstrictor responses to topically applied cold (ice pack) were of similar magnitude in hypertensives and controls. However, the reactivity of the terminal arterioles in hypertension to directly applied epinephrine was approximately 10 × that found in healthy subjects. In the various normotensive diseases, a similar elevation of arteriolar epinephrine reactivity occurred only with Laennec's cirrhosis, and with cases of hyperglobulinemia. In these illnesses, however, the vessels did not display vasoconstriction, increased spontaneous vasomotion, nor abnormal capillary patterns. These latter phenomena, with associated elevation of epinephrine reactions, were noted only in subjects with hypertensive disease.

Glycine Metabolism in Rheumatoid Arthritis. HENRY M. LEMON, JOSEPH M. LOONEY and WILLIAM C. CHASEN, Boston, Mass. (Introduced by Chester S. Keefer).

Collagen and elastin are unique among tissue and serum proteins in containing 20–25 per cent glycine, the metabolism of which has been observed by utilizing the methods of Alexander for estimation of serum glycine and alanine, before and one hour after the intravenous injection of 1.77 gm. of sodium benzoate. The conjugation of glycine with benzoate and its rapid urinary excretion provides a physiologic load upon the hepatic and peripheral pool of this amino acid. Over thirty ambulatory control subjects including normals, metastatic cancers, and osteoarthritics showed a mean fall in serum glycine one hour after injection of 6.9 ± 1.3 (S.E.) per cent, with a mean hippurate excretion of 0.87 ± 0.03 (S.E.) gms. In more than 44 patients with diseases of collagen, of whom nearly 90 per cent were ambulatory rheumatoid arthritics with elevated sedimentation rates, the fall of serum glycine was three times that of the controls, 20.4 ± 2.3 (S.E.) per cent, with some values over 50 per cent. This greater fall was not produced by increased hippurate excretion, which averaged 0.93 ± 0.05 (S.E.) gm. in these cases, in whom 14 values were under 0.60 gms. Serum alanine fell 14.9 ± 3.5 (S.E.) per cent in controls and 18.7 ± 3.2 (S.E.) per cent in rheumatoids. Urinalysis, phenolsulfonphthalein excretion, bromsulfalein excretion, serum bilirubin, serum proteins, cholesterol ester ratios, thymol and cephalin flocculation were normal in nearly all cases, so that in no case could serum glycine alterations be attributable to disturbed hepatic or renal function.

Serial observations of serum glycine response correlated well with subjective and objective evidence of rheumatoid activity, including sedimentation rate, but not with the duration or extent of the disease. Some of the greatest reductions in serum glycine occurred early in the disease before marked radiologic changes. Both spontaneous and ACTH or cortisone induced remissions effected a return to a normal pattern, but there was no immediate alteration induced by hormone therapy. Diets low in glycine may re-activate rheumatoid activity, but supplementation of dietary glycine with 60–90 gms. daily has not as yet produced definite therapeutic benefit. The test has proven to be a useful diagnostic adjunct, and may indicate defective peripheral glycine metabolism in rheumatoid arthritis.

Effect of Cortisone and ACTH on Body Water and Electrolyte Distribution. MARVIN F. LEVITT and MORTIMER E. BADER, New York, N. Y. (Introduced by Alexander B. Gutman).

Serial determinations of extracellular volume (inulin space of distribution), plasma volume (T-1824 space), serum electrolyte concentrations, glomerular filtration rate and renal plasma flow were made before and during cortisone (100–200 mgms./day) or ACTH (100 mgms./day) therapy. All patients were maintained on a salt-free rice diet for 3–7 days prior to and during the course of hormone treatment. Throughout the control and treatment periods all urine was collected and analyzed for sodium, potassium, and chloride content.

Despite the rigid salt restriction, both cortisone and ACTH induced in each patient a progressive increase in approximately isotonic extracellular volume of 20–40 per cent. In 3 patients studied at frequent intervals during the course of hormone therapy, this increase in extracellular volume reached its maximum 8–9 days after the onset of treatment and then gradually decreased to control values despite the continuation of therapy. Neither the progressive increase nor subsequent decrease of extracellular fluid and total extracellular electrolyte was reflected in body weight changes or over-all sodium and chloride balances. The observed increase in plasma volume was much smaller and averaged 9 per cent. In each patient so studied, there was a gradual increase and later decrease in glomerular filtration rate and filtration fraction. Peak changes in extracellular volume and renal function coincided in time.

Previous evidence indicates that inulin is excluded from the cell and that its space of distribution affords the best method available for the measurement of extracellular volume. Provided that this premise is true, these data suggest that cortisone and ACTH produce a transient but significant shift of water, sodium, and chloride into the measurable extracellular space.

The Relation of Mitral Valve Area to Clinical Status in Patients with Mitral Stenosis. BENJAMIN M. LEWIS, FLORENCE W. HAYNES and RALPH J. SPIEGL, Boston, Mass. (Introduced by Reginald Fitz).

Thirty patients with mitral stenosis were studied by cardiac catheterization and from these data mitral valve areas and pulmonary arteriolar resistances were calculated by previously published formulae.

From analysis of history, physical examination, electrocardiogram, and x-ray done prior to catheterization, major symptoms and signs were found to begin at a valve area of 1.0 cm.^{2} (normal 4–6 cm.^{2}). Patients with larger valve areas had few and variable symptoms. Hydraulically, if a normal flow is to be maintained at a stenosis of 1.0 cm.^{2} or less, left atrial (and therefore pulmonary "capillary") pressure must rise to levels which approach plasma osmotic pressure, with the resultant threat of pulmonary edema. The one compensatory mechanism left such a patient is an increase of pulmonary arteriolar resistance, which, by checking the pumping ability of the right ventricle, would tend to prevent sudden rises in pressure in the capillaries of the lung. Such an increase occurs to a variable degree when the mitral valve area is 1.0 cm.^{2} or smaller. Thus, as the mitral valve area approaches 1.0 cm.^{2} in size and before resistance in the pulmonary arterioles rises appreciably, hemoptysis and paroxysmal nocturnal dyspnea are often noted. As the valve narrows farther and the resistance does rise appreciably, cardiac enlargement and signs of right ventricular failure usually follow. Significant linear correlations are found between cardiac enlargement and mitral valve area and between cardiac enlargement and pulmonary arteriolar resistance. Therefore, when the mitral valve area is 1.0 cm.^{2} or less, the following clinical manifestations become increasingly prominent: disabling exertional dyspnea, edema, hepatomegaly, cardiac enlargement, enlargement of the left auricle and the pulmonary artery, auricular fibrillation and electrocardiographic evidence of right ventricular hypertrophy.

Thus the mitral valve, like most organs of the body, has a reserve capacity to the extent of 80 to 85 per cent before important signs of functional inadequacy appear.

The Effect of Cortisone Acetate on Idiopathic Acquired Hemolytic Anemia. ALLYN B. LEY and FRANK H. GARDNER, Boston, Mass. (Introduced by William B. Castle).

Observations were made in three patients with acquired hemolytic anemia. Each originally presented an enlarged spleen, an anemia with an erythroblastic marrow, reticulocytosis and hyperbilirubinemia, and a positive antiglobulin (Coombs) test. In only one patient were there increases in red cell osmotic and mechanical fragilities. Two of the patients were treated initially with cortisone acetate: in one this was preparatory to splenectomy; in the other the drug has been continued in gradually decreasing dosage. The third patient was first treated with ACTH and then continued on cortisone acetate as maintenance therapy.

In each of the first two patients a significant therapeutic effect was observed. The red cell, hemoglobin and hematocrit values gradually rose and the reticulocyte counts subsided as the serum bilirubin values returned to more normal levels. The Coombs test titers became weaker or negative. In the patient who demonstrated increased red cell fragilities, these abnormalities disappeared. The third patient, whose initial improvement was induced by the use of ACTH has since maintained on cortisone acetate normal blood values and a normal serum bilirubin for 2 months. However, the initial Coombs test titer has shown no change.

It is concluded that cortisone, like ACTH, is an effective agent in the treatment of some patients with acquired hemolytic anemia.

Immunization Against Scrub Typhus by Combining Living Vaccine with Chemoprophylaxis. HERBERT L. LEY, JR. and JOSEPH E. SMADEL,* Washington, D. C.

Earlier studies by Army Research Units in Malaya showed that chloramphenicol given prophylactically suppressed overt scrub typhus in volunteers exposed by sitting in the grass in hyperendemic areas. Typical disease subsequently developed when the last dose was given three weeks following initial exposure but volunteers remained well if prophylaxis was continued for four weeks.

Since inactive scrub typhus vaccines have proved useless in the field, a combined procedure consisting of intradermal inoculation of "attenuated" living *R. tsutsugamushi* followed by intermittent prophylactic administration of 3.0 gram oral doses of chloramphenicol was studied. All eight volunteers receiving vaccine without chemoprophylaxis developed clinical scrub typhus eight to ten days after inoculation. The six given drug on the 8th, 12th and 16th day sickened on the 20th to 23rd day. None of the groups of eight who received their last dose on the 21st, 27th, or 34th day subsequently developed clinical scrub typhus. Eschars appeared at the site of vaccination in all volunteers and rickettsemia occurred in the control and test volunteers.

Intermittent chemoprophylaxis from the 8th to the 21st day following inoculation of rickettsiae suppressed overt disease but permitted a smoldering infection.

Quantitative Studies in Man of the Cardiac Effects of Reflex Vagal Stimulation. ARTHUR J. LINENTHAL, Boston, Mass. (Introduced by A. Stone Freedberg).

Certain quantitative aspects of reflex vagal action on the heart, not heretofore described in man, have been studied in order to clarify the role of this reflex in the genesis and treatment of cardiac arrhythmias.

Changes in sino-auricular rate, auriculo-ventricular conduction time and site of impulse formation were determined electrocardiographically following manometrically-controlled carotid sinus pressure in patients with angina pectoris or paroxysmal arrhythmias.

Significantly, this visceral reflex follows well-established principles of neurophysiology applying to various motor and sensory phenomena. Stimulus-response curves for

effects on rate and auriculo-ventricular conduction are S-shaped. In the same individual these two cardiac responses, frequently simultaneous, are independent, having different thresholds and different rates of increase and decline. Rapid accommodation occurs if constant pressure is maintained for more than a few seconds. The slowed heart rate rises rapidly, leveling off below the control rate as pressure is continued.

Right and left vagal stimulation may have marked action on both sino-auricular and atrio-ventricular nodes. Varying combinations of the two effects occur in different individuals.

During a two-year period in a patient with angina pectoris, the action on auriculo-ventricular conduction increased markedly, without change in the effect of carotid sinus pressure on the sino-auricular node and the cardiac rate; this was traced to alterations in the heart.

Atrio-ventricular beats may occur during maximum sino-auricular slowing. Increased sino-auricular rate above the control level, supra-ventricular and atrio-ventricular tachycardias and ventricular extra-systoles have been observed after reflex vagal stimulation has been discontinued, during rebound from reciprocal sympathetic inhibition.

Atropine and quinidine may block reflex vagal action on the atrio-ventricular node without altering its effect on the sino-auricular node.

These findings afford a new quantitative approach applicable to the clinical and pharmacological study of this important reflex.

The Effect of Graded External Pressures on the Vascular Volume of the Leg. JULIUS LITTER and J. EDWIN WOOD, III, Boston, Mass. (Introduced by Robert W. Wilkins).

Quantitative plethysmographic measurements were made of the vascular volume of the leg, *in vivo*, with the circulation under normal conditions of rest. The blood was first expressed from the leg and thigh by the application of air-pressure (250 mm. Hg) successively to the water-filled plethysmograph on the leg, and to overlapping blood pressure cuffs on the thigh. After the circulation was restored, the increase in leg volume was recorded until reactive hyperemia had subsided and the resting vascular volume was constant. These measurements were repeated over a range of external water pressures on the leg from minus 5 to plus 55 mm. Hg referred to heart level.

A marked decrease in vascular volume of 2.5 per 100 cc. of leg (from 4.0 to 1.5 cc.) was produced by increasing the external water pressure only 10 to 15 mm. Hg. This was attributed to a large decrease in the volume of the veins (and perhaps the venules) due to the reduction of the effective venous pressure to barely greater than zero. When the external water pressure was further increased from 15 to 55 mm. Hg, only a small additional decrease in volume of 0.5 cc. occurred. This was interpreted as being due to decreases in the volume of the capillaries, arterioles and arteries, the venous volume and effective pressure remaining constant.

These results give quantitative information about the volume of different blood vessels *in vivo*, and confirm the *in vitro* studies of other workers showing that the volume of veins depends upon the effective venous pressure, and is independent of absolute pressure. The data also have a practical bearing on the use of elastic compression to accelerate venous flow and prevent thrombo-embolic complications in hospitalized patients.

Some Studies of Posterior Pituitary and Adrenal Cortical Interrelationships. C. W. LLOYD, J. F. HARRIS and J. LOBOTSKY, Syracuse, N. Y. (Introduced by R. H. Lyons).

Two normal subjects and five patients with liver disease were injected daily with Pitressin tannote in oil while they were maintained on standardized salt and fluid intakes. Frequent observations of body weight, serum sodium, and urine volume, sodium and corticosteroid, were made. Very little change in any of these measurements occurred in the normal subjects. The patients with liver disease had striking changes, developing hyponatremia and edema as results of the excretion of increased amounts of sodium and the retention of water. Decreases of serum sodium to values as low as 106 m.Eq/1 and weight gains as great as 20 pounds occurred in these patients during Pitressin administration. When the serum sodium remained within normal limits, no change in urinary corticosteroid occurred. The urinary corticosteroid rose to high values when hyponatremia had been produced, suggesting the occurrence of an adrenal cortical response. In the presence of sodium depletion and water retention, administration of adrenal cortical extract in addition to the Pitressin caused diuresis and sodium retention.

Studies on the Metabolism of Cholesterol in Normal Man and in the Nephrotic Syndrome. IRVING M. LONDON,* GLORIA F. SABELLA and MARTHA M. YAMASAKI, New York, N. Y.

The rate of synthesis of serum cholesterol may be measured by determining the rate at which deuterium is incorporated into the serum cholesterol when an elevated deuterium concentration is maintained at a steady level in the body water. The rise in deuterium concentration in the serum cholesterol represents the appearance in the circulation of newly synthesized cholesterol molecules formed from precursors which have derived their deuterium from the labeled body water. On prolonged maintenance of a steady deuterium concentration in the body water, the isotope concentration in the serum cholesterol rises to a maximum value. The time required to reach 50 per cent of this maximum deuterium concentration is the half life time, $t_{\frac{1}{2}}$, of serum cholesterol, i.e., the time required for half the serum cholesterol molecules present in the circulation to be formed.

A study in a normal adult male revealed a value of about 8 days for the half life time of serum cholesterol. This represents a turnover time, or mean survival time, of about 12 days ($t_{\frac{1}{2}}/\ln 2$).

A similar study has been conducted in a patient with

the nephrotic syndrome having marked hypercholesterolemia. The rates of formation of cholesterol of the serum and of the erythrocytes have been determined. The results indicate that the half life time of the serum cholesterol is prolonged. These findings will be discussed in terms of the pathogenesis of the hypercholesterolemia of the nephrotic syndrome.

Studies have been performed on the interrelationships of serum and erythrocyte cholesterol. The incubation of normal mature human erythrocytes with C^{14} labeled acetate reveals that these cells have no significant capacity for cholesterol synthesis. The origin of erythrocyte cholesterol will be discussed in the light of these findings.

Entero-Hepatic Circulation of Bromsulphalein: Studies in Man and Dog. STANLEY H. LORBER, HARRY SHAY, M. J. OPPENHEIMER and HERMAN SIPLET, Philadelphia, Pennsylvania (Introduced by Richard A. Kern).

Previously demonstrated intestinal absorption of bromsulphalein was further invèstigated in dogs employing a catheterization technique. Significant serum BSP concentrations in hepatic vein, portal vein and peripheral artery were usually observed within 5 minutes of its intraduodenal instillation; being highest in portal blood and dye appeared in bile within 10–15 minutes. For one minute, following administration of BSP into the portal vein, dye concentrations in the hepatic vein exceeded those in peripheral artery. From 2 to 15 minutes, BSP concentrations in peripheral artery exceeded those in hepatic vein but at 30 and 45 minutes little difference between the two was observed.

To correlate these observations with the clinical BSP test, cholecystectomized patients were administered the 2 and 5 mg./kg. doses intravenously with and without common duct aspiration. Significant correlation between dye removal and change in serum BSP levels was observed only after the 5 mg./kg. dose. Marked decreases in serum BSP concentrations occurred when a significant percentage of the intravenously administered dye was recovered in the bile. The effect of reabsorbed dye upon serum levels was more readily evident after the intravenous administration of BSP than after its intestinal instillation. This may be due to "saturation" of the excretory mechanism under the former conditions.

We conclude from these data that the influence of an enterohepatic circulation upon the BSP test is a quantitative one and suggests that the smaller dose is preferable for clinical use. The relationship of these observations to EHBF will be discussed.

The Syndrome of Short P–R Interval, Normal QRS Complex and Paroxysmal Rapid Heart Action. BERNARD LOWN and FRANK GANONG, Boston, Mass. (Introduced by Samuel A. Levine).

Although the Wolff-Parkinson-White syndrome has been widely reported, the association of paroxysmal tachycardia and a short P–R interval without QRS abnormality has not been recognized.

From the electrocardiographic files of the Peter Bent Brigham Hospital two hundred cases with a P–R interval of 0.12 or less were compared with a control group of 200 cases with P–R intervals of 0.16 and 0.18 seconds. Cases on digitalis or with conditions known to predispose to paroxysmal tachycardia were excluded from both groups.

Among the 200 cases with the short P–R intervals, there were 16 cases with anomalous A–V conduction. Four of these had paroxysms of rapid heart action. Of the remaining 184 cases with normal QRS complexes, 19 had paroxysmal tachycardia. In the 200 control group there was only one such case with tachycardia. Thus there is a markedly greater incidence of rapid heart action in the short P–R group.

These cases differ from the Wolff-Parkinson-White syndrome in several ways. Their usual P–R interval is 0.12 second, rarely less, the QRS duration is less than 0.09 and the P–J interval is seldom more than 0.20 second. Slurring of the initial portion of the R wave is absent. Unlike the Wolff-Parkinson-White they do not exhibit "normalization" of the P–R and QRS even after many years follow-up.

In this study it was noted that cases of Addison's disease have P–R intervals longer than 0.15, while cases with Cushing's syndrome have P–R intervals less than 0.14 second; furthermore massive doses of cortisone seem to shorten the P–R interval. Whether endocrine factors are operative in the genesis of this entity is as yet uncertain.

These cases constitute an easily recognizable clinical group consisting of paroxysmal tachycardia, preeminently in females, starting in middle life, with a snapping mitral first sound, who have a constantly short P–R and a QRS devoid of slurring or prolongation.

