

**PROCEEDINGS OF THE FORTY-FIFTH ANNUAL MEETING OF  
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION HELD  
IN ATLANTIC CITY, N. J., MAY 4, 1953**

*J Clin Invest.* 1953;**32**(6):548-615. <https://doi.org/10.1172/JCI102771>.

Research Article

**Find the latest version:**

<https://jci.me/102771/pdf>



PROCEEDINGS OF THE FORTY-FIFTH ANNUAL MEETING OF  
THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION  
HELD IN ATLANTIC CITY, N. J., MAY 4, 1953

PRESIDENTIAL ADDRESS

By EUGENE A. STEAD, JR.

I have the honor to address the membership of this Society and its guests in my role as President. I am not wise enough to bring to you the philosophies of the ages nor am I old enough to assume the role of an elder statesman. I can bring to you the philosophy which has guided me in my own development. This philosophy has been developed over the years by contact with many men, but in a great measure it has resulted from the various Chiefs under whom I have worked. I would like to share with you today some of the things which they have taught me.

My first Professor of Medicine was Dr. James Edgar Paullin of Atlanta. He was also our family doctor. He first showed me in his work that the family doctor of the future was going to be the intelligent, interested internist. He made home calls rarely, but he knew every member of my family, and no medical attention was ever sought without first reviewing the problem with him. Dr. Paullin showed me that the community was interested in the growth of its doctors. Every Tuesday and Wednesday morning he was at Grady Hospital. His patients respected his desire for continued growth as a doctor and never begrudged him this time. He was interested in medical politics and repeatedly demonstrated that all human group endeavor involves some type of political activity. He was a medical politician in the best sense of the word and all of American medicine profited by his broad interests.

My second chief was Henry Asbury Christian. Under his guidance the Peter Bent Brigham medical resident staff developed many leaders in Medicine. The atmosphere of the Brigham was one that gave honor to scholarship. There I first learned that, just as many people liked to fish or play golf, I liked to work with my head. Dr. Christian was successful in the development of leaders because he fostered in his residents a willingness to take the initiative. His attitude was not that the Brigham service was good because of the senior staff, but that it was good in spite of the senior staff. He relied heavily on the fact that he had pulled into his net many bright boys from all sections of the country, and he expected them to produce. I have never seen men more conscious of their ability to learn for themselves than those resident groups assembled by Uncle Henry.

My third chief was Elliot Cutler, under whom I served a 16-months surgical internship. He was loved and honored by all of his staff—even the lowly intern. On his service I discovered how hard surgeons work, and I learned that those long hours in the operating room use up the time which the internist loves to spend talking with his patients and teaching.

My fourth chief was Marian Blankenhorn of Cincinnati. He gave to me my basic interest in clinical observation. Under his guidance, the history and physical examination came alive. His teaching centered around the patient and he destroyed once and for all my interest in dry clinics. He gives his resident a very free hand in running the service, and from him I learned not only to do my own work but how to get other people to work.

My fifth and last chief was Soma Weiss. By that time, the clay was better worked and more ready for the molding, and Soma taught me many things. He demonstrated the importance of the undergraduate student in our own learning. Repeated efforts to explain to the student the basic mechanisms of health and disease kept before us the extent of our own ignorance and made us examine critically the premises on which we based our glibly quoted clinical aphorisms. We learned the importance of appreciating what is not known about a condition as well as what is known. I have never ceased to drink from this well of undergraduate naïveté and skepticism.

This same use of teaching as a learning device was employed with the resident staff. Every intern and resident taught students, not because there was no other way to teach the student but because of the learning value to the resident group.

From Soma I learned the importance of keeping down artificial barriers which interfere with learning. Whoever knew the most about the problem—be he second-year student, instructor or visitor—was cock-of-the-walk for the moment. Soma achieved remarkable give-and-take with everyone contributing to the learning pot and everyone taking knowledge back out. He realized that the goal of the medical student was the opportunity for the correlation and consolidation of his knowledge which can be achieved only in the fourth year. He was never willing to sacrifice that period of high learning of the fourth-year student, stimulated to its fullest by an alert resident staff, for ease of operation of the outpatient clinic. The student was a doctor taking care of his patient under supervision. Therefore, as a doctor, therapeutic and diagnostic decisions could be made only with the student present. This made joint ward rounds with students, interns and residents imperative and greatly increased the cohesiveness of the group.