A Comparison of the Effects of ACTH and Cortisone in Nephrosis. JOHN A. LUETSCHER, JR.,* QUENTIN B. DEMING and BEN B. JOHNSON, San Francisco, Calif.

Twenty-nine patients with nephrosis, with or without clear evidence of glomerulonephritis, have received cortisone or ACTH for 5 to 16 days for a total dose of 500 to 2100 mg. Of 18 courses of treatment with cortisone, 10 resulted in complete elimination of edema, 3 led to partial diuresis, and 5 cases had no beneficial result. Of 14 cases treated with ACTH, 10 lost all edema, while 3 showed no significant diuresis. Treatment with either agent usually increases the clearances of creatinine or inulin and causes retention of sodium and water during the initial days of treatment, followed by release on later days. After treatment, there is usually diuresis and more or less reduction of proteinuria, with increased plasma protein and albumin levels. ACTH may initiate an impressive elimination of water and sodium during the latter days of treatment, and proteinuria may decline at this time: such changes are rarely seen until after discontinuance of cortisone treatment. Reduction in serum cholesterol usually follows ACTH treatment. A reduction in the abnormal sodium-retaining activity of urinary corticoids is observed whether diuresis occurs during or after treat-

ment, but fails to occur when edema is unaffected. Improvement in urinary sediment is often observed when a reduction in proteinuria occurs.

The duration of a remission following ACTH or cortisone varies greatly. It may be interrupted by an intercurrent infection or there may be a gradual return of proteinuria or edema without obvious cause. Sustained improvement lasting many months is possible.

Protein Studies on Treponemal Immobilizing Antibodies. HAROLD J. MAGNUSON,* HENRY TAUBER, CHARLOTTE MCLEOD and WARFIELD GARSON, Chapel Hill, N. C.

Cohn Method X for the fractionation of normal human plasma has been adapted to the fractionation of plasmas or serums from approximately 50 patients with untreated early syphilis in whom treponemal immobilizing antibodies could be demonstrated. The chemically separated fractions were further studied by electrophoresis. These studies indicate that the immobilizing substances are present in Precipitate I + III − 1,2.3 associated with the high molecular weight isoagglutinins and a small amount of prothrombic protein; and in Precipitate I + III − 3 associated with the high molecular weight antihemophilic globulin, cold insoluble globulin, and a small amount of plasminogen. These precipitates which contain nearly all of the spirocheticidal substances represent a small percentage of the blood proteins. Unsuccessful attempts have been made to demonstrate binding of these spirocheticidal substances to pathogenic *T. pallidum* through the use of I^{131} tagged and fluorescein tagged serums and serum fractions.

The Effect of a Series of Bacteria and Their Products on the Human Leukocyte. SAMUEL P. MARTIN, IVAN L. BENNETT and S. CHAUDHURI, Durham, N. C. (Introduced by David T. Smith).

The effect on human leukocytes of a series of bacteria and their carbohydrate products has been tested. Morgan has noted that leukocytes from splenic implants of guinea pigs did not migrate toward the carbohydrate extracted from S. typhosa. With the same carbohydrate, Delauncy noted marked inhibition of diapedesis of leukocytes, and Miles and Nivens explained the paucity of leukocytes in injured tissue on the inability of the animal to maintain adequate blood pressure. In our studies leukocytes were observed by a slide cell technique after in vitro and in vivo administration of various bacterial products.

With the use of the intact organism in vitro, S. typhosa and Ps. aeruginosa inhibited the migration of leukocytes in concentration of 6×10^6 organisms/ml (approximately one per leukocyte). M. aureus and D. pneumoniae had no inhibitory effect in concentrations of 6×10^8 organisms/ml. The purified carbohydrate of S. typhosa (Morgan), P. aeruginosa (Baxter), and S. Marcesens (Shear), as well as filtrates, inhibited the migration of leukocytes. As the dosage was decreased, stimulation was noted. Polysaccharides from D. pneumoniae type III showed no inhibition.

In the study of blood drawn before and after the administration of heat killed S. typhosa, a moderate stimulation of leukocytes is noted during the first two hours after the material is given. This stimulation is prevented by the administration of ACTH.

It would appear that the alteration of migration of leukocytes may reflect a change in the cell and that this may be important in the distribution of leukocytes following the administration of these compounds.

Thyroid Hyperplasia Disproportionate to Function. GEORGE B. MCADAMS, New Haven, Conn. (Introduced by William T. Salter).

In distinguishing between toxic and non-toxic goiter, a single determination over the gland of radioactive pick-up (due to I^{131}) may be misleading. Such a measurement is more likely to indicate thyroid hyperplasia than thyroid hyperfunction. If repeated determinations are made with the Geiger-Mueller counter (following the test dose of I^{131}), however, it is possible to estimate the rate at which iodide is transformed into thyroxine and secreted. This TMR (thyroid metabolic rate) correlates well with the concentration of serum thyroxine or "hormonal" iodine. In smouldering or burned-out Graves' disease (*forme fruste*), the TMR usually lies within normal limits; whereas the maximal uptake of radioactivity in the gland is extremely high (due to hyperplasia). The TMR normally is between 0.18 and 0.32 mg. per day *per square meter* of *body surface,* in terms of L-thyroxine. In myxedema the TMR approaches zero; and in brisk hyperthyroidism it exceeds half a milligram of L-thyroxine per day per square meter. Evidently the best way to determine thyroid function is to estimate the amount of L-thyroxine produced per day. One should not be misled by the size or mass of thyroid parenchyma.

The Action of Protoveratrine on Renal Function in Hypertension. E. MEILMAN, Boston, Mass. (Introduced by S. L. Gargill).

The ability of protoveratrine to lower blood pressure in a variety of conditions characterized by hypertension affords the opportunity of studying such patients during periods of elevated blood pressure and lowered or normal blood pressure. Since this fall in blood pressure is the result of a decreased peripheral resistance, it was important to determine kidney function during this period.

Renal clearance studies (inulin and PAH) have been carried out 40 times in 35 patients with hypertension of varied etiology before and after protoveratrine. In most of these, clearance materials were administered with a constant speed infusion pump. In ten studies after single i.v. injections of protoveratrine, a moderate to marked fall in GFR and a lesser fall in ERPF usually occurred during the most marked blood pressure fall; both functions recovered toward but not above control levels as the blood pressure rose to former levels. Since such measurements were made during a constantly changing blood pressure level, further studies during a continuous i.v. infusion of protoveratrine were made while the blood pressure was being maintained at a steady low level for 2–4 hours.

These studies demonstrate that GFR usually approaches or reaches control levels 1–2 hours after the first lowering of blood pressure, while ERPF reaches or exceeds control values in this time. These observations are consistent with the hypothesis that the kidney in essential hypertension as well as chronic renal disease is capable of undergoing vasodilatation following the administration of protoveratrine.

The vasodilatation is accompanied by a marked decrease in the resistance of the kidney.

The Causes of Constriction of the Digital Vascular Bed. MILTON MENDLOWITZ,* New York, N. Y.

In the digital circulation the sympathetic nervous component of vasoconstriction can be eliminated by the application of heat indirectly supplemented by the intravenous administration of TEAC. When this is done in hypertensive patients, residual vasoconstriction, the mechanisms for which remain obscure, can often be revealed.

The possibility that metabolic factors are responsible for this residual vasoconstriction was investigated by studying oxygenation and hydration. In severe anemia, when the slight decrease in viscosity was incorporated in the calculations, there was no evidence of intrinsic vasodilatation. In clubbed fingers, moreover, and in coarctation of the aorta, intrinsic vasoconstriction was not a response to increased perfusion pressure in the digital arteries.

Hydration was studied by comparing the circulation in contralateral digits in patients with unilateral lymphedema, usually following radical mastectomy. No significant differences were found beyond those in a control group. In one patient with malignant hypertension, studied repeatedly, salt depletion produced a gradual decrease in non-neurogenic or intrinsic digital vascular resistance. The factors of chemical composition of intra- and extra-cellular fluids were not concomitantly studied.

In every patient subjected to dorso-lumbar sympathectomy some return of neurogenic vasoconstriction in the toe could be demonstrated. In addition, in at least one-third of these patients, an increase in non-neurogenic intrinsic vascular resistance followed sympathectomy. Infusion of benzodioxane after prior release of residual sympathetic nerve tone by indirect heat and TEAC failed to demonstrate any relationship between this intrinsic narrowing and circulating epinephrin. The available evidence suggests disuse atrophy and consequent shortening of arteriolar circular muscle as the probable cause of this decrease in vascular caliber.

Observations on the Effect of Intravenous Human Serum Albumin in Hypersensitivity Reactions and Various Arthritic States. WILLIAM R. MERCHANT, THOMAS MCP. BROWN,* LUCILLE B. ROBINSON and RUTH H. WICHELHAUSEN, Washington, D. C.

The association of serum protein alterations with various disease states is being recognized more frequently with the increased application of electrophoresis and other techniques. In those diseases with an infectious etiology or a possible hypersensitivity mechanism, attention has usually been directed toward abnormalities in the globulin fractions. The significance of the relation of the albumin to the globulin fractions has been obscure.

Relatively minute amounts of human serum albumin interfere with the zinc turbidimetric measurement of gamma globulin. In patients with rheumatoid arthritis human serum albumin given intravenously produced temporary variations of clinical effect from marked symptomatic improvement to mild exacerbation of symptoms. The latter was usually associated with larger quantities of albumin. A transient rise in albumin and depression of gamma globulin fractions, as determined electrophoretically, was noted following single injections of albumin. These changes paralleled those noted in the sera of patients receiving cortisone.

To correlate the comparative clinical and serum protein effects further, known sensitivity reactions were studied. In three patients with serum sickness and in seven patients with reactions due to penicillin, prompt relief of their symptoms was obtained when small quantities of albumin were administered intravenously. One patient with aplastic anemia had four successive transfusion reactions. Three subsequent transfusions were given without reaction when administered concomitantly with albumin. Tuberculin hypersensitivity and the precipitin reaction in serum sickness could also be modified by human serum albumin.

Three patients with gout who were completely refractory to colchicine were studied. Albumin alone produced no effect in these patients. However, when albumin administration was immediately followed with colchicine marked clinical improvement was obtained.

These observations would suggest that serum albumin independently, or in its interrelationship with globulin, may modify the manifestations of known hypersensitivity and various arthritic states.

Observations on the Pressor Response Occurring During Dialysis with the Artificial Kidney. JOHN P. MERRILL, WALTER T. GOODALE and ROY C. SWAN, Boston, Mass. (Introduced by Henry A. Christian).

The pressor response accompanying dialysis with the artificial kidney is characterized by a rise in both systolic and diastolic pressure with a slight increase in pulse pressure and pulse rate. The etiology of this response is at present not clear but conclusions as to the dynamics of the response and its clinical significance can now be made. The possible contribution of the glucose content of the bath, of changes in plasma volume, in extracellular fluid, and the mechanical effect of transport of blood through the apparatus have been eliminated by controlled experiments. An increase in cardiac output, as measured by the technique of cardiac catheterization, has been implicated in the majority of these responses. Certain hypertensive patients may show no increase in cardiac output, but a marked increase in peripheral resistance and mean arterial blood pressure. One such patient, however, given

the peripheral vasodilator, protoveratrine, at the height of the pressor response maintained her mean arterial blood pressure by doubling the cardiac output in the face of a halved peripheral resistance. In all cases where measured, pulmonary artery pressures were unchanged.

Two patients with hypotension refractory to infusion of large amounts of plasma and blood have been studied. In neither of these patients was there evidence of peripheral vasoconstriction. Both patients were jaundiced and anuric. Initial cardiac output was low, and in one patient the pressor response occurring during dialysis was accompanied by a 35 per cent increase in cardiac output and resumption of urine flow. In one nephrotic patient, without nitrogen retention, dialysis was accompanied by a marked increase in glomerular filtration rate from 75 cc/minute to 170 cc/minute and a marked increase in urine flow and sodium excretion. A second patient with long-standing Laennec's cirrhosis and edema showed a marked increase in cardiac output with a similar increase in urine flow and sodium excretion.

The Effects of Experimental Pericardial Tamponade on Pressures in the Pulmonary Circulation. JAMES METCALFE and JOHN W. WOODBURY, Boston, Mass. (Introduced by C. Sidney Burwell).

Peripheral venous, peripheral arterial, pulmonary venous (in four cases "pulmonary capillary") and pulmonary arterial pressures have been measured in a series of dogs subjected to progressive pericardial tamponade by a method permitting normal respiration with a closed thorax.

The well documented rise in peripheral venous pressure and fall in peripheral arterial pressure is again confirmed. With increasing intrapericardial pressure, pulmonary venous pressure (or "pulmonary capillary") is found to rise in every case. In spite of this consistent rise in pulmonary venous pressure, the filling gradient of the left ventricle (pulmonary venous pressure minus intrapericardial pressure) gradually becomes zero. At this point in the procedure peripheral arterial pressure falls precipitously. Depending on its resting level, mean pulmonary arterial pressure rises or falls to approximate that level of pulmonary venous pressure which is reached at the height of tamponade.

These experiments, therefore, for the first time make it clear that in tamponade there is congestion of the pulmonary vascular system as well as of the systemic vascular system. The significance of these observations will be discussed.

Observations on Renal Mechanisms for Sodium and Potassium Excretion in ACTH-induced Diuresis of Nephrotic Edema. JACK METCOFF, C. P. RANCE and N. NAKASONE, Boston, Mass. (Introduced by C. A. Janeway).

The renal capacity to excrete osmotic loads delimits some aspects of tubular function. Insulin and para-aminohippurate clearances with coincident loads of sodium salts of the non-reabsorbable anions $S_2O_3^=$ and PAH$^-$ were obtained initially in three edematous nephrotic children and subsequently before, during, and after ACTH-induced diuresis. Comparable load studies were done in normal untreated children.

Relative osmotic diuresis occurred in nephrotics despite edema or ACTH therapy. Patterns of fixed-cation excretion varied. Sodium plus potassium excretion usually exceeded that of non-reabsorbable anion. Cation excess was largely balanced by diminished chloride reabsorption.

The aliquot of infused sodium contributing to the tubular load (fractional filtered load) was estimated from retention, concentration and apparent volume of distribution. Normal children excreted the total fractional filtered load plus additional quantities of filtered sodium to satisfy obligatory anion demands. Edematous nephrotic children reabsorbed 50–90 per cent of the fractional filtered sodium load. This reabsorption appeared asymptotic. ACTH-induced diuresis began during therapy. Coincidentally, and in the non-edematous state thereafter, reabsorption of the sodium load was markedly curtailed.

Osmotic diuresis of potassium occurred with $Na_2S_2O_3$ and NaPAH loads during edema. Potassium excretion exceeded amounts simultaneously filtered in over seventy periods. During and after ACTH-induced diuresis, potassium excretion was largely independent of urine flow, simulating the normal load response.

These observations suggest that during nephrotic edema, renal tubular cells excessively but not maximally reabsorb sodium. Further reabsorption of a superimposed sodium load occurred during ACTH therapy, unless diuresis of edema fluid had begun. With diuresis, sodium reabsorption was markedly reduced. Potassium excretion, relatively unaltered by ACTH therapy, conformed with the hypothesis of tubular cation exchange. Potassium secretion suggested a more distally located mechanism for rapid mobilization of cation.

ACTH-induced diuresis may be mediated by relative sodium rejection and concomitant restoration of normal potassium transfer by renal tubular cells.

Relationship of Allantoin and Endogenous Creatinine Clearances to Glomerular Filtration Rate in Man. BENJAMIN F. MILLER,* ALEXANDER LEAF, AUDLEY R. MAMBY and ZELMA MILLER, Boston, Mass.

The allantoin, the endogenous creatinine chromogen, and the specific creatinine clearances have been proposed for the determination of the glomerular filtration rate to obviate the intravenous infusion required by the inulin clearance. In this study we have compared these three clearances with the inulin clearance to assay their reliability for filtration measurements.

In seven patients with a variety of renal lesions, clearance ratios were determined in a total of twenty-two periods. The allantoin clearance equalled the inulin clearance in seven periods, and was lower in the other fifteen. In eight comparisons, the ratio of allantoin to inulin clearance fell below 0.8. Ratios of 0.73, 0.75, and 0.64 were observed in a patient with severe cardiac failure.

The clearances of *creatinine chromogen* ("apparent cre-

atinine") and of *specific creatinine* did not prove acceptable as true measures of filtration rate. *Creatinine chromogen*/inulin ratios as high as 1.64 were obtained in a patient suffering from long-standing glomerular nephritis, and as low as 0.56 in the cardiac patient mentioned above. The highest *specific creatinine*/inulin clearance ratio, 1.74, was obtained in the patient with the lowest renal function (average inulin clearance of ten cc.). The lowest ratio, 0.98, was found in the patient with severe cardiac failure. The endogenous *creatinine chromogen* was determined by the Bonsnes and Taussky modification of the Jaffe reaction. *Specific creatinine* was measured by the Miller-Dubos enzymatic method.

The variations in clearance ratios of allantoin/inulin and endogenous creatinine/inulin are interesting because they may eventually lead to a better understanding of tubular function in various renal syndromes. We feel, however, that the variations are so large that the allantoin and creatinine clearances should be considered unreliable measures of filtration rates in disease.

Erythrocyte and Serum Cholinesterase in Ulcerative Colitis. HUGO C. MOELLER, J. ALFRED RIDER and JOSEPH B. KIRSNER,* Chicago, Ill.

Hyperactivity of the parasympathetic nervous system with resultant hypermotility of the bowel has been implicated in the pathogenesis of ulcerative colitis. The neurogenic hyperfunction may be accompanied with an increased concentration of acetylcholine, and, correspondingly, cholinesterase, at the myoneural junction. Whereas serum cholinesterase parallels the nutrition of the individual, erythrocyte cholinesterase may reflect cholinesterase at the myoneural junction.

Cholinesterase activity was measured by the Warburg manometric technique in 50 normal individuals and in 35 patients with ulcerative colitis; results are expressed as microliters of CO_2 liberated during an average 5-minute period by 0.1 cc. of serum or erythrocytes. In the normal group, erythrocyte cholinesterase varied from 71 to 102, averaging 86; serum cholinesterase ranged from 27 to 46, averaging 36. In active ulcerative colitis erythrocyte cholinesterase tended to be higher, ranging from 79 to 137, averaging 99; serum cholinesterase was lower than normal, varying from 11 to 36, averaging 23. Erythrocyte cholinesterase was normal in patients with myasthenia gravis, gastric carcinoma and polycythemia vera. However, high values were observed also in peptic ulcer and pernicious anemia, indicating that the increased erythrocyte cholinesterase in ulcerative colitis is not specific for this disease.

Twenty-four patients with moderate to severe colitis received ACTH or cortisone; the clinical response was good in most cases. During the active stage, erythrocyte cholinesterase ranged from 81 to 137, averaging 108; serum cholinesterase varied from 8 to 34; averaging 22. At the time of greatest improvement during ACTH therapy, erythrocyte cholinesterase ranged from 72 to 104, with a mean of 85; serum cholinesterase varied from 17 to 44, averaging 27. Fluctuations in erythrocyte cholinesterase paralleled the recurrences and remissions of ulcerative colitis.

Studies on in vitro Potassium Accumulation. GILBERT H. MUDGE, New York, N. Y. (Introduced by John V. Taggart).

Thin slices of rabbit kidney cortex lose K during preparation in chilled isotonic NaCl and reaccumulate K from a low external concentration (10 mEq./l.) during incubation under optimal conditions. The process of reaccumulation was studied with simultaneous measurements of tissue respiration and electrolyte composition. K accumulates as a net exchange for Na. Depression of aerobic oxidation by drug action, changes in oxygen tension, temperature, etc., produces a simultaneous depression in K uptake.

All metabolic inhibitors which depress respiration likewise depress the accumulation of K. Anoxic anoxia, cyanide and arsenite depress these functions in a parallel manner. A larger group of compounds (including azide, mercuric chloride, organic mercurials and Benemid) have a marked effect on electrolyte metabolism at concentrations significantly lower than those required to depress oxygen consumption. Studies of 2,4-dinitrophenol and related compounds showed a good correlation between depression of K uptake and inhibition of the generation of high energy phosphate bonds. Inhibitors of carbonic anhydrase, cholinesterase and alkaline phosphatase had no effect on K accumulation.

Tissue hydration was slightly increased by anoxic anoxia but more so by specific inhibitors. Tissue osmotic pressure (measured as the sum of Na plus K) remained essentially constant, regardless of changes in water content, in every experiment with a constant external osmotic pressure. Depression of metabolic activity is, therefore, associated with an isosmotic increase in tissue hydration. No evidence was found to support the concept that the cells are normally hypertonic to their environment.

K accumulation is regarded as an example of active transport. Conditions affecting net accumulation are now being examined with the use of radioactive K.

An Estimation of Portal Venous Pressure by Occlusive Catheterization of an Hepatic Venule. J. D. MYERS* and W. JAPE TAYLOR, Durham, N. C.

Knowledge of portal venous pressure in man is confined largely to measurements at laparotomy. A ready estimate of portal venous pressure in intact man would be valuable in diagnosis and in correct assessment of the therapeutic procedures such as porto-caval shunts. Dexter's experience that pulmonary "capillary" pressure is reflective of pulmonary venous pressure suggested similar exploration of the more complex hepatic circulation.

In 18 observations on 10 cats, selected because of similarity of the feline and human hepatic circulations, pressures, measured through an intravenous catheter passed occlusively into a distal hepatic venule, ranged from 6 to 13.5 mm. Hg (mean 8.6). Almost simultaneous direct determinations of portal venous pressure agreed closely

(mean difference 0.6 mm. Hg; range −3.5 to +2.5). Epinephrine raised both pressures to a similar degree. Ligation of the hepatic artery caused no significant reduction in either pressure.

In 12 humans without liver disease, the mean pressure was 4.8 mm. Hg in an hepatic venule occluded by a catheter passed via an antecubital vein. The free pressure in a large central hepatic vein averaged 3.3 mm. Hg, there being no significant gradient between the two pressures. Blood withdrawn from occluded hepatic venules had essentialy identical glucose and oxygen contents with hepatic venous blood indicating no massive circulatory obstruction from the catheterization.

Seven patients with portal hypertension from Laennec's cirrhosis gave pressures in the occluded venule from 11.5 to 32 mm. Hg (mean 20.1). These pressures agree in magnitude with portal venous pressures recorded by others at laparotomy in portal hypertension. The pressures between the control and hypertensive groups did not overlap. The pressure gradient from the occluded venule to a central hepatic vein in the cirrhotics averaged 14.4 mm. Hg in contrast to the small normal gradient.

These preliminary studies suggest that the pressure recorded from an occluded hepatic venule is indicative of portal venous pressure and can be used to estimate portal hypertension in man.

The Crystallization of "Direct" and "Indirect" Bilirubin from Human Serum and Their Respective Properties. VICTOR A. NAJJAR* and BARTON CHILDS, Baltimore, Md.

Serum (0.1 − 1.0 ml.) from patients with jaundice is brought to pH 6.0 extracted into ether and from ether into pyrophosphate buffer pH 9.0. The pH is then lowered to 7.4 and cooled to 4°C. Crystals from "indirect" serum form readily and are long thin needles with occasional plates, whereas, crystals from "direct" serum are slow forming and are short blunt rods. The extracted bilirubins retain their respective "direct" and "indirect" diazo characteristics, are free of protein, dialyzable, and have an absorption maximum at 440 mμ in pyrophosphate buffer pH 8.2. Under appropriate conditions they can recombine with normal non-jaundice serum, retain unaltered diazo characteristics, become non-dialyzable at pH 7–8.2, and the absorption maximum shifts back to 470 mμ where it was in the original jaundice serum. "Indirect" bilirubin combines equally well with albumen and γ globulin on a molar basis. It becomes "direct" reacting after mild alkali treatment pH 10.5 with a release of acidic groups. This reaction is not reversible. Upon fractionation with ammonium sulfate the original "indirect" jaundice serum and the "indirect" pigment combined with normal serum behave identically. Bilirubin begins to come down with the proteins at 0.28, reaches a maximum at 0.40 and is completely precipitated at 0.48 saturation. Similarly, "direct" jaundice serum and "direct" bilirubin recombined with normal serum behave the same. The "direct" bilirubin, however, begins to come down at 0.38, reaches a maximum at 0.44, and precipitates completely at 0.56 saturation.

The Effects of Pitressin on the Excretion of Water and Electrolytes in Normal Subjects and Patients with Cirrhosis of the Liver and Ascites. WILLIAM P. NELSON, III, and LOUIS G. WELT, New Haven, Conn. (Introduced by David M. Kydd).

Normal subjects and patients with cirrhosis of the liver and ascites have been compared with respect to the intensity and duration of their response to pitressin. An infusion of 5 per cent glucose in water was administered so as to attain and maintain a positive balance of one liter of water throughout the entire study. This promotes a relatively steady maximal rate of excretion of water, due, presumably, to a continuous suppression of secretion from the posterior pituitary gland. Two and one-half or one hundred milliunits of pitressin were then administered intravenously, which was followed by a sharp antidiuresis. The rates of excretion of electrolytes and water were observed until the latter returned to the original maximal level.

There was no difference between the normal and the cirrhotic groups in the time required to attain a maximal rate of excretion of water from the original hydropenic state although these rates were strikingly lower in certain of the cirrhotic patients. The per cent decrease in the rate of excretion of water was greater with the larger dose of pitressin but did not differ in the two groups. In addition, the time required for recovery from the antidiuresis was the same in the normal subjects and patients with cirrhosis. In all except one instance the rates of excretion of sodium and chloride decreased.

There is no evidence of a delayed inactivation or excretion of endogenous antidiuretic hormone in patients with cirrhosis of the liver as compared with normal subjects, nor is there evidence of increased sensitivity, delayed inactivation or excretion of administered pitressin in these doses. Pitressin did not promote an increased rate of excretion of sodium or chloride.

Changes in Plasma and Erythrocyte Sodium, Potassium, and Water During Recovery from Diabetic Coma. GEORGE NICHOLS, JR. and NANCY NICHOLS, Boston, Mass. (Introduced by Alexander Marble).

Changes in plasma and erythrocyte sodium, potassium, and water in ten cases of recovery from diabetic coma were studied. No patients had evidence of intrinsic renal damage.

Values obtained were compared with those found by the authors in thirty-seven normals. Total plasma and red cell volumes were calculated by a new method presented by the authors.

On admission, plasma was depleted of sodium and water and total potassium. Erythrocytes showed normal water, low sodium and potassium.

During the early stages of treatment, where large amounts of 0.85% sodium chloride and insulin were given, plasma water and sodium were restored rapidly to normal values. Plasma potassium dropped precipitously in concentration and also showed a drop in total quantity.

The red cells became dehydrated and showed a loss in total potassium during this period.

In the latter stages of recovery, when patients had resumed normal, oral diets, erythrocyte water and potassium slowly returned to normal, often requiring eight to ten days. Plasma potassium reached normal concentrations in four to ten days.

Red cell sodium values were slightly low on admission, fell during therapy, and returned towards normal with the potassium. In some cases, this return was slower than that of potassium.

No correlation between these electrolyte and water changes and the level of the blood sugar, either at the outset or during therapy, was apparent.

Effect of Sodium Depletion and Repletion on Renal Function and Body Fluids During Uremia. J. F. NICKEL, P. LOWRANCE and E. LEIFER, New York, N. Y. (Introduced by Joseph C. Turner).

"Azotémie par manque du sel" is generally attributed to diminished glomerular filtration secondary to a change in body fluid volume during salt deprivation. Studies of the effect of sodium depletion in four patients with renal insufficiency due to malignant nephrosclerosis, chronic diffuse glomerulonephritis and polycystic disease have revealed that hypofiltration may develop under these conditions without change in arterial pressure or in composition and volume of body fluids. Following control determinations, rapid depletion of body sodium was achieved by salt-poor diet and ingestion of a cationic exchange resin (Lilly), 45 to 60 Grams per day for six days. After this period, filtration (inulin clearance) had fallen by 45 per cent, on the average, although it did not change in one normal subject. P-aminohippurate clearances fell to approximately the same extent in all, presumably indicating intra-renal vasoconstriction. The blood urea nitrogen rose sharply in two during sodium withdrawal. Sodium and chloride output diminished markedly but not to the same extent as in the normal. Potassium excretion increased somewhat in the patients with renal insufficiency but fell in the normal. Despite these changes serum electrolyte concentrations were not significantly affected and there was no appreciable alteration in total body water (antipyrine), extracellular water (inulin space), and plasma volume (T-1824). Maximal PAH excretion diminished in every instance, roughly to the same extent as electrolyte excretion. PAH loading resulted in augmented output of sodium; the urinary hydrogen ion concentration rose in the normal under these conditions but not in the others. In the latter, sodium depletion appeared to exaggerate potassium output during PAH loading so that potassium clearances exceeded inulin clearances. These alterations appeared to be reversible and cleared on repletion with sodium.

Clinical Evaluation of Various Types of Ballistocardiographs. JOHN L. NICKERSON, JAMES A. L. MATHERS and DOUGLAS S. SJOBERG, New York, N. Y. (Introduced by Robert L. Levy).

In view of the differing physical characteristics of the numerous ballistocardiographs in current clinical use a series of observations has been made on a group of normal individuals and a group of patients with cardiovascular disorders using representative instruments of the various types. In the normal group, pattern similarity with the various instruments is more frequent than in the cardiovascular group where one may, with certain instruments, fail to detect obvious cardiovascular abnormalities. Observations made on patients in whom the clinical status was improved by surgical or drug therapy have also shown the inability of certain instruments to detect changes towards normal. In an attempt to explain the divergences between the patterns obtained with the various ballistocardiographs, theoretical and experimental studies have been made and will be discussed.

Postural Changes in the Cerebral Circulation, Studied by Continuous Oximetric and Pressure-Recording Techniques. JOHN L. PATTERSON, JR., and JOHN L. CANNON, Atlanta, Ga. (Introduced by A. J. Merrill).

Under conditions in which the oxygen uptake of the brain remains essentially constant, the reciprocal of the cerebral arteriovenous oxygen difference, $1/(A\text{-}V)_{o_2}$, will vary directly with the cerebral blood flow (CBF). Advantage can be taken of this relationship to measure changes in CBF continuously. Studies with the nitrous oxide technique have shown that the mean cerebral oxygen consumption during 65° head-up tilting does not change in non-fainting subjects.

In the present studies continuous measurements of cerebral $(A\text{-}V)_{o_2}$ difference were made with two absolute-reading oximeters. Per cent arterial oxyhemoglobin saturation was obtained with an earpiece, and internal jugular venous saturation with a cuvette. Blood was withdrawn at constant slow rate through the cuvette with a syringe-pump. Arterial and jugular venous pressures were recorded from strain gauges mounted at ear level.

In ten non-fainting subjects, tilted head-up to 65°, the fall in CBF, indicated by changes in $1/(A\text{-}V)_{o_2}$, averaged 20 per cent, but varied from 0 to 40 per cent. In four other subjects, smaller angles of tilt produced smaller changes in CBF.

The CBF began to fall within one minute after tilt and on return to recumbency promptly rose to or above baseline levels. Jugular venous and arterial pressure changes showed directional correlation. In most subjects jugular pressure fell to subatmospheric levels after tilt, contributing materially to the net cerebral perfusion pressure (NCPP = arterial-internal jugular venous pressure).

Both in postural hypotension and vasodepressor syncope (four subjects) fainting occurred when the NCPP approached 15 mm. Hg. The NCPP and $1/(A\text{-}V)_{o_2}$ correlated closely until syncope became imminent. In a postural hypotensive the NCPP and CBF progressively fell until the faint occurred, whereas in normals both functions were readjusted at a lower level after tilt and later fell precipitously just before the faint.

The Cerebral Circulation and Metabolism in Chronic Pulmonary Emphysema with Observations on the Effects of Inhalation of Oxygen. JOHN L. PATTERSON, JR., ALBERT HEYMAN * and T. WHATLEY DUKE, Atlanta, Ga.

Reduction in arterial oxygen tension or elevation of carbon dioxide tension have dilator effects upon cerebral blood vessels. Similar changes in blood gas tensions often develop in chronic pulmonary emphysema. The administration of oxygen to such patients alleviates the anoxemia but may result in untoward reactions, such as drowsiness and convulsive seizures.

The cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$) were determined by the nitrous oxide technique before and during the administration of oxygen in nine patients with chronic pulmonary emphysema, seven of whom had cor pulmonale, and in eight control subjects.

The CBF in the patients with emphysema was elevated to a mean value of 68 cc., compared with 50 cc./100 Gm. brain/min. in the control subjects, while the $CMRO_2$ was slightly below the control value. There was a tendency for patients with the lowest values for arterial pO_2 and the highest values for pCO_2 to show the highest blood flow values, and the converse was also true. During inhalation of 85–100 per cent oxygen, the mean CBF in the patients with emphysema rose to 79 cc., but in contrast, fell by 12 per cent in the control subjects. The maximum CBF attained during oxygen inhalation was 113 cc./100 Gm./min. Two patients who had the lowest flow values while breathing air showed a decrease in CBF with oxygen. The mean $CMRO_2$ did not change and mental aberrations did not develop during the 30 minutes of oxygen administration.

The increased CBF found in the emphysematous patients breathing air was probably a reflection of the high mean arterial pCO_2 of 58 mm. Hg and low pO_2 of 39 mm. Hg. Oxygen inhalation, by depressing pulmonary ventilation, caused a further increase (12 mm. Hg) in pCO_2. This increase in pCO_2 apparently more than offset the much larger rise in pO_2, and thus produced an increase in cerebral blood flow.

Comparative Studies of Cortisone Administered Orally and Intramuscularly to Patients with Rheumatoid Arthritis. M. PATTERSON, C. H. ADAMS and C. STEVENSON, New York, N. Y. (Introduced by Richard H. Freyberg).

During one year, in approximately sixty patients with rheumatoid arthritis, the effects of cortisone administered orally have been compared to effects of this hormone injected intramuscularly. Usually cortisone was given by both routes at different times in the same patients.

Most effects were strikingly similar whether the hormone was given orally or parenterally. There were some significant differences. Cortisone is absorbed more quickly from the gut than from the muscles, as shown by a quicker antirheumatic effect and an earlier reduction in circulating eosinophiles. The same single dose of hormone produced quicker antirheumatic effects when given orally, but the effects disappeared more quickly than when it was given intramuscularly.

Many schedules of administration were studied and compared. Usually the most effective manner of administration of cortisone orally was in three doses at approximately six hour intervals, beginning on arising, with one third to one half of the daily amount given in the first dose, and one third or one fourth given in the second and third doses. When so administered, equivalent clinical effects were produced orally by 1.2 times the daily intramuscular dose—a surprisingly low ratio. No important undesirable effects resulted from oral administration. Cirrhosis of the liver, gastric anacidity and partial gastrectomy did not alter the effectiveness of orally administered cortisone. Smoother effects were usually obtained by the hormone orally.

Effectiveness of Corticotropin Suspensions. R. W. PAYNE, I. N. ROSENBERG, M. S. RABEN, A. P. CLEROUX and E. B. ASTWOOD,* Boston, Mass.

Partly purified corticotropin made in this laboratory by adsorption on cellulose was found to be therapeutically effective in a dose of 1 mg. given every eight hours. This preparation facilitated studies on different methods of administration; it could be injected intravenously and could readily be incorporated in small volumes of various media designed to prolong absorption from subcutaneous sites.

The subcutaneous injection of 1 mg. in saline was followed by a maximal urinary ketosteroid excretion in three to five hours and a return to normal or subnormal values after six to eight hours; there was a parallel clinical response. A single dose of 4 mg. suspended in 0.5 cc. of sesame oil produced an increase in ketosteroid excretion lasting 36 to 48 hours with therapeutic effectiveness over this length of time.

Used in the treatment of rheumatic and allergic diseases in a dose of 4 to 8 mg. every 24 or 48 hours, it was found to be effective in all of twelve trials when given once daily, and in six of nine trials when injected once every two days. Severely ill patients required daily injections; ambulatory patients showed satisfactory responses to 48 hourly doses for as long as treatment was given (four to six weeks).

The purified corticotropin preparation was found to be adsorbed strongly by small amounts of powdered oxidized cellulose and the aqueous suspension could be given subcutaneously. Although optimal conditions have not thus far been established, observations on seven patients indicated therapeutic effectiveness for 18 to 24 hours.

A Comparison of the Metabolic Effects of the Adrenal Cortical Steroids, Compounds A, E, F and S Acetate. OLOF H. PEARSON, LEONARD P. ELIEL and VINCENT P. HOLLANDER, New York, N. Y. (Introduced by C. P. Rhoads).

Microcrystalline, aqueous suspensions of Compounds A, E, F (Kendall) and S (Reichstein) acetate were administered intramuscularly to 4 patients with chronic lymphatic leukemia. Metabolic balances of N, P, K, Ca,

Na and Cl were obtained. The steroids were given at intervals of 6 hours in a daily dosage of 100 to 800 mgs. for periods of 10 to 24 days. The effects of Compounds E and F were compared in 2 patients, Compounds E and S in one patient. Compound A only was studied in one patient.

Compound E in a daily dosage of 100 to 200 mgs. regularly produced negative balances of N, K, P and Ca, and positive balances of Na and Cl.

Compound F in a daily dosage of 100 to 200 mgs. produced negative balances of N, K, P and Ca of approximately the same order of magnitude as that obtained with Compound E. However, Compound F in amounts up to 400 mgs. per day failed to induce retention of Na and Cl.

Compound A in a daily dosage of 100 mgs. produced marked retention of Na and Cl, but in daily amounts up to 800 mgs. was without significant effect on N, K, P and Ca balances.

Compound S in a daily dosage of 200 mgs. produced slight retention of Na and Cl which was of less magnitude than that produced by similar amounts of Compound E. Compound S produced no significant effect on N, K, P and Ca balances.

Compounds E and F induced shrinkage of lymphoid tumor masses and a rise in white blood count, whereas Compounds A and S were without effect on lymphoid tissue.

These observations indicate qualitative as well as quantitative differences in the physiologic effects of the adrenal cortical steroids, as indicated by the separation of effects on protein and Na and Cl metabolism.

An In Vivo *"Oxygen Dissociation Curve."* RAYMOND PENNEYS, Philadelphia, Pa. (Introduced by Hugh Montgomery).

By means of the Millikan oximeter and the oxygen (polarographic) electrode, simultaneous measurements of the arterial oxygen saturation of the blood and oxygen tension of warm skin were made *in vivo*, in normal human subjects. It has been shown previously that skin having a fast circulation has an oxygen tension approximately that of the arterial blood. The electrode used was the stationary open type platinum micro-electrode which measures relative changes in oxygen tension. The arterial saturation was decreased, or increased, by the inhalation of gases of varying oxygen concentration. A total of twenty-one experiments were done.

When the saturation was lowered to 85 per cent, the tension fell to 51 per cent of the original reading, obtained while the subject was still breathing room air. At 80 per cent saturation it was 42 per cent; at 75 per cent, 39 per cent; and at 70 per cent saturation, 35 per cent. When the arterial saturation was increased to 98 per cent, the oxygen tension rose to 135 per cent. At 99 per cent saturation it was increased to 180 per cent, and at 100 per cent saturation, to 460 per cent. When these oxygen tension and arterial saturation values are plotted against one another, a curve is evolved which is almost identical to the *in vitro* oxygen dissociation curve for blood.

The oximeter and oxygen electrode therefore allow measurements related to the dissociation of blood to be made *in vivo*. These methods require considerably less time and skill than the *in vitro* methods and give continuous, dynamic information. Applications are being made at the bedside of critically ill patients.

Altered Metabolic Response Prior to the Development of Hypertension in a Patient with Hypertensive Vascular Disease. GEORGE A. PERERA,* New York, N. Y.

Evidence has been obtained previously pointing toward variations in pressor response and disturbances in salt and water metabolism in patients with essential hypertension.

A 27-year-old woman, selected because of a strong family history of hypertension and a positive cold pressor test, was found to have repeated casually-determined blood pressure values ranging between 116/72 and 134/84. Placebo injections failed to modify the arterial tension, whereas the administration of desoxycorticosterone, 10 mgs. daily for five days, was associated with a rise in pressure to hypertensive levels. In addition, the rigid restriction of salt for 24 hours while on a constant diet resulted in weight loss of but 0.28 Kilos. Followed closely thereafter, persistent hypertension developed rather abruptly three months after the above tests, with evidence by exclusion that it was on the basis of hypertensive vascular disease.

The failure to lose significant weight on salt withdrawal has been associated principally with uncomplicated hypertensive disease. An early pressor response to desoxycorticosterone has been noted before only in hypertensives. Treated for similar brief periods, no modification of arterial tension occurred in over 20 normotensive adults, including a few subjects with vasomotor hyperreactivity.

This study suggests that an altered metabolic response exists before the development of hypertension, but gives no indication as to whether such changes precede the onset of overt disease by more than a short margin. Nevertheless, it provides a further argument that hypertension is but a manifestation of disease and that further search is warranted for a metabolic factor in advance of the first outward signs of hypertensive vascular disease.

The Determination of Cardiac Output by a Continuous Recording System Utilizing the Injection of Iodinated (I^{131}) Human Albumen. W. H. PRITCHARD, W. J. MACINTYRE, R. W. ECKSTEIN and H. L. FRIEDELL, Cleveland, Ohio (Introduced by Joseph M. Hayman, Jr.).

In an effort to find a reliable method for determination of cardiac output which could be conveniently performed at repeated intervals of several days or weeks, calculation of cardiac output involving dilution of an injected substance as a function of time has been used. Previous studies by Hamilton and Nylin have established the validity of the theory.

Utilizing iodinated (I^{131}) human serum albumen in small amounts (75–150μc), studies have been carried out

in dogs and man to test the accuracy of application of the method based on this theory.

During the rapid injection of a few ccs. of the radioactive material, arterial blood was allowed to flow slowly through a small properly shielded tube over a scintillation counter with the gamma activity recorded continuously by a Berkeley counting rate computer used in conjunction with a scaler and Esterline Angus graphic meter.

By calculation from the dilution curve so inscribed, cardiac output was determined, and following a subsequent 10 minute interval the final dilution (blood volume) level also recorded. Specific activity of the 10 minute arterial whole blood sample was measured *in vitro* in terms of μc/cc., and the flow system calibrated by the observed response of the system *in vivo* to this known activity at final dilution.

In eight experiments on dogs the dilution method gave average values 15 per cent higher than corresponding simultaneously recorded aortic rotameter flow values. If 5 per cent is added to the rotameter values to allow for the unmeasured coronary flow, an average agreement of + 10 per cent is obtained with a range of + 5 to + 16 per cent.

Preliminary studies on nine patients using cardiac catheter technique for comparison has given satisfactory results in eight with an agreement within ± 9 per cent.

In the method discussed no phase appeared critical except that of ensuring a sufficiently rapid flow past the counter.

The Rate of Utilization of Radioiron as a Measure of Bone Marrow Damage from Irradiation. J. E. RALL, LEON HELLMAN, WENDELL PEACOCK and RULON W. RAWSON,* New York, N. Y.

Fourteen patients have been studied before and after isotopic radiation from I^{131} calculated to have delivered from 33 rep (Roentgen equivalents physical) to 1300 rep to the blood as determined by measuring the level of circulating I^{131} and calculating the rep with Marinelli's formulae. The degree of radiation damage as measured by changes in the peripheral blood was better correlated with the rep delivered to the blood than with the amount of I^{131} administered. The doses of I^{131} were inconsistently correlated with the rep delivered to the blood: a 300 millicurie dose of I^{131}, for example, delivered 188 rep and a dose of 254 millicuries delivered 765 rep to the blood. One death due to irradiation occurred after a total of 1300 rep delivered by five doses of I^{131} in 14 months. Changes in the formed elements of the peripheral blood were observed similar to those described by others.

By studying radioiron utilization a decrease in the rate of hemoglobin synthesis following relatively small amounts of irradiation has been demonstrated. Four patients were given Fe^{55} before and after radiation and the rate of hemoglobin synthesis measured by frequent determinations of the hemoglobin radioiron levels. The curve of increase in radioactivity of hemoglobin was a simple exponential curve from which the time for half build up ($T\frac{1}{2}$) in normal patients was 1.5 to 4 days. After irradiation $T\frac{1}{2}$ increased to 3 to 6 days. A marked decrease in the rate of hemoglobin synthesis after as little as 100 rep was noted. This occurred in spite of the fact that there were no significant changes in the hemoglobin, red blood count or lymphocyte count.

The change in the rate of hemoglobin synthesis as measured by Fe^{55} was the most sensitive single index of radiation damage.

Antihyaluronidase Response Following Hemolytic Streptococcal Infection in Childhood. LOWELL A. RANTZ * and JOSEPH M. DI CAPRIO, San Francisco, California.

The heat-stable serum streptococcal antihyaluronidase titers have been measured following Group A hemolytic streptococcal infection in a large number of infants and children using the mucin clot inhibition technique, and compared with the levels of antistreptolysin O simultaneously determined.

Three distinct patterns of antistreptolysin response in childhood have been previously reported. In one, the production of antibody was feeble and, in a second, brisk but poorly maintained. Children who exhibited either of these patterns failed to form measurable antihyaluronidase.

A third group made large amounts of antistreptolysin O and levels were well maintained for long periods of time. Moderate to very high titers of antihyaluronidase were discovered following infection in many but not all of these individuals.

Evidence can be presented which indicates that the changing patterns of antistreptolysin and of antihyaluronidase response result from the conditioning of the antibody-forming apparatus by repeated infection by hemolytic streptococci.

Antihyaluronidase may be a more feeble antigen or be produced in less effective amounts by certain strains.

This information suggests the role of repeated infection by hemolytic streptococci in the pathogenesis of rheumatic fever, since antihyaluronidase is regularly present in the serum during the acute phase of the disease.

Studies on Intravenous ACTH Administration. ALBERT E. RENOLD, GEORGE MAISTEREENA and PETER H. FORSHAM,* Boston, Mass.

ACTH has been administered intravenously to normal subjects and to patients with and without adrenal pathology. Quantitative relationships between dosage, duration of administration and the adrenocortical stimulation obtained have been investigated. A standard intravenous ACTH test and an assay method for ACTH in man have been evolved.

Confirming previous reports, the intravenous administration of relatively small amounts of ACTH was found to afford a rapid and maximal adrenal cortical stimulation, as measured by a fall in circulating eosinophils and a rise in urinary 17-ketosteroid excretion. No response was demonstrated in five patients following bilateral adrenalectomy. In normal subjects a straight-line relationship was established between ketosteroid excretion, ACTH

dosage (over a range from 0 to 15 mg. LA-1-A) and the duration of infusion for a constant dose.

As the intravenous ACTH test, 20 mg. Armour ACTH were infused in 500 ml. diluent (saline or 5 per cent dextrose) over eight hours. The ten hour eosinophil fall was always 90 per cent or more in patients without adrenal insufficiency and the rise in twenty-four hour ketosteroid excretion (7–22 mg.) afforded an estimate of the acute functional reserve of the adrenal cortex. No anaphylactic phenomena occurred with the slow intravenous infusion of commercial ACTH.

The minimal amounts of ACTH and the quantitative relationships mentioned suggested use of the intravenous method as an assay of ACTH in man. Highly purified polypeptide mixtures and various commercial preparations have thus been compared.

Four patients "resistant" to ACTH after repeated courses of intramuscular administration showed no response either to intramuscular ACTH in doses of 25 to 50 mg. every six hours for one to four days or to a continuous intramuscular drip. They did, however, respond normally to the intravenous test. Increased tissue inactivation apparently plays an important part in acquired adrenocortical unresponsiveness.

The Effect of an Inhibitor Fraction from Human Sputum on the Multiplication of Influenza Virus. HARRY M. ROSE,* New York, N. Y.

Previous studies have indicated that human sputum contains different factors which a) inhibit hemagglutination by influenza virus (PR8 strain), b) reduce infectivity of the virus for mice and chick embryos, and c) enhance infectivity of the virus for mice under certain circumstances. A fraction has been obtained from sputum which inhibits infection of mice and chick embryos but possesses no enhancing properties; the inhibitor substance in this fraction deteriorates spontaneously unless frozen or lyophilized, is heat labile and is not specific antibody. Chick embryos were inoculated with 1000 EID50 of PR8 virus by the chorioallantoic route and received one injection of the inhibitor fraction by the same route from 1 to 6 hours later; in some experiments a second injection of inhibitor was made 24 hours after the first. The amounts of infective virus and viral hemagglutinin in the chorioallantoic fluids and membranes of treated and control chick embryos were determined at daily intervals for 4 days after primary inoculation. In treated embryos the multiplication of virus in the chorioallantoic membrane was delayed and decreased and the release of virus into the chorioallantoic fluid was reduced or entirely prevented. These effects were obtained in embryos given one injection of inhibitor as late as 6 hours after infection and were magnified by two injections of inhibitor. It is concluded that human sputum contains a substance which may restrain the multiplication of influenza virus within infected host cells, and that this substance may also modify the release of virus from these cells. The action of the inhibitor substance may be compared with the effect of Friedlander bacillus polysaccharide on mumps and PVM viruses, as reported by Horsfall and Ginsberg. The chemical nature of the inhibitor is unknown but other studies suggest that it may consist at least in part of carbohydrate.

The Influence of Cortisone on Water Diuresis in Man. JACK D. ROSENBAUM, ROBERT K. DAVIS and BRUCE C. FERGUSON, Framingham, Mass. (Introduced by Maurice B. Strauss).

The renal excretion of water and solutes has been studied in 9 adult male patients free of renal or cardiovascular disease before, during, and after the parenteral administration of 200 or 500 mg. of cortisone daily. With the subject sitting, water was ingested in the morning to establish and maintain a load of 1500 ml. for 3 to 8 hours. During cortisone administration, rates of urine flow reached 34 ml. per minute, exceeding control values by 50 to 200 per cent. The excretion rates of sodium, potassium, chloride, urea and creatinine after water loading were similar in treatment and control studies. The augmentation of diuresis during therapy could be diminished by decreasing the dietary intake of sodium chloride. The rate of diuresis during successive collection periods declined if the excretion rate of sodium (and chloride) also fell; after such a decline, if assumption of the recumbent posture resulted in an increased rate of sodium excretion, the rate of diuresis rose again.

During therapy, endogenous creatinine clearance often increased by as much as 100 per cent, but the magnitude of this increment bore a variable relationship to the increase in urine flow observed. Preliminary studies suggest that after therapy is instituted, C_{Cr} may increase several days before augmentation of diuresis occurs and that after therapy is discontinued C_{Cr} may remain elevated after the rate of diuresis has returned to control values.

Sensitivity to exogenous pitressin (15 milli-units intravenously) was unaltered.

Striking changes in the diurnal rhythm of the renal excretion of water, sodium, potassium and chloride were regularly observed. The normal nocturnal suppression of water and electrolyte excretion was often completely eliminated and always diminished. Ambulatory subjects showed an actual reversal of the rhythm; the nocturnal rates of water and electrolyte excretion exceeded those of the day.

The Mechanism of Anemia in Leukemia and Malignant Lymphoma. J. F. ROSS,* C. L. CROCKETT, JR. and C. P. EMERSON, Boston, Mass.

The anemia developing in patients with leukemia, lymphoma and other neoplastic diseases is obscure as to mechanism and pathogenesis. The usually accepted concept that it is due to the mechanical crowding out of erythropoietic tissue by neoplastic cells is not, in the majority of cases, confirmed by anatomic study. Neither does blood loss, an overt hemolytic process, or "toxic" bone marrow depression adequately explain the observed anemia. We have investigated the possibility that it may be caused by an increased rate of destruction of red cells.

We have evaluated the rate of blood destruction by simultaneously: 1) determining the quantitative fecal urobilinogen and calculating the hemolytic index from P^{32}-determined red cell mass; 2) and by measuring the rate of disappearance of transfused normal erythrocytes in leukemic patients by methods of selective red cell agglutination.

In ten patients with leukemia or lymphoma it has been demonstrated that the rate of destruction of erythrocytes is markedly increased. The differential agglutination studies indicated a rate of disappearance of transfused cells two or three times more rapid than normal, and the hemolytic index was elevated considerably in the majority of subjects. Evidence was also obtained that the rate of formation of red blood cells was often increased. Only one of this group had the usual evidences of hemolytic disease such as reticulocytosis, hyperbilirubinemia or a positive Coombs test.

These observations indicate that the anemia of leukemia and malignant lymphoma is due in large part to an increased rate of red blood cell destruction, and in many instances, in contrast to the generally accepted concept, the rate of red cell formation, instead of being diminished, may actually be accelerated.

Relation of Abnormal Serum Components to Bence Jones Protein in Multiple Myeloma. R. W. RUNDLES,* G. R. COOPER and R. W. WILLETT, Durham, N. C.

The significance of the abnormal protein components in the sera of patients with multiple myeloma has been studied in a series of 30 patients with the disease. Methods used included electrophoresis, diffusion and ultracentrifugation.

Abnormal serum components, recognizable in the patterns of 25 of the 30 patients, varied from minute increments to as much as 7–9 gm. per 100 cc. The electrophoretic mobility of the serum components, 0.3–2.6, was equal to or less than that of the Bence Jones protein excreted by the same patient. Differences in mobility were confirmed by adding urinary protein to serum and by studying mixtures of Bence Jones proteins.

There was no relation from patient to patient between the amount of abnormal protein in the serum and the amount of Bence Jones protein excreted in the urine, but in individual patients the serum increment remained proportional to the amount of Bence Jones protein in the urine as plasma cell growth was suppressed by therapy or as relapse occurred.

Abnormal serum components had diffusion rates $D^{\alpha}_{20,w} = 3.1–5.0$ (in 10 patients) and sedimentation velocities $S^{\alpha}_{20,w} = 6.19–6.76$ (in 14 patients). Calculated molecular weights ranged from 120,000 to 200,000, the majority between 140,000 and 160,000.

Bence Jones urinary proteins were variable in diffusion rate, $D^{\alpha}_{20,w} = 4.7–9.8$, and sedimentation rate, $S^{\alpha}_{20,w} = 2.44–4.40$. The calculated molecular weight of Bence Jones proteins excreted by 10 patients was 24,000 (1), 32,000–42,000 (4), 45,000–52,000 (3), 60,000 (1), and 90,000 (1).

Our findings indicate that the serum increments in myeloma are homogenous abnormal proteins of high molecular weight. Bence Jones proteins are moieties of low molecular weight, filterable through the glomeruli, apparently derived from the abnormal serum components.

A Safe Immunologic Adjuvant for Enhancing the Height and Persistence of Antibody Response to Influenza Virus Vaccines in Man. JONAS E. SALK,* MARY LYNCH BAILEY, and ANGELA M. LAURENT, Pittsburgh, Pa.

The results of field trials on influenza virus vaccines indicate the need for enhancing further the effectiveness of this immunizing agent. Various methods have been employed in attempts to accomplish this, i.e., multiple inoculations, intracutaneous rather than subcutaneous injections, increasing virus concentration in the vaccine, and the addition of certain immunologic adjuvants. Of these, only the use of the mineral oil adjuvant of Freund has yielded data sufficiently worthwhile to pursue further. When the Henles tried such adjuvants in humans, using the emulsifying agent Falba and mineral oil, they encountered abscesses in two out of eighty subjects and persistent nodules in the remainder. This discouraged further use of this preparation in man. However, in the course of other studies in this laboratory, using a different mineral oil (Bayol F) and another emulsifying agent (Arlacel A), it was observed in monkeys that when such mixtures were inoculated intramuscularly, no undesirable reactions were observed clinically. This prompted the extension of these studies to the problem of influenza virus vaccine, both in monkeys and in man, with very dramatic results in terms of enhancement and persistence of antibody response without undesirable side reactions. In addition, it was found that the amount of antigen could be reduced to very low levels and when combined with adjuvant is still effective in inducing the formation of high levels of antibody. As of this writing, more than two hundred individuals have been inoculated with such preparation without untoward effect and immunologic data over a six month period, in some, indicate a high degree of persistence of antibody well beyond the range achieved with vaccines in use at present. A further effect has been the enhancement of antibody response over a broad range of the antigenic spectrum, especially for the type A strains that vary so widely in antigenic composition.

Some Observations on the Mechanism of Delirium in Pernicious Anemia. DONALD C. SAMSON, SCOTT N. SWISHER, RICHARD M. CHRISTIAN and GEORGE L. ENGEL,* Rochester, N. Y.

Psychological disturbances in pernicious anemia are well known. Examination of 12 consecutive patients admitted to our hospital revealed delirium to be the most common disturbance, occurring in 11. One of the 11 was also schizophrenic.

In delirium the basic psychological disturbance is in level of awareness presumably due to some disturbance in cerebral metabolism. The purpose of this paper is to examine the relationship between the pernicious anemia process and the delirium.

Techniques used were serial mental status examina-

tions particularly directed toward evaluation of the level of awareness; serial EEG's with quantitative frequency analysis; and the usual hematological studies.

Results: (1) 11 of 12 patients were delirious by clinical criteria at some time; (2) 8 of these 11 had slowing of the EEG consistent with delirium, 2 had records at lower limit of normal frequency but which became faster with clinical improvement. One had low voltage fast type record; (3) all 12 patients responded hematologically, some suboptimally, to treatment. One of these patients had improvement in mental status, EEG and RBC, but with no reticulocyte response, on transfusions alone. Of the 11 clinically delirious patients, 9 recovered completely from delirium, one improved, and one was unchanged. Because their clinical condition became much worse two days after admission, 2 patients did not have serial EEG's. Of the 10 patients who improved, there was a correlation between onset of mental status improvement and the reticulocyte response in 8. In the 7 cases in which quantitative EEG studies could be done, EEG improvement correlated with the reticulocyte response and preceded the RBC rise in 5; one patient had clinical improvement without EEG change; one patient responded to transfusion alone without reticulocyte response.

From this study the authors suggest the following: (1) that disturbance in cerebral function is common in pernicious anemia; (2) that this disturbance is a primary metabolic one and not necessarily secondary to the anemia; (3) that it is reversible with specific therapy in most instances.

Studies of Coronary Disease in the Experimental Animal. III. Polarographic Studies of Intramyocardial Oxygen Availability in Dogs with Acute Coronary Occlusion and Narrowing, Correlated with Epicardial Electrocardiograms. JOHN J. SAYEN, WARNER F. SHELDON, HARRY F. ZINSSER, P. T. KUO, ORVILLE HORWITZ and DAVID P. MCCALLIE, Philadelphia, Pa. (Introduced by Charles C. Wolferth).

Local oxygen availability in exposed dog hearts has been studied by an improved technique permitting readings from ten platinum electrodes per minute or continuous readings at individual sites. Based on electrode values during coronary occlusion, three myocardial zones could be distinguished: 1) "Central" areas, showing rapid falls in electrode readings to less than a quarter of control values, 2) "Border" areas, falling more slowly and to a lesser degree, 3) "Remote" areas which show no significant change during occlusion. This physiological classification of myocardial areas by electrode values was consistent with the gross appearance of the heart when the ischemic regions' borders could be discerned. The earliest epicardial electrocardiographic changes, even at the center of ischemic areas, were much less definite than the conspicuous changes in oxygen electrode values, which appeared within ten seconds.

Responses to pure oxygen inhalation before release of coronary occlusion were as follows: 1) electrode rises in central areas were rare, slight and transient, 2) border area electrode values regained more than a third of their initial fall in 68 per cent of instances, 3) remote electrodes regularly recorded large increases characteristic of normal muscle.

Acute coronary narrowing had effects similar to occlusion but permitted more time to study the ischemic areas. Larger areas of the ischemic muscle behaved like "border" zones and consequently electrode rises were more frequent during oxygen administration before release of the narrowing. Electrocardiographic abnormalities in the border area sometimes tended to diminish when the oxygen electrode showed a rise in the same area. Fifty per cent oxygen inhalation produced much smaller increases than did pure oxygen.

Conclusion: Platinum electrodes provide a fairly precise, very sensitive means for studying localized myocardial ischemia and responses to oxygen inhalation. These experimental data support the inference that human myocardial infarction should be treated by measures producing maximal alveolar oxygen concentrations.

Leptospiral Meningitis. Investigation of a Water-Borne Epidemic Due to L. pomona. MORRIS SCHAEFFER, Montgomery, Ala. (Introduced by Paul B. Beeson).

In July of 1950, in the vicinity of Geneva, Alabama, a rural, peanut growing area, there occurred an epidemic of an influenza-like disease, attacking about 50 of approximately 80 adolescents and young adults who had been swimming in the creek. An artificially enlarged portion of it was frequented as the local swimming hole. The summer had been hot and dry and the water shallower and slower moving than usual. Dead hogs had been seen floating in the creek and its branches. On July 4 the hole was crowded with swimmers. Two to 10 days later they began to develop chills and fever, headache, nausea, vomiting, arthralgia, myalgia, stiff neck and back. Many had conjunctivitis and photophobia, some chest pain, abdominal pain, constipation or diarrhea, a few transient maculo-papular rashes. None showed jaundice or other liver involvement. Spinal fluid findings on several were typical of aseptic meningitis. The duration of illness was 5–14 days with occasional relapses but no complications or sequelae.

Because of the abundance of rats and hogs on the outlying farms, agglutinations for leptospira were run on the paired serum specimens obtained from 22 of the involved individuals after possible viral and rickettsial agents were ruled out. Of these 18 showed antibody titer increases against *L. pomona* in a magnitude attributable to recent infection with this agent. A control group of sera from individuals of the same age group and locality but not swimming nor ill was negative.

Since leptospirosis was not suspected at the outset, no attempts at isolation of leptospira were made and the water samples, frozen for virus isolation, were unsuitable. Animal surveys could not be made until 3 months later when there had been considerable turnover especially among the hogs. However, some positive sera were found among the few remaining hogs as well as cows, horses and mules.

An extensive rat survey yielded negative cultures and serum agglutinations for *L. pomona.*

This is the first description of such an epidemic in the United States. A similar outbreak due to *L. grippotyphosa* was reported recently to have occurred in a group of military personnel in France.

The Effects of Intravenous Papaverine and Procaine on Cerebral Circulation and Metabolism. P. SCHEINBERG, H. W. JAYNE, M. RICH and M. S. BELLE, Coral Gables, Fla. (Introduced by Frank L. Engel).

Adequate therapy of many types of cerebral vascular accidents will depend upon agents which produce dilatation of cerebral vessels. In this study the nitrous oxide technique for determining cerebral blood flow was used to measure the effects on the cerebral circulation of large intravenous doses of papaverine in one group of patients and procaine in another group. All age groups are represented in the study; patients with known cerebral vascular disease were not included. Control studies were done prior to administration of the drug.

Intravenous papaverine (180 mgm.) resulted in a 24 per cent increase in cerebral blood flow, a 21 per cent decrease in cerebral arterio-venous oxygen difference, a 28 per cent decrease in cerebral vascular resistance, and a 12 per cent decrease in mean arterial pressure. Cerebral oxygen consumption was unchanged. Cerebral venous oxygen tension was slightly increased. The decrease in cerebral vascular resistance and increased blood flow are indicative of dilatation of cerebral blood vessels, though the mechanism for this is not yet known. It should be noted that facial flushing was insignificant in these subjects. It was found that administration of papaverine must be accomplished over at least a 15 minute interval. Too rapid infusion of the drug may cause extreme drops in blood pressure and great apprehension on the part of the patient, with changes in cerebral circulation that cannot be attributed directly to the drug.

Procaine (750–1000 mgm.) administered intravenously over a period of 12 to 15 minutes produces no significant alteration in any of the cerebral metabolic functions, though our data indicate that further studies may show an increased cerebral vascular resistance following procaine.

The Effects of Adrenocorticotropic Hormone (ACTH) on Cerebral Blood Flow and Metabolism. JAMES F. SCHIEVE, PERITZ SCHEINBERG and WILLIAM P. WILSON, Durham, N. C. (Introduced by W. M. Nicholson).

While patients receiving adrenocorticotropic hormone (ACTH) usually show an increased sense of well-being and mild euphoria, a small number become psychotic. These clinical observations led to a study of cerebral blood flow by the nitrous oxide method. The oxygen consumption and glucose utilization per 100 gm. brain were obtained by multiplying the blood flow by the respective arterial-cerebral venous differences.

A total of 44 observations on 14 patients was made (15 before, 23 during, and 6 following ACTH). ACTH was given in full therapeutic doses (60–100 mgs. Armour or 30–40 mgs. Wilson daily). One 12 year old child received 25 mgs. Armour daily. Measurements were made on therapy at least once and as often as four times at intervals varying from 7 to 56 days.

On ACTH a significant reduction in cerebral blood flow occurred (61 to 50 ml./minute/100 grams brain). This was accompanied by a significant widening of arterial-cerebral venous oxygen (5.8 to 6.5 vols. %) and glucose (8.2 to 10.1 mgs. %) differences. These were of sufficient magnitude to result in essentially unchanged cerebral oxygen consumption (3.5 to 3.2 ml. O_2 per 100 gms. of brain per min.) and glucose utilization (4.9 to 4.9 mg. glucose per minute per 100 gms. of brain). A 32 per cent increase in cerebral vascular resistance occurred, but only a 9 per cent increase in mean arterial blood pressure.

Six patients with initially low flows (below 50) with low $CMRO_2$ (2.8) and CMR Gl. (3.6) showed no change in cerebral blood flow or metabolism on ACTH in spite of the usual clinical response.

Two patients who became psychotic on ACTH revealed no change in cerebral metabolism or blood flow as measured by this method.

Comparative Studies of the Biochemistry of Heart and Skeletal Muscle. GERHARD SCHMIDT, MARIA FULD, RICARDO CUBILES and SAMUEL PROGER,* Boston, Mass.

Both the skeletal and heart muscle of higher animals resemble each other with regard to their content of some characteristic constituents, for example, myosin and adenyl-pyrophosphate. A study of the literature, however, reveals that they differ strikingly in many important aspects of metabolism and of chemical composition. As examples there are the well-known differences in content of substances such as cytochrome C, aldolase, and phosphocreatine, as well as the striking difference in the behavior of these tissues under anaerobic conditions.

Because of the possible relationship of demonstrable biochemical differences to the special functional characteristics of heart and skeletal muscle a comparative study of both tissues has been undertaken. Thus far our studies have dealt with the content of phosphorylase and phosphoglucomutase of both tissues (in rats, rabbits, and dogs) as well as the content of bound and free β-alanine in the heart and skeletal muscle of rats and beef.

The phosphorylase content of heart muscle was found to be roughly about half that of skeletal muscle while the phosphoglucomutase content showed a much greater difference in the two tissues.

Bound β-alanine in the form of carnosine and anserine constitutes, next to phosphocreatine, the predominant component of the nonprotein nitrogen fraction of skeletal muscle. On the contrary the heart muscle was found to contain only negligible quantities, if any, of free or bound β-alanine.

Since the total nonprotein nitrogen fraction is quantitatively similar in both tissues and since the heart muscle contains only about $\frac{1}{10}$ the amount of phosphocreatine as is found in skeletal muscle as well as practically no

β-alanine compounds, it must be concluded that there exists a large content of nonprotein nitrogen substances peculiar to the heart muscle. We are at present attempting to determine the nature of these substances.

Relative Viscosity and Clotting Time of Whole Blood in the Healthy and in Those with Vascular Disorders. ROBERT A. SCHNIEDER, New York, N. Y. (Introduced by Harold G. Wolff).

Although the importance of an increase in blood viscosity coexisting with a shortened clotting time would be readily recognized, no definite studies of such simultaneous measurements in human subjects are available. Accordingly such measurements were undertaken on individuals subjected to a variety of stresses including 20 healthy subjects, 20 patients with essential hypertension, 8 patients with structural heart disease undergoing congestive failure and 5 patients with thrombophlebitis. Clotting time was measured in siliconized tubes and relative viscosity was determined using a capillary viscosimeter.

In healthy subjects shortened clotting time was uniformly accompanied by a variable increase in blood viscosity. In certain cases hematocrits were determined and the increase in viscosity appeared related to an increase in red cell mass. Twenty hypertensive patients were studied because of their known proclivity for thrombosis. These subjects reacted uniformly during stressful interviews productive of a pressor response with a shortening of the clotting time (average 15%) and an increase in viscosity (average 7%). Five hypertensive subjects, in whom no pressor response occurred, showed no changes in viscosity or clotting time. Patients with heart disease showed shortening of clotting time and increase in blood viscosity during periods of congestive failure which values approached normal with compensation. In contrast, patients with thrombophlebitis showed shortened clotting time but usually no increase in viscosity during bouts of venous thrombosis. These patients were anemic and this may have accounted for their failure to show a rise in blood viscosity.

The factor of viscosity is ignored in calculating peripheral resistance, the assumption being that it is constant. This study indicates that this convention should be reexamined and suggests that variations in blood viscosity may be pertinent to the hemodynamics of hypertension. Shortened clotting time is often accompanied by increased viscosity and this may bear upon the problem of thrombosis.

Studies on the Sulfhydryl Content of the Serum Proteins. EMANUEL B. SCHOENBACH * and NORMAN WEISSMAN, Baltimore, Md.

The biological significance of sulfhydryl containing compounds has been explored since glutathione was first isolated from yeast in 1921. Evidence has been presented that the sulfhydryl groups are important for the structure of proteins and various metabolic processes such as cellular division, and enzyme activity.

An amperometric method has been adapted to the determination of sulfhydryl groups in the serum proteins. This technique has been shown to be quantitative and specific for the $-SH$ group. This determination is not affected by the presence of creatine, desoxyribonucleic acid, penicillin or cystine. Previous studies on sera from normal individuals and patients suffering from various types of illness have shown that the protein sulfhydryl was significantly reduced among the latter group. A further investigation was therefore initiated in which the sera were fractionated by the Pillemer procedure into albumin and globulin components and these components were analyzed with respect to their nitrogen, peptide and sulfhydryl content.

The sera from patients with carcinoma, multiple myeloma, lupus erythematosus, nephritis, cirrhosis, and one cystinuric, when thus studied, showed that the albumin was usually reduced while the globulin was normal or increased as measured by their nitrogen or peptide content. The ratio of peptide per mg. of protein nitrogen for these components did not deviate markedly from the normal. The sulfhydryl per gram of albumin nitrogen or per peptide bond was below the normal value among 86–87% of the samples and the sulfhydryl per gram of globulin nitrogen or peptide bond was low among 76–79%. It is suggested that a common metabolic abnormality related to protein formation which results in a qualitative change in both albumin and globulin components occurs in these disease states which is reflected in the low sulfhydryl values.

Effects on Hypertension of Sulfhydryl and Hydrazine Compounds. HENRY A. SCHROEDER, St. Louis, Mo.

Pherentasin, a sustained pressor substance found in human hypertension, is a primary amine containing a carbonyl group, the latter apparently being necessary for prolonged activity. This formula suggests that two metabolic disturbances may be associated with hypertension: imperfect deamination of amines and incomplete reduction of certain ketones. To test the latter hypothesis, a group of sulfhydryl-containing compounds were injected in brief experiments in normotensive and hypertensive rats. Differential prolonged depressor action was exhibited in the hypertensive animals by substances such as cysteine, dimercaptopropanol, β-mercaptopropionic acid, mercaptosuccinic acid, thioglycollic acid and others of similar configuration. When the sulfhydryl group was on a ring structure no such action was demonstrated; sulfur containing compounds such as cystine, methionine, oxidized glutathione and the like exhibited no action. The effects lasted 2 to 3 hours in rats and dogs; in man dimercaptopropanol likewise exerted a brief (2 hr.) hypotensive action. These substances abolished the pressor action of arterenone but not of arterenol, and probably destroyed pherentasin in like manner.

Because hydrazine compounds also may attack carbonyl groups, two substances, 1-hydrazino-phthalazine and 1-hydrazino-4-methyl-phthalazine were tested for their hypotensive properties in renal hypertensive dogs; prolonged chronic lowering of blood pressure was observed. In 30

patients with hypertension these agents given by mouth exerted continuous hypotensive effects for days or weeks; the most pronounced actions occurred in neurogenic hypertension, the least in renal hypertension. Adverse renal effects were not observed. Previous sympathectomy enhanced the action considerably. Tachyphylaxis occasionally occurred, but was not persistent. Toxic manifestations of headache and nausea could be controlled by anti-histaminic drugs and soon disappeared. Postural hypotension accompanied initial administration. It is believed that these two types of compounds are probably anti-hypertensive agents.

Crystalline Calcium Chondroitin Sulfate. MAXWELL SCHUBERT and JULIA EINBINDER, New York, N. Y. (Introduced by Colin M. MacLeod).

Mucopolysaccharides are major components of connective tissue and information that will help to characterize and to distinguish them is needed. Of particular interest for the chemistry of joints is the chondroitin sulfate of cartilage.

Using mild isolation methods previously worked out and described, it has now been found possible to separate chondroitin sulfate from the cartilage of nasal septum as a calcium salt into fractions differing slightly in solubility and optical rotation. The least soluble fraction is amorphous, the more soluble fractions are crystalline. All have the same composition as regards hexosamine, hexuronic acid, calcium and sulfur content. This is the first time any connective tissue mucopolysaccharide has been crystallized and it is the first indication that chondroitin sulfate from cartilage may not be a single chemical substance.

Analytical data on these fractions show that the values for hexosamine, hexuronic acid, calcium and sulfur are all lower than would be expected on the basis of the known components of chondroitin sulfate. This evidence may indicate the presence of an as yet unidentified component of chondroitin sulfate.

A study has been made of the consumption of periodate by the two crystalline fractions of chondroitin sulfate. There is a slow and progressive reduction of periodate over a period of 80 hours which shows no break that could be related to the end groups, though this method has been used by Meyer, Odier and Siegrist.

The Effect of Pituitary and Adrenal Hormones on the Metabolism and Excretion of Potassium. DONALD W. SELDIN, LOUIS G. WELT and JOSEPH CORT, New Haven, Conn. (Introduced by Charles H. Burnett).

In experimental animals and man, administration of ACTH or adrenal steroids may be followed by three changes in potassium metabolism: potassium excretion increases; the serum concentration falls; and hypochloremic alkalosis develops. The following study attempts to ascertain whether these changes are caused by direct action of adrenal hormones, either on tissue cells or renal tubules, or whether they are the passive consequence of sodium retention which these hormones sometimes produce.

Patients with and without edema, maintained on diets nearly constant in potassium, but containing only negligible quantities of sodium, were given prolonged courses of DOCA (20 mg per day for one month), cortisone (100–300 mg per day for 33 days), and ACTH (160 mg per day for 19 days). No change in serum concentration of potassium, sodium, bicarbonate, or chloride was detected. Potassium excretion was not accelerated by DOCA, cortisone, or ACTH, except for a transient increase during the first day of administration of the latter two hormones.

The study was extended to rats maintained on sodium-free diets containing adequate quantities of potassium. Prolonged injections of DOCA produced no change in potassium excretion, muscle composition, or serum concentrations of bicarbonate, chloride, potassium, or sodium.

The following hypothesis is therefore proposed. DOCA has no *direct* effect on internal exchanges or renal excretion of potassium; if subjects have access to sodium salts, however, a potassium diuresis may follow DOCA administration as a passive consequence of sodium retention; when potassium excretion is thus accelerated, potassium is lost as potassium chloride, thereby producing a hypochloremic alkalosis. Cortisone and ACTH appear to behave like DOCA in promoting potassium excretion by sodium retention, although these hormones in addition accelerate potassium losses by direct effect on tissue cells as well. In the doses used in these studies this cellular effect was only transitory.

The Role of Somatotrophic Hormone (STH) in the Production of Malignant Nephrosclerosis, Periarteritis Nodosa and Hypertensive Disease. HANS SELYE,* Montreal, Canada.

Experiments on female piebald rats revealed that electrophoretically pure somatotrophic hormone (STH) produces nephrosclerosis with variable degrees of nephritis, marked polyuria, myocarditis, pancreatic periarteritis nodosa and hypertension. The experimental animals were sensitized to mineralo-corticoid actions by unilateral nephrectomy and an excess NaCl intake, but this sensitization in itself did not produce such changes.

The type of the toxic manifestations, as well as the sensitization of the animals to these STH effects by unilateral nephrectomy and NaCl, parallel our previous findings in animals receiving excesses of desoxycorticosterone acetate (DCA), or lyophilized anterior pituitary (LAP) material.

It is assumed that STH is the active principle responsible for the toxic actions of LAP upon the kidney and the cardiovascular system. The lesions caused by STH are considered to be, in all likelihood, secondary to a sensitization of the tissues to DCA-like mineralo-corticoids. In addition STH may also increase the production of mineralo-corticoids by the adrenals.

The cardiovascular and renal damage normally caused by STH overdosage is prevented if cortisone is simultaneously administered in doses adequate to produce adrenocortical atrophy.

STH increases the sensitivity of the rat to the production of experimental arthritis. Simultaneous treatment with cortisone exerts a contrary effect. However, large doses of cortisone are required to inhibit an experimental arthritis in the STH-treated than in the otherwise not treated animal. Apparently the anti-arthritic effect of cortisone is inversely proportional to the amount of STH present in the organism.

In the production of the so-called "disease of adaptation" STH appears to play a rôle equally as important as that of ACTH. The former is responsible for the activity of mineralo-corticoids, which stimulate defensive granuloma formation, while the latter regulates the secretion of gluco-corticoids, which inhibit such defense reactions.

Some Effects of a Single Intravenous Dose of Hypertonic Sodium Bicarbonate in Man. R. B. SINGER, J. K. CLARK and A. P. CROSLEY, JR., Philadelphia, Pa. (Introduced by W. C. Stadie).

Three male subjects were given 7.5 per cent sodium bicarbonate solution intravenously (2.3 milliequivalents per kilogram in 10 minutes). During a control period and for two hours after the injection data were obtained on acid-base factors of arterial blood and urine, on inulin, creatinine (GFR) and PAH (ERPF) clearances, and on inulin space. The average maximum changes in the blood were: pH, +0.17; plasma CO_2 content, +12.8 mM/L; sodium, +9 mEq/L; chloride, −5 mEq/L; red cell fraction, −2 per cent; hemoglobin −0.5 gm/100 ml; CO_2 pressure, ±3 mm. of Hg. The maximum urinary changes were noted in the first 10 minute period after the bicarbonate: increase of pH from 5.9 to 8.0; increase of sodium output from 0.13 to 0.85 mEq/minute; transient increase of chloride excretion followed by a drop below the control rate. The rise in Na excretion rate was paralleled by a rise of ERPF to 39 per cent above the control, but GFR showed no significant change.

The renal elimination of sodium bicarbonate was estimated from sodium excretion in excess of chloride. An average of 17 per cent of the dose given was accounted for in the urine at the end of one hour, and only 34 per cent to 78 per cent in 24 hours. The extracellular sodium bicarbonate was estimated from the inulin space and either (a) the change in plasma (Na − Cl) concentration from the control level, or (b) the change in plasma CO_2, with an additional amount allowed for red cell CO_2 increase and hemoglobin buffering. By the latter method, in which the experimental error was smaller, the amount of the dose remaining in the inulin space at 20 minutes was 64 per cent and at 60 minutes, 50 per cent. The unaccounted-for residue, 26 per cent at 20 minutes, and 33 per cent at 60 minutes, is considered to represent intracellular transfer of sodium.

Studies of Portal Venous Oxygen Content in Unanesthetized Man. C. MCC. SMYTHE, H. F. FITZPATRICK and A. H. BLAKEMORE, New York, N. Y. (Introduced by Franklin M. Hanger).

The recent development of methods of "regional heparinization" has provided a means by which blood may be obtained directly from the portal vein in man. A small-bore plastic catheter is introduced 3 or 4 cm. into a portal tributary during construction of a portacaval anastomosis to relieve portal hypertension due to cirrhosis. It may be maintained *in situ* for as long as one week post-operatively by a constant infusion of heparin that serves both to keep the catheter open and to prevent thrombosis at the site of anastomosis. Samples of portal venous blood have been obtained on 13 occasions from 9 patients who were resting quietly in bed at least 3 days post-operatively. The fasting arterial-portal venous oxygen difference was 1.9 vol. per cent on the average and ranged from 0.4 to 3.3 vol. per cent. Corresponding values were obtained in measurements made on blood sampled from the portal vein during operation in 4 additional subjects. The site at which blood was sampled in the portal venous system (gastro-epiploic, mid-colic, inferior mesenteric, and portal veins) did not affect these values. Digestive activity appeared to augment the arteriovenous oxygen difference, on one occasion from 3.2 to 5 vol. per cent and on another from 3.4 to 5.1 vol. per cent about one hour after a small meal. Since relatively little oxygen appears to be taken up by the gastrointestinal tract, the portal venous blood must provide a substantial portion of the oxygen brought to the liver in man.

The Lipid Composition of Ultracentrifugates of Normal Human Serum in the Post Absorptive State. J. R. SNAVELY, W. H. GOLDWATER, M. L. RANDOLPH, C. C. SPRAGUE and W. G. UNGLAUB, New Orleans, Louisiana (Introduced by R. H. Turner).

To the study of the lipid and protein composition of serum we have applied a quantity ultracentrifugal technic based on chemical analyses of serial levels of the column of ultracentrifugate after the exposure of serum samples to high gravitational fields.

It was found that positively sedimenting proteins produced a density gradient of logarithmic shape ranging from 1.003 to 1.100, and that along this gradient lipid aggregates were reproducibly accumulated. At the very top a cream-like layer was rich in neutral fat, but also contained phospholipid and free and esterified cholesterol. Near the middle a faintly turbid zone showed high concentrations of free and ester cholesterol and phospholipid; below this a clear zone contained phospholipid and cholesterol as esters only, and near the bottom phospholipids occurred, but no cholesterol.

When correlation graphs were constructed for the free cholesterol-containing segments of centrifugate relating the concentrations of this substance to those of phospholipids, and cholesterol esters, it was found that linear interrelationships obtained in the upper segments of the tube. The relationship between phospholipid and free cholesterol was expressed by the formula $PL = 2.5\ FC + a$, and that between free cholesterol and cholesterol esters by the formula $CE = 2.3\ FC$ in the creamy layer rich in neutral

fat, and by the formula $CE = 4.5\ FC + b$ in the deeper layers.

Below the cream layer, the values for a and b in the phospholipid and cholesterol ester equations increased progressively with increasing depth in the column, and represent, we believe, moieties of cholesterol esters and phospholipids not combined with free cholesterol.

It is suggested that in the quantity ultracentrifuge a stratification of groups of lipid aggregates occurs, some of which contain phospholipids, free, and esterified cholesterol in definite proportions, and that these aggregates constitute a quantitatively important part of the lipid transport mechanism.

A Study of Failures in the Use of Quinidine for Conversion of Chronic Auricular Fibrillation. MAURICE SOKOLOW,* San Francisco, Calif.

Approximately 80 per cent of 75 cases of chronic auricular fibrillation were converted to sinus rhythm by the use of quinidine without important toxicity and often with considerable therapeutic benefit. However, 20 per cent failed to convert, and it is the purpose of this paper to evaluate the factors causing the failure.

Twenty-four final failures were observed (including 9 studied through the courtesy of Dr. Mervyn Goldman at the Veterans Hospital, Oakland). In 15 of these (60%), quinidine was stopped because of nausea and vomiting. In the remaining 9 failures, quinidine was discontinued because of adverse cardiac effects. Widening of the QRS complexes was observed in 4 cases, runs of ventricular premature beats occurred in 3 cases and Stokes-Adams attacks appeared in 2 cases.

The most common cause of initial failure was inadequate dosage, although doses exceeding 3 to 4 grams per day and blood concentrations exceeding 10 mg/L gave less likelihood of success and more possibility of toxicity. Successful conversion was accomplished in 50 per cent of cases requiring doses exceeding 3 grams per day and in 30 per cent requiring 4 grams per day. Particular care and frequent observation was required with these large doses. Relatively low blood concentrations with relatively high doses is a favorable situation in which to pursue use of the drug. In 15 cases in which blood concentrations exceeding 9 mg/L were required, 50 per cent converted; in 10 cases in which levels exceeding 10 mg/L were required, 40 per cent converted; in 9 cases with levels of 11 mg/L or more, 30 per cent converted.

Initial failures in conversion resulted from the continued use of fixed dose schedules beyond the time when the blood concentration on such a dose was found to rise. Increasing the size or frequency of the dose corrected the fault. Similarly, initial failures occurred because of hesitancy to continue the drug after auricular flutter developed.

In an occasional case when failure occurred because of nausea and vomiting due to fairly rapid administration (every 2 hours for 5 doses), successful conversion resulted when the drug was given four times a day, and the size of the dose increased every 3 to 4 days.

The Significance of Hyponatremia in Congestive Heart Failure. RUSSELL D. SQUIRES and J. RUSSELL ELKINTON,* Philadelphia, Pa.

The serum sodium concentration was studied in 54 samples from 44 patients with peripheral edema due to congestive failure. The mean value for all the samples was 132.8 ± 8.1 m.eq. per liter, and the mean for those without vomiting or renal insufficiency was 134.7 ± 7.2 m.eq. per liter, compared to a mean of 139.2 ± 2.5 m.eq. per liter in a group of 21 normal control subjects. The hyponatremia appeared to be most closely correlated with the frequency and duration of administration of mercurial diuretics.

Fourteen balance studies were then carried out in twelve edematous cardiacs, most of whom were hyponatremic, and all of whom had become refractory to mercurial diuretics. The majority were given hypertonic sodium solution, some received potassium, and a few were given both ions. During control periods in six patients the serum sodium concentration fell to even lower levels when the patient was in sodium equilibrium or was retaining sodium. The administration of hypertonic sodium solution resulted in some degree of diuresis in only four of ten patients; in only one was the edema completely cleared. Most of the patients developed thirst while still hyponatremic. The administration of potassium, with or without sodium, to a few of the patients was not associated with any diuresis nor with any consistent loss of intracellular sodium, although potassium was retained in the cellular phase.

It is concluded that while systemic sodium depletion may be present, may be depressing the circulation, and may be treated successfully with hypertonic sodium solution, other factors are involved in this syndrome leading to retention of water rather than to loss of salt. Alteration in intracellular osmolarity may be one of these, and appears to be at least quantitatively of greater importance than net transfers of cellular sodium and potassium.

The Effect of Thyrotropin on the Release of Hormone from the Human Thyroid. JOHN B. STANBURY, RICHARD E. GOLDSMITH and GORDON L. BROWNELL, Boston, Mass. (Introduced by Paul C. Zamecnik).

While thyrotropin (TSH) increases the over-all activity of the thyroid gland, it has not been clear which of the various stages in the metabolic pathway of iodine through the thyroid are primarily stimulated by TSH and which may be seemingly stimulated only because the preceding step was altered. This study concerns the effect of TSH on iodine stored in the gland of patients receiving 2-methyl-1-mercaptoimidazole (MMIA).

Six patients with typical Graves' disease were given tracer doses of radioactive iodine. The biological half life of the labeled iodine of the thyroid was then determined. When this was established the subjects were given varying doses of MMIA. The biological half life became appreciably shortened in two subjects. When TSH was added to this regime the biological half life was appreciably shortened in all. It was shown that the excretion of radioiodine was increased in all patients when they were

given MMIA. These results are interpreted to mean that TSH induces a release of thyroid hormone from the gland independently of its effect on the accumulation of iodide.

From the change in RaI excretion with and without MMIA and the change in biological half life an estimation of the re-utilization fraction of iodide can be made. This is somewhat less than the RaI uptake of the unblocked gland. The implications of these results are discussed.

A Clinical Study of Intravenous Procaine Amide (Pronestyl): Its Value and Hazards. NORMAN S. STEARNS and EDMUND J. CALLAHAN, Boston, Mass. (Introduced by Laurence B. Ellis).

Intravenous pronestyl was administered to 33 patients with ventricular and/or supraventricular arrhythmias. Arterial blood pressure and electrocardiographic changes were followed. Initial pronestyl infusion rates between 25 and 100 mg. per minute were employed. Infusions were slowed or stopped when indicated by hypotensive responses or conversion of arrhythmias.

Ventricular tachycardia was abolished in 7 of 8 patients. Multiple ventricular ectopic beats were eliminated in 8 of 12 patients and decreased in 3. A–V nodal tachycardia was abolished in 3 of 4 patients. Effective drug doses ranged from 60 to 800 mg. Thirteen of 15 patients with ventricular or nodal arrhythmias attributed to digitalis toxicity responded. Pronestyl, in the dose range employed, was not effective in converting auricular fibrillation or in slowing associated rapid ventricular rates. Supraventricular arrhythmias, other than nodal tachycardia, were affected less strikingly than ventricular arrhythmias.

Systolic and diastolic blood pressure fall associated with signs and symptoms of collapse occurred in 14 of 33 patients. Blood pressure fall, usually to levels below 100 mm. Hg, occurred in patients with high, normal, and low control levels; and, both in patients with ventricular and supraventricular tachycardias. Blood pressure fall occured with pronestyl infusion rates of 25, 50, and 100 mg. per minute in response to total doses averaging less than 500 mg. Neosynephrine in 6 patients successfully counteracted pronestyl induced hypotension in 5.

Pronestyl administered to 4 patients with bronchial asthma produced profound hypotension with convulsive movements or precipitated asthmatic attacks.

Easily recognized electrocardiographic changes indicative of toxicity were rarely produced by pronestyl. Blood pressure determinations were a more important guide to safe pronestyl infusion rates. Because significant hypotension was produced in spite of slow pronestyl infusion rates, the drug should be administered by this route only to patients with susceptible arrhythmias where immediate conversion is highly desirable.

Rate of Platelet Survival in Thrombocytopenia. MARIO STEFANINI and JYOTI B. CHATTERJEA, Boston, Mass. (Introduced by William Dameshek).

The survival time of transfused platelets was studied in 13 cases of idiopathic thrombocytopenic purpura (ITP) and in 7 cases of thrombocytopenia due to acute leukemia or aplastic anemia.

Platelet counts, clot retraction, serum prothrombin activity (SPA), bleeding time and capillary fragility were determined at regular intervals following direct transfusion of high platelet level polycythemic blood using siliconized apparatus. To limit errors due to possible utilization of platelets by platelet famished tissues, a preliminary fresh blood transfusion was given. In ITP transfused platelets disappeared from the circulation in 0–3 hours; in secondary thrombocytopenia longer survival times (12–96 hours) were observed. In both groups clot retraction and SPA closely paralleled platelet level, while bleeding time and capillary fragility returned to original values much later. Platelet survival times before and after unsuccessful splenectomy in one case were identical.

Experiments were performed in patients with ITP during splenectomy: (1) samples of blood obtained from splenic artery and vein in 10 patients showed no significant differences in platelet count, clot retraction or SPA. (2) in one case responding favorably to splenectomy, polycythemic blood was injected into the antecubital vein; blood samples collected simultaneously during splenectomy from splenic artery and vein showed no appreciable differences. (3) in two cases responding favorably to splenectomy, polycythemic blood was injected directly into the splenic artery at the time of operation and blood recovered from the splenic vein. All blood values remained essentially unchanged. Adrenalin tests before and after splenectomy revealed no essential differences. Thus, no evidence of platelet sequestration by the spleen was found.

The rate of platelet survival in ITP was strikingly decreased. The mechanism of this effect may involve several factors which are being investigated at present.

The Nature of the Blood Vessel Damage in the Shwartzman Phenomenon. CHANDLER A. STETSON, New York, N. Y. (Introduced by Maclyn McCarty).

In contrast to the extensive studies which have been made of the bacterial products which elicit the Shwartzman phenomenon, the ability of such non-bacterial substances as glycogen, agar, and antigen-antibody complexes to produce the reaction has received comparatively little attention. Because of the relative rapidity with which these materials induce the local hemorrhagic reaction, however, it seemed possible that they might be producing directly some effect which occurs only secondarily following the injection of the more slowly acting bacterial endotoxins. Both classes of materials have been found to produce a profound leucopenia and thrombocytopenia when injected intravenously in rabbits. This action can, in the case of the non-bacterial substances mentioned above, be correlated with the ability of these agents to produce *in vitro* an immediate and striking aggregation of platelets and leucocytes into large clumps which exhibit marked adhesive properties. Evidence has been obtained which indicates that the leucopenia and thrombocytopenia which

follow the intravenous injection of these agents is due to the formation of such clumps of formed elements *in vivo*, with subsequent removal of the aggregates in the capillary beds of internal organs. The development of the characteristic local hemorrhagic necrosis of the Shwartzman phenomenon appears to be due to the plugging of capillaries and veins in the prepared skin areas by leucocyte-platelet thrombi, with resulting interruption of blood flow and necrosis of the involved vessels. A similar mechanism appears to be operating in the experimental production of hemorrhagic necrosis in mouse sarcoma 180 by the intravenous injection of materials capable of eliciting the Shwartzman phenomenon.

The Relationship of Streptolysin S Inhibitor to Phospholipid Levels in the Serum of Human Beings and of Rabbits. GENE H. STOLLERMAN, BERNARD B. BRODIE, BETTY LEVY and YETTA POROSOWSKA, New York, N. Y. (Introduced by J. Murray Steele).

The fall in the serum levels of streptolysin S inhibitor (SSI) which usually occurs during the acute phase of rheumatic fever suggested a disturbance of lipoprotein metabolism in this disease. Previous studies indicated that the inhibition of this streptococcal hemolysin by serum is associated with alpha and beta lipoproteins rather than with specific antibody. Streptolysin S is inhibited by saline suspensions of phospholipids but not by cholesterol or neutral fats. In addition, a large proportion of the SSI is destroyed by the action of lecithinase on serum. It therefore seemed possible that variation in SSI might reflect a parallel variation of serum lipoproteins rich in phospholipid.

The relationship of the serum levels of total phospholipid, lecithin and sphingomyelin to the SSI titer was studied in patients with a variety of diseases. Fluctuations in total phospholipid, and particularly in lecithin levels, were associated with similar variations in SSI. Diseases associated with the most marked increase of beta lipoprotein (biliary obstruction, xanthomatosis, nephrosis, etc.) showed the highest levels of SSI and phospholipids, while in rheumatic fever, and in occasional patients with protracted febrile states, subnormal phospholipid levels were accompanied by depression of the SSI titers. There was, however, a relatively wide range of lecithin values for any given titer of SSI.

An increase in streptolysin S inhibition was induced in the serum of rabbits by producing a marked hyperphospholipemia in these animals by intravenous injection of the non-ionic detergent Triton WR 1339. Repeated doses of this detergent (1.25 ml/Kg of a 12½% solution three times weekly for nine weeks) resulted in a significant rise in SSI after serum phospholipid had increased to more than five times its initial level. When the injections of triton were omitted, both phospholipid and SSI fell promptly and rapidly rose again when injections were resumed.

The Practical Importance of the Diurnal Variations in Number of the Circulating Eosinophils. J NORRIE SWANSON, Boston, Mass. (Introduced by Marian W. Ropes).

The circulating eosinophil cells were counted throughout the day in a group of arthritic and healthy persons, in order to assess the importance of diurnal variations. Without such data, the interpretation of a decrease in the number of these cells as a measure of adrenal-cortical responsiveness to certain agents is difficult.

In most subjects the cells decreased in number from early to late morning, increasing around midday, decreasing slightly in the early afternoon and increasing again late in the day. These changes were of such a magnitude as to make it apparent that isolated eosinophil counts must be carried out at the same time of day to be comparable.

On thirteen occasions, in four subjects, the morning fall was greater than 50 per cent, usually reaching its maximum at the fourth hour. In contrast, the early afternoon fall was, with one exception, never greater than 40 per cent, and greater than 30 per cent in only two persons. The latter fall was usually maximum at the second hour and never lasted longer than two hours. These data suggest that tests, such as the four-hour-ACTH and epinephrine tests, aimed at determining induced eosinopenia should be done in the afternoon, because at this time the spontaneous fall is slight and of shorter duration than in the morning. Furthermore, an induced afternoon fall, four hours after the administration of a given agent, would be of more significance because normally a rise would be anticipated. The effect on the cells of the midday meal was found to be insignificant.

Three patients did not show an eosinopenic response to repeated injections of ACTH, but did following the usual dose of epinephrine. The interpretation of these latter observations is not apparent.

A Plasma Factor Sensitive to Variations in Prothrombin Conversion. MORRIS TAGER* and ANNE L. LODGE, Cleveland, Ohio.

Staphylocoagulase reacts with a plasma globulin, the coagulase-reacting factor (CRF), to convert fibrinogen to fibrin. The reaction takes place in the absence of calcium, in the presence of heparin and of an excess of citrate and oxalate ions. CRF cannot be identified with prothrombin, with Seegers' AC globulin, nor with any other factors at present known to be essential for physiological clotting.

Although the physiological and the coagulase clotting processes appear to be dependent on different mechanisms, a link has been found indicating a relationship between the two systems. When plasma is converted to serum, CRF activity is diminished. The present study has sought to elucidate the mechanism of this reaction. It was observed that maximal CRF loss is incurred when fresh plasma is converted to serum, while the loss is far less marked when older plasma was used. The addition of the thermolabile prothrombin-conversion accelerator (Factor V of Owren) to old plasma strikingly enhances CRF loss, and heating Factor V abolishes this effect. The CRF loss will not take place in the absence of prothrombin, and

in agreement with Duthie, calcium is essential for this reaction. No evidence was found that the CRF loss results from passive adsorption on the fibrin clot.

CRF consumption during the conversion of plasma to serum is therefore a sensitive indicator of prothrombin conversion: effective prothrombin conversion is reflected by maximal CRF loss, while impairment of prothrombin conversion spares the coagulase-reacting factor.

Colorimetric Measurement of the in vitro *Action of Human Liver on Alpha-Estradiol.* H. J. TAGNON,* PHYLLIS SCHULMAN, ALEXANDER BRUNSCHWIG and SEYMOUR LIEBERMAN, New York, N. Y.

It has been known for several years that liver slices incubated *in vitro* inactivate estrogens. Inactivation studies were based on the bioassay method of measuring estrogens because the colorimetric methods of assay do not yield satisfactory results in the presence of tissues. The quantitative aspects of the metabolism of estrogens by liver tissue *in vitro* are not completely defined and there are few data concerning the ability of the human liver to inactivate estrogens *in vitro*.

A colorimetric method is presented here which permits measurements of natural estrogens in the range 10 to 100 micrograms, in the presence of biological material. It is a modification of that of Talbot et al. using tetrazotized dianisidine as the color producing reagent on a benzene extract after prolonged alkaline hydrolysis. The color is read at 425 mμ. The hydrolysis is necessary for good recovery. Recovery experiments in the presence of liver averaged a 94 per cent value with a 2 sigma deviation of 7 per cent.

With this method it was shown that 300 mg. of rat liver slices metabolize an average of 45 micrograms of alpha-estradiol in 3 hours at 37.8° C. and pH 7.4. Measurements after 1, 2 and 3 hours showed a linear progression of the reaction. The reaction proceeded optimally between pH 6 and 8. Boiled liver tissue was found to be completely inactive.

The method proved to be equally applicable to the study of human liver. Fresh human liver slices obtained at laparotomy and immediately incubated metabolized alpha-estradiol at the same rate as rat liver. This is the first demonstration of the extent with which human liver is able to metabolize alpha-estradiol *in vitro*. Impairment of this activity in disease is being investigated.

The Effect of Cortisone on Nontropical Sprue (Idiopathic Steatorrhea). ASHTON B. TAYLOR, MANDRED W. COMFORT, ERIC E. WOLLAEGER and MARSCHELLE H. POWER, Rochester, Minn. (Introduced by Hugh R. Butt).

This study was stimulated by previous suggestions relative to the possible role of the adrenal cortex in the pathogenesis of nontropical sprue and the recent availability of cortisone. Studies of metabolic balance on 2 patients and clinical studies on 3 other patients have been conducted. Observations were made concerning (1) fecal fat and nitrogen, (2) concentration of electrolytes in serum, urine and fecal material, (3) blood prothrombin and (4) serum proteins. Cortisone was administered in periods either by the intramuscular or oral routes in dosages varying from 25 to 100 mg. daily. These periods were preceded by and alternated with control periods during which either no medication or a similar appearing placebo was administered.

After the administration of cortisone a number of significant changes were observed. These included a subjective sense of improvement and increased appetite, a marked increase of prothrombin in the blood (return of prothrombin time to normal) and elevation of the concentration of albumin in the serum and of the total protein. There was a significant decrease in fecal fat and nitrogen excretion, though in no case was there a return to absolute normal levels. In addition to these effects the balance of electrolytes in the blood, urine and fecal matter showed alterations similar to those previously observed after the administration of cortisone to patients having other conditions. During control periods and periods when placebos were administered a return toward pretreatment levels was noted.

Development of edema and elevation of blood pressure were encountered coincident with the administration of doses of 100 mg. With this exception the clinical courses of these patients were beneficially affected by the administration of cortisone during these relatively short term studies.

The Effects of Cortisone on Experimental Bacterial Infection and on the Tissue Damage Produced by Bacterial Toxins. LEWIS THOMAS,* WILLIAM J. MOGABGAB and ROBERT A. GOOD, Minneapolis, Minn. and New Orleans, La.

It was previously shown that Cortisone treatment of rabbits caused marked enhancement of infection by hemolytic streptococci, and also increased the local tissue damage resulting from an intradermal injection of meningococcal endotoxin. The present study represents an extension of these observations.

Streptococcal infection of Cortisone treated rabbits results in septicemia and death, with doses of streptococci which produce no demonstrable systemic infection in untreated rabbits. Massive deposits of streptococci are present in the interstitial tissue of the heart in animals which die while receiving Cortisone; when death occurs after cessation of Cortisone treatment microorganisms are not seen, but numerous foci of inflammatory cell infiltration appear throughout the myocardium.

When the rabbits are immunized with streptococci prior to Cortisone treatment and infection, bacteremia occurs briefly or not at all and the animals survive, indicating that enhancement of infection by Cortisone is probably not caused by interference with the immune mechanism. However, these immunized animals develop extensive inflammatory reactions in the heart following infection and Cortisone treatment; photomicrographs which illustrate the lesions will be presented.

The intradermal or intraconjunctival injection of endo-

toxin from gram-negative microorganisms causes a reversible local inflammatory reaction in normal rabbits. In Cortisone treated rabbits inflammation is inhibited, and extensive hemorrhage and necrosis occurs in the injected area. The *intravenous* injection of toxin in Cortisone treated rabbits results in bilateral cortical necrosis of the kidneys, within 24–72 hours. An identical renal lesion occurs in Cortisone treated hamsters following the intraperitoneal injection of toxin. With Cortisone alone, or toxin alone, no hemorrhage or necrosis of the kidneys occurs.

Observations which may bear upon the mechanism involved in these properties of Cortisone will be described.

Chronic Renal Acidosis and Severe Osteomalacia with Symmetrical Pseudofractures: The Renal Impairment as Related to Changes in Mineral Metabolism. BEVERLY T. TOWERY, Nashville, Tenn. (Introduced by R. H. Williams).

Albright and associates have reported that impairment of renal conservation of base may result in hypercalciuria and osteomalacia. An elderly woman ill with florid osteomalacia and chronic acidosis provided an opportunity to investigate further the relationship between the renal insufficiency and bone disease.

Preliminary studies revealed serum pH and electrolyte changes characteristic of moderately severe acidosis with minimal nitrogen retention, whereas the serum inorganic phosphorus concentration was low. This finding, together with low serum calcium and high alkaline phosphatase concentrations, corroborated radiographic evidence of osteomalacia with multiple pseudofractures (Milkman).

The urine specific gravity and pH were fixed within narrow limits; the excretion of titratable acid and ammonia remained low; urinary bicarbonate concentration remained relatively high despite low plasma bicarbonate levels. The available evidence supported the diagnosis of pyelonephritis without nephrocalcinosis or nephrolithiasis. Achlorohydria was demonstrated but not steatorrhea.

The renal calcium excretion was exceedingly low and moderately positive balances of calcium and phosphorus were found, presumably on the basis of the extreme mineral deficit. Observations were repeated after an interval of five months, virtually complete healing of the osteomalacia having been accomplished with sodium citrate and vitamin D therapy. Following the interruption of alkali, sodium and potassium balances became negative as acidosis recurred but the anticipated hypercalciuria and negative calcium balance were not observed. Clearance studies revealed a marked depression of the glomerular filtration rate, ($C_{thiosulfate}$). The low rate of excretion of titratable acid was not increased significantly when the phosphorus load was augmented progressively by the intravenous infusion of phosphate buffer.

It is suggested that the absence of hypercalciuria at acidotic levels after healing of the osteomalacia, was contingent, in part, upon the severity of the renal insufficiency. The pathogenesis of the bone disease will be discussed in relation to the observed abnormalities of renal function.

Amyotonia Congenita: A Symptom Complex. FRANK H. TYLER, W. J. POLGLASE and W. KRIVIT, Salt Lake City, Utah. (Introduced by M. M. Wintrobe).

Oppenheim in 1900 described a clinical syndrome present at birth or appearing shortly thereafter in which reduction in muscular tone was the cardinal finding. The close relation if not identity of the majority of these cases with infantile muscular atrophy was pointed out subsequently by several authors. We have observed that an identical clinical syndrome may result from excess glycogen storage in muscle. This disorder is differentiated from von Gierke's cases by the early onset and lack of such signs of hepatic dysfunction as hypoglycemia and ketosis. On the basis of preliminary data it would appear that both the neural atrophy and the glycogen storage syndrome are inherited in the same fashion but that each results from a different mutation. A strikingly different microscopic appearance of biopsy material serves as the only definitive means of recognizing the amyotonia associated with glycogen storage in muscle.

Study of glycogen from one of our cases has shown that it differs in its physical and chemical properties from normal human glycogen. These findings are probably related to the demonstration that two enzyme systems are responsible for the hydrolysis of glycogen. Our evidence suggests that mechanical damage to the muscle occurs as the result of the accumulation of excessive amounts of glycogen. The need for biopsy study to identify the different forms of amyotonia congenita is emphasized.

Biochemical Studies on Leukocytes. WILLIAM N. VALENTINE * and WILLIAM S. BECK, Los Angeles, Calif.

The emerging metabolic patterns of leukocytes in health and disease make imperative the investigation of the many parameters characterizing each set of circumstances. Quantitative data are presented on alkaline and acid phosphatase activity in isolated leukocytes and on leukocyte histamine content in health, physiologic leukocytoses and leukemia. In normal subjects, alkaline phosphatase activity (expressed as mg. of P liberated per hour by 10^{10} leukocytes) averaged 25.8 (range 13.4–58.0). In neutrophilic leukocytosis, the mean was 119.1 (range 35.4–276.5). In chronic myelocytic leukemia, excepting one patient, the mean was 4.0 (range 0–14.4). Alkaline phosphatase was consistently low to normal in chronic lymphatic leukemia and low in blastic leukemias. Acid phosphatase varied less, being in the normal range in leukocytosis and slightly elevated in chronic myelocytic leukemia. Acid phosphatase was low in predominantly blastic leukemias and somewhat low in chronic lymphatic leukemias. Histamine was markedly reduced per 10^{10} cells in neutrophilic leukocytoses and leukemoid reactions, and averaged 2–3 times normal values in chronic myelocytic leukemia. Histamine of cells per ml. of blood was tremendously elevated in chronic myelocytic leukemia, and normal to low in leukocytosis. Low values were found in chronic lymphatic leukemia and very low values in predominantly blastic leukemias.

Other biochemical parameters are being explored. In

terms of histamine and phosphatase content, the various groups studied show little overlap, and the metabolic patterns appear characteristic of the disease rather than the cell count or cell immaturity. This was true after therapy and in instances where the peripheral blood was temporarily indistinguishable from normal. These and similar metabolic patterns offer diagnostic aid in the occasional case but, of more importance, suggest a basis for future investigations into the fundamental nature of the derangements and their correction.

The Intravenous Administration of Emulsions of Vitamin K_1. THEODORE B. VAN ITALLIE, ROBERT P. GEYER and FREDRICK J. STARE,* Boston, Mass.

The superiority of vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) over other vitamin K preparations in the treatment of dicumarol-induced hypoprothrombinemia is well established. However, its intravenous administration by a convenient and rapid procedure has only recently become clinically feasible. A 5 per cent emulsion of vitamin K_1 suitable for intravenous use in man has been prepared by means of the same general techniques developed in this laboratory for the manufacture of caloric fat emulsions. It is sterile and non-pyrogenic and has a particle diameter below 1 micron.

The preparation has been given intravenously without difficulty to thirty-five patients, in amounts providing up to 1000 mg. of vitamin K_1 in a single injection. Ten patients with hypoprothrombinemia associated with hepatic disease and twenty-five patients in various stages of dicumarolization received amounts of from 5 to 10 mg. per Kg. body weight. Prothrombin times were determined at frequent intervals after injection of the emulsion.

No improvement in prothrombin time occurred in the group with liver disease. However, the effect of the intravenously administered vitamin K_1 on the dicumarolized patients was invariably dramatic. Effective increases in prothrombin activity occurred in 1 hour and increases of as much as 50 per cent were observed in 5 hours.

Recovery curves representing as a function of time prothrombin activity of hypoprothrombinemic subjects given intravenous vitamin K_1 are useful in the study of prothrombin production. When the supply to the liver cells of vitamin K_1 is no longer the limiting factor, it becomes easier to appraise other variables involved in prothrombin manufacture. Accordingly, it is believed that intravenous emulsions of vitamin K_1 will be of special value both in the treatment of dicumarol-induced hypoprothrombinemia and in the study of coagulation mechanisms.

Studies of the Volume and Composition of Sweat During Diuresis in Patients with Nephrosis. AAGE WARMING-LARSEN and WILLIAM M. WALLACE,* Boston, Mass.

The volume and composition of active sweat produced in response to a thermal stimulus has been studied in twenty-three normal children and twelve children with the nephrotic syndrome before ACTH therapy, during therapy and after cessation of therapy. All children were on approximately the same sodium, potassium and chloride intake and all were acclimatized to mild summer heat.

Normal children produced from 100 to 200 grams/m^2./hour of sweat in response to a thermal stimulus. The sodium concentration ranged from 15–55 mEq./l.; the potassium concentration from 6–12 mEq./l. and the chloride concentration from 10–50 mEq./l. During the phase of oliguria, edema and sodium retention the nephrotic children produced, in response to an equal thermal stimulus, approximately one-fifth to one-tenth the volume of sweat produced by the normals. The sodium concentration of this sweat varied from 90–120 mEq./l. while the potassium concentration was 4–5 mEq./l. When diuresis occurred, the sweat response to the thermal stimulus became identical with the normal, both with regard to the volume and electrolyte concentrations. The return to normal pattern began and was complete before subcutaneous edema had disappeared. The increment in volume of sweat was great enough to counteract the fall in concentration so that the sodium and chloride lost per unit of time from the sweating skin was greater after diuresis than before. The sweating performance in one child with a spontaneous diuresis was identical with that of children diuresing after ACTH therapy. The data suggest that extrarenal factors may play a role in the sodium retention of the nephrotic syndrome.

Patterns of Pulmonary Hemodynamic Responses in Man. JAMES V. WARREN,* JOSEPH T. DOYLE, E. HARVEY ESTES and JOSEPH S. WILSON, Atlanta, Ga.

Observations on intracardiac and pulmonary pressures, general and "pulmonary" blood volumes in a group of approximately 100 patients suggest that several patterns of altered pulmonary hemodynamics can be delineated.

Under most circumstances, the pulmonary circulation appears to function as a passive vascular bed. Experimental acute expansion or reduction of blood volume fails to alter the ratio of blood in the pulmonary and systemic circuits but produces a considerable increase or decrease in pulmonary vascular pressures. Indeed, a linear relationship is demonstrable between pulmonary vascular pressures and the blood volume. However, the pressure gradient between pulmonary artery and capillary remains remarkably constant. Changes in flow are not responsible for the observed pressure alterations. In the normal resting subject, therefore, volume changes in the pulmonary circuit appear to alter the pressure levels but do not appreciably change the pressure gradients.

In some patients with congestive heart failure and hypervolemia, the pulmonary pressures are proportionately increased, pressure gradients are unaltered and the pressure-volume relationship is maintained just as in normal subjects receiving a saline infusion acutely. On the other hand, in certain patients with pulmonary disease, mitral stenosis, thyrotoxicosis and severe anemia associated with heart failure, there is pulmonary hypertension and a high arterio-capillary pressure gradient with deviation from the normal pressure-volume relationship.

These results suggest that factors other than pulmonary congestion are operative in producing the pulmonary hypertension; the elevated pressure apparently being the summation of variable degrees of functional and organic pulmonary vascular resistance, related to such factors as hypoxia and structural vascular disease.

The observations indicate that from an analysis of the pulmonary pressure-volume relationship and the arterio-capillary pressure gradient certain deductions can be made regarding the relative distribution of blood in the pulmonary and systemic circuits, and the mechanisms producing the pulmonary hypertension.

Studies on the Mechanism of Edema Formation in Patients with Low Serum Electrolytes. CHRISTINE WATERHOUSE, E. HENRY KEUTMANN and LEONARD D. FENNINGER, Rochester, N. Y. (Introduced by W. S. McCann).

Low serum electrolyte concentration without dehydration or decreased extracellular fluid volume has been reported in various chronic diseases. This study concerns the development of edema in some such subjects.

The nitrogen, calcium, phosphorus, potassium, sodium and chloride balances were studied in 4 patients for periods of 40 to 110 days. The volume of body fluid compartments was determined and muscle biopsies performed. Two subjects with pituitary myxedema and one with rheumatic heart disease were edematous and had low serum electrolytes when the experimental period began, while a patient with advanced carcinoma developed this clinical picture during study.

The following observations were made: 1) Despite sodium levels of approximately 125 meq. and chloride 80–90 meq. per liter, no evidence of dehydration was found. In fact, frank edema was often present. 2) Skeletal muscle showed consistently less potassium and more sodium than usual. 3) The administration of salt by mouth or by intravenous injection of hypertonic saline caused a slight transient rise in serum electrolyte concentration. The extracellular fluid volume increased markedly as did intracellular sodium. Testosterone propionate produced a similar effect. A low salt intake or mercurial diuresis caused reduction in extracellular fluid and lowered serum sodium and chloride. 4) Treatment with desiccated thyroid increased the serum electrolyte level in a patient with pituitary insufficiency. The effect was poorly sustained. 5) ACTH produced no effect on the serum electrolytes of the patient with heart disease. 6) It is postulated that a cellular defect, perhaps of the protein, leads to a low cellular osmotic pressure in some patients with chronic debilitating illness. The lowered osmotic pressure in the extracellular fluid is apparently a result of this phenomenon, and can only be transiently changed by artificial manipulations of the extracellular fluid. Hormonal therapy (thyroid, DCA, testosterone, ACTH) has thus far, in our hands, proven ineffective in correcting the defect, as long as the primary disease process remains uncontrolled.

The Relationship of Pyridoxine Deficiency to Adrenal Function in the Production of Leucocytes. DAVID R. WEIR and JOHN F. MUELLER, Cleveland, Ohio (Introduced by Joseph T. Wearn).

Previous work has shown that pyridoxine deficiency induced in mice by feeding the analogue, desoxypyridoxine hydrochloride, causes granulocytosis, lymphopenia and myeloid metaplasia in the spleen. Entirely similar changes occur when mice are treated with cortisone.

Experiments designed to determine the relationship between pyridoxine deficiency and adrenal function gave the following results: 1) In adrenalectomized mice pyridoxine deficiency causes the same hematologic changes as in normal animals indicating that the effect of pyridoxine deficiency is not mediated through the adrenal cortex. 2) In pyridoxine-deficient mice, treatment with cortisone produces a summation of hematologic effect. The granulocytosis and lymphopenia are more marked than with pyridoxine deficiency alone or cortisone treatment above. 3) The hematologic effect of cortisone treatment cannot be prevented by the administration of large amounts of pyridoxine, pyridoxal, and pyridoxamine.

It is concluded that a relationship between the hematologic effect of pyridoxine deficiency and that of cortisone treatment has not been demonstrated.

On the Chemical Structure of Compounds Capable of Preventing Leukopenia Induced by Nitrogen Mustard. AUSTIN S. WEISBERGER, ROBERT W. HEINLE* and BENNETT LEVINE, Cleveland, Ohio.

Cysteine protects animals against lethal amounts of irradiation. Administration of cysteine prior to the injection of nitrogen mustard (HN_2) also prevents the severe leukopenia induced by HN_2. The mechanism of this protection is not known, but it may be postulated that the effect is due either to chemical inactivation of HN_2, to a sparing action on sulfhydryl-containing enzymes, or to some other specifie protective effect.

A study of the relation of structure to action was made by comparing the leukopenia-preventing effect of various amino acids and sulfhydryl-containing compounds. All compounds investigated prevented leukopenia when incubated with HN_2 prior to injection into rabbits. However, when administered *in vivo* prior to the injection of HN_2, only cysteine, homocysteine and glutathione possessed activity; the two latter were approximately one-half as active as cysteine. Other compounds with a high reducing potential, such as thioglycolic acid, BAL and ascorbic acid have exerted no protective effect, nor have such amino acids as methionine, serine or alanine. It would appear that a very specific structure may be required for high activity, and studies of the possible functional roles of the carboxyl, amino and sulfhydryl groups and the spatial configuration of the molecule, are now in progress. Preliminary clinical tests reveal that the therapeutic effect of HN_2 is not abolished when leukopenia is prevented by prior administration of cysteine.

The Effects of the Pituitary and Adrenal Hormones on the Metabolism and Excretion of Sodium and Water. L. G. WELT, D. W. SELDIN and J. H. CORT, New Haven, Conn. (Introduced by John P. Peters).

The administration of DOCA, lipo-adrenal extract, and cortisone to patients with cirrhosis, rheumatic fever and heart failure, and rheumatoid arthritis, on a salt-restricted regime, provided strikingly negative results. In none of these studies was there any alteration in the extrenal exchange of water, its internal distribution, nor in the concentrations of electrolytes in the serum.

Rats fed a sodium free diet and treated with DOCA did not differ from their controls with respect to the external or internal exchange of water, nor in the concentration of sodium in the serum. Administration of cortisone promoted no alterations in the concentration of sodium in the serum, nor in the volume or distribution of muscle water. They excreted more sodium and nitrogen than their controls.

The administration of a preparation of ACTH with low pressor activity promoted the retention of water in the patients with cirrhosis and rheumatic fever and heart failure but not in the arthritic. This same preparation also promoted water retention in a patient with Addison's disease. A different preparation of ACTH did not lead to water retention in the cirrhotic. Withdrawal of ACTH was followed by a period characterized by a negative balance of sodium and weight loss. This reaction was not dependent on the presence of edema, nor the retention of water during ACTH administration.

DOCA, cortisone, and lipo-extract appear to have no primary effect on the external and internal exchanges of water. Retention of water during administration of ACTH is not mediated via the adrenal cortex, but is due to a contaminant, presumably posterior pituitary. The response to the latter is variable and appears to be greater in those with a disordered Starling equilibrium. ACTH withdrawal is followed by a period of relative adrenal cortical insufficiency with salt-wasting, diuresis, and weight loss.

Comparison of the Antityphoid Activities of Aureomycin and Chloramphenicol on Bacteria Artificially localized in Vivo. CHARLES A. WERNER, VERNON KNIGHT and WALSH MCDERMOTT,* New York, N. Y.

A technique has been developed whereby bacterial populations can be artificially localized *in vivo,* where they subsist entirely on the host, are subject to chemotherapy, yet remain accessible for periodic observations. Triple-layered unenriched agar wafers are inserted into the peritoneal cavity of a suitable host. The middle layer of agar is seeded with a measured number of microorganisms. Substances in the extracellular fluid easily penetrate the wafers; although multiplication occurs, the bacteria do not become invasive. Wafers can be removed at intervals for determinations of the concentrations of bacteria and drugs.

This method has been used to investigate the discrepancy between the antityphoid activities of aureomycin and chloramphenicol.

Despite chemical dissimilarities, these two compounds in equal doses have comparable activities over a wide range of infections including the predominantly intracellular infections typhus and brucellosis. Although both compounds inhibit *S. typhosa in vitro,* only chloramphenicol has exerted impressive antityphoid activity *in vivo.*

Explanations of this discrepancy have included the following: 1) Unlike brucellosis or typhus, insufficient aureomycin might reach the parasites in typhoid fever; 2) *S. typhosa in vivo* might be insusceptible to aureomycin unlike *S. typhosa in vitro;* 3) the lesions of typhoid fever, aside from intracellular location of the parasite, might selectively protect the bacilli from aureomycin.

S. typhosa in vivo was susceptible to both drugs penetrating from the extracellular fluid but neither drug was eradicative. Both drugs were comparably active at high serum concentrations and comparably inactive at low concentrations. Concentrations necessary for substantial antityphoid activity were essentially the same for either drug but were considerably higher than those usually attained with aureomycin in humans.

It appeared that the drug-parasite relationship of both was essentially the same *in vivo* as *in vitro,* but that the drug-host relationship of chloramphenicol assumes an importance in typhoid fever not revealed in other infections.

The Use of Dextran in the Treatment of Shock. JOSEPH S. WILSON, E. HARVEY ESTES, JOSEPH T. DOYLE and WALTER L. BLOOM, Atlanta, Ga. (Introduced by F. William Sunderman).

Dextran is a glucose polymer, prepared by bacterial fermentation of a sucrose solution with subsequent hydrolysis to obtain a molecular size comparable to human albumin. An American product with a relatively narrow range of molecular size has been studied as an agent to expand the plasma volume. Injection of 500 cc. or more of a 6 per cent solution into human subjects produces an increase in plasma volume which is maintained over a period of 6 to 12 hours. No evidence of renal or hepatic dysfunction has been detected. Many patients have received repeated injections without untoward reactions.

Five normal human subjects were subjected to blood loss of 500–900 cc. and then given Dextran solution. Following therapy, there was no significant deviation of hemodynamics from the control state except for the diminished number of red blood cells. Dextran has now been used in the treatment of 50 patients with various types of shock. A majority of these were individuals with shock due to blood loss, the remainder had various disorders such as extensive trauma, burns, and infectious diseases. The Dextran produced hemodynamic improvement in all instances of hemorrhagic shock when blood loss could be controlled. Following therapy, there was evidence of hemodilution, increased blood pressure and improved

peripheral circulation. The clinical results in all patients were indistinguishable from those that might be expected from the use of plasma. There has been no evidence of untoward immediate or late reactions nor evidence of the storage of Dextran. Rouleaux formation is not so enhanced as to interfere with blood typing.

Dextran appears to be a useful substitute for plasma in the treatment of shock. The relative ease of production and stability of the solution suggest that it may be a satisfactory material for stockpiling for use in the event of large-scale disaster.

Kinetics of Hemodialysis. A. V. WOLF, DONALD G. REMP, JOHN E. KILEY and GORDON D. CURRIE, Albany, N. Y. (Introduced by Richard T. Beebe).

Studies using a Brigham-Kolff artificial kidney show that the circulating fluid volume in the cellophane casing is proportional to the rate of flow, ranging approximately from 240 cc. volume at zero flow to 1060 cc. at a flow of 500 cc./min. The rate of exchange of individual compounds across the dialyzing membrane may be expressed by the equation $(A-U)_t = (A-U)_0 e^{-(B+b)Dt/Bb}$, where A and U are concentrations of particular solutes in plasma and bath water, respectively, t is time in minutes, B is volume of bath fluid, b is volume of distribution of solute excluding volume of bath fluid, and D is a value defined as the net rate of exchange of material per minute per unit concentration gradient between plasma and bath fluid. D is called the "dialysance" because of a mathematical parallel to the "clearance." Its value is characteristic for different molecular and ionic species, but for any one species it increases with blood flow and cellophane surface area. Using the urea dialysance as a reference, relative dialysances have been determined under various conditions for creatinine, uric acid, glucose, sucrose, phenol red, alanine, glutamic acid, tryptophane, and several ions. In two experiments where prepared solutions were used as "blood," the relative dialysances at a flow of 300 cc./min. were as follows: chloride, 1.21; urea, 1.00; potassium, 0.91; sodium, 0.87; bicarbonate, 0.74; creatinine, 0.65; uric acid, 0.46; phosphate, 0.35; glucose, 0.35; and phenol red, 0.17.

Rates of water exchange have been determined for various gradients of glucose and sucrose.

Antibiotic Therapy of Leptospirosis with Discussion of Effects upon Leptospiremia. T. E. WOODWARD,* H. E. HALL, J. A. HIGHTOWER and E. PONS, Baltimore, Md.

Leptospirosis contracted by seventy-eight patients in Puerto Rico was investigated through collaboration of the Tropical Disease Research Laboratory, the United States Army, the University of Maryland and the Puerto Rican medical authorities. Approximately two weeks treatment with various antibiotics was administered to the following groups: chloramphenicol 19, aureomycin 15, streptomycin 12, penicillin 8, terramycin 11 and aureomycin-streptomycin combination 13. Fifty patients in this series were seriously ill and thirty-five (44%) had clinical jaundice. Mean values in days for the time treatment was started was 6.7 and duration of fever after beginning therapy 2.5. Fourteen clinical relapses (18%) occurred in some of each group. It is significant that seven patients developed recrudescent symptoms while receiving antibiotic treatment but none of these were in the chloramphenicol and aureomycin-streptomycin groups. One fatal case occurred in the chloramphenicol series.

Prior to initiating treatment leptospiremia was demonstrated in forty-four (56.4%) of the patients; the diagnosis was proved in the remainder by serological test. Complete data regarding the duration of leptospiremia after beginning treatment will be available in time for the final report but the following results are now available: penicillin two cases positive, six and sixteen days after treatment started; terramycin two cases, two days; aureomycin two cases, one day and two days respectively; chloramphenicol three cases, first, second and eighth days respectively; streptomycin and aureomycin-streptomycin combination no data.

The data now available warrant the following conclusions: 1) early treatment greatly enhances the rapidity of recovery; 2) the ideal antibiotic chemotherapeutic agent for leptospirosis is not yet available, based upon the failure of the antibiotics tested to eradicate the leptospiremia and prevent relapses; and 3) leptospirosis is a disease of protean nature making clinical evaluation of a therapeutic regimen quite difficult.

Physiology of Hemorrhagic Shock in Man. Bromsulphalein Retention, Circulating Eosinophiles, and Skin Reactive Hyperemia. NORMAN ZAMCHECK, Boston, Mass. (Introduced by Clark W. Heath).

Physiology of shock in patients with gastrointestinal hemorrhage was studied by means of bromsulphalein retention (5 milligram per kilogram dose) determined 45 minutes after dye injection, circulating eosinophiles (Thorn) and reactive hyperemia (Di Palma).

Nine patients without liver disease showed bromsulphalein retentions during shock of less than 10 per cent including one in severe shock with 1 per cent retention. Within 24 hours on recovery from shock all values were below 5 per cent. Seven patients with liver disease other than cirrhosis had retentions during shock of 3–33 per cent with fall in 24 hours to less than 15 per cent, and continued fall with recovery. Four non-jaundiced patients with cirrhosis showed retentions of 20–46 per cent during shock with little or no fall in the first 24 hours, followed by slow improvement on recovery.

Eleven of 12 patients showed fewer than 70 circulating eosinophiles per cubic millimeter immediately following admission for hemorrhage. Seven had no eosinophiles on one or more counts. The eosinophile level rose promptly as bleeding ceased. Despite severe shock one patient failed to show a low eosinophile count (180 per cu. mm.). He died on the second hospital day, 12 hours following gastrectomy. Adrenal-cortical insufficiency could not be excluded.

All persons in deep shock showed marked prolongation

of the time required to produce a definite hyperemic reaction on application of standard pressure (threshold time), which was always greater than 120 seconds and usually indefinitely prolonged, as well as equal delay in the time of disappearance of hyperemia (clearing time). In early shock and in severe dehydration less prolonged threshold and clearing times were obtained.

These results suggest that hemorrhagic shock 1) may cause only slight reductions in blood flow through the normal liver, 2) initiates prompt adrenal-cortical reaction as evidenced by fall in eosinophiles.

Transfer Rates Across Muscle Cell Membranes in Man: Measurement of Blood Flow and Fluid Compartments in Skeletal Muscle. KENNETH L. ZIERLER, REUBIN ANDRES and JOSEPH L. LILIENTHAL, JR.,* Baltimore, Md.

The Stewart principle has been adapted to measurement of peripheral blood flow in the forearm of man. A suitable substance is infused into a brachial artery at *constant* rate, I mg. per min., by a motor-driven syringe delivering about 0.1 ml. per min. Blood is collected continuously from a catheter placed in a deep ipsilateral vein in the forearm. If P mg. per ml. is the concentration of infusate in venous plasma at equilibrium (corrected for recirculation), then plasma flow equals I/P. Theory does not require that infusate be confined to intravascular space, and simultaneous infusion of T-1824 and inulin have yielded identical rates of plasma flow. The method is sensitive to rapid changes in blood flow and permits continuous flow measurements. Measurements in normal man yielded flow rates of an order similar to but larger than values reported from plethysmography.

With this method it has been possible to measure rates of transfer of substances into or out of forearm muscles. Net transfer of a metabolite is a function of the product of its arterio-venous concentration difference and the rate of arterial flow to the part. Although these two variables define alterations in transfer rates induced during the course of an experiment, a reference base is essential for comparison of one individual with another.

Theoretical considerations indicate the feasibility of using the volume of muscle water in the forearm as a reference base. Muscle water might be measured as the difference between inulin and antipyrine spaces, determinable from the exponential rate of disappearance of infusates from venous effluent after halting the injection. Similar treatment of rate of disappearance of T-1824 permits estimation of plasma volume in the part; in normal individuals it constitutes some 3.5 per cent of the forearm with the wrist and hand occluded.

Body Water, Body Chloride and Plasma Volume Changes in Patients Receiving ACTH Therapy. MORRIS ZIFF and JEROME SIMSON, New York, N. Y. (Introduced by Currier McEwen).

In the course of adrenal cortical hormone therapy, a syndrome of hypochloremia with alkalosis has been frequently observed. It was of interest, therefore, to study changes in body chloride content during ACTH therapy. Simultaneous measurements of total exchangeable body chloride, total body water and plasma volume were made on six patients with rheumatic diseases receiving ACTH. Body water was measured by the antipyrine method and exchangeable body chloride by the distribution of bromide. Studies were carried out on each individual before, during and after treatment.

All patients retained water promptly. Two subjects, who developed edema, retained water and chloride in a parallel manner, assuming that chloride was retained in the concentration present in the extracellular fluid. Two patients, in whom the balance of chloride to water gained, calculated on this assumption, was initially positive, developed negative balances after several weeks of therapy. One patient, studied three weeks after starting therapy, demonstrated a marked negative chloride to water balance. One patient showed a persistently negative chloride to water balance throughout therapy.

Evidence of circulatory decompensation occurred in one individual, with marked increase in plasma volume. Plasma volume increased moderately in two others without overt signs or symptoms. In three, the plasma volume fell during therapy although there was a tendency for serum K^+ and Cl^- to fall, and HCO_s^- to rise, no definite metabolic alkalosis was observed.

Changes in plasma volume appeared to be dependent on repair of anemia during ACTH therapy and expansion of extracellular fluid volume. Factors influencing the relationship between changes in body water and body chloride during ACTH administration are discussed.