Soma never forgot that the function of a university service was to train men and the output of research was always secondary to this main objective. He carried on research with the resident staff for its effect on their thinking. Research with the older staff was a means of keeping their minds alert and their teaching interesting.

In the laboratory, Soma taught us the value of always

carrying out some observations each day on a patient. Even if the experimental procedure proved useless, we always learned something from the patient. He was never uneasy about development of the specialized knowledge of the physicist, the biochemist, or the physiologist. He always believed that men with sound clinical training who spent time in learning the ways of sick patients could hold their own in the research field. He emphasized the time and hard work that went into the understanding of patients and the mechanisms of disease, and never begrudged the years necessary for the development of clinical skills.

He taught us the value of not giving up too easily and yet he kept us from beating our heads against a stone wall on a problem for which methods then known were inadequate. He brought new methods into the laboratory and was always searching for collateral evidence to buttress any thesis he entertained. This willingness to approach any problem from many angles accounted for the soundness of his final conclusions and for his amazing ability to write many papers with so few errors in basic concepts.

Soma maintained a great interest in the symptoms which patients present. He realized that the learning of medicine from the practice of medicine was dependent entirely upon an accurate evaluation of the cause of the patient's complaint. A patient with heart disease who complains of shortness of breath may have congested lungs which are causing the dyspnea, or he may be short of breath from anxiety, or he may have independent lung disease. If the doctor mistakes emotional dyspnea for congestive failure, he learns nothing from treating the patient.

In his study of symptomatology, Soma appreciated the complexity of medicine in modern society. He was aware of the frequency with which multiple factors operate to produce disease and symptoms. He became interested in the mechanisms by which emotional reactions

cause the patient to feel abnormal. The beautiful series of experiments carried out in his laboratory on carotid sinus fainting remain of primary importance because they demonstrate reflex ways of feeling unreal, light-headed, and even of becoming unconscious. These observations were not important in curing patients with fainting by denervating the carotid sinus: they were important in demonstrating reflex mechanisms for feeling abnormal.

Soma not only knew what he wanted to do, but he knew how to get it done. He didn't rail at the political moves necessary to keep the city fathers happy. He knew that all the ruling powers were people and he enjoyed handling people. It made little difference whether they were sick folk or people with power who needed education. He showed that administration was a necessary function and could be fun in its own way.

Soma Weiss had a unique degree of interest and enthusiasm for what we did which went far beyond our professional activity. He had an understanding of our personal lives and of our hopes and ambitions. He was interested in our clinical problems and in any observation we made in the laboratory. He shared with us the thrill of those first observations which are so exciting and also so apt to be not repeatable. He enjoyed any of our little triumphs as much as he enjoyed his major ones. No wonder we worked long hours and tried to think great thoughts. Here I learned the secret of running a successful medical department: the chief must assemble about him a number of people, any one of whom can outdistance him in some field. If this can be accomplished without arousing anxiety and jealousy, there will develop an excellent department whose chief enjoys tremendous satisfaction from the growth of his staff.

These, then, are the lessons learned from some of my teachers and I am grateful for the opportunity to pass them on to you.

## ABSTRACTS

*The Cardiac Output in Cirrhosis of the Liver, at Rest and on Exercise.* WALTER H. ABELMANN and HENRY J. KOWALSKI, Boston, Mass. (Introduced by Laurence B. Ellis).

The cardiac output at rest may be elevated in chronic hepatic disease as previously reported. Of 29 patients with Laennec's cirrhosis and chronic alcoholism, studied on 40 separate days by means of the dye injection method of Hamilton, 10 showed a high resting cardiac output on 15 occasions. When high, the cardiac output was elevated out of proportion to the oxygen consumption and was associated with an increased stroke volume. Peripheral vascular resistance, generally normal or low, was inversely related to blood flow.

Nine patients responded normally to moderate exercise by an increase in cardiac output in proportion to the oxygen consumption and by a decrease in peripheral resistance. Two patients with very high output and low peripheral resistance at rest failed to increase cardiac output or decrease peripheral resistance during exercise.

In general, serial observations revealed no predictable relation of blood flow to stage of disease. Normal as well as high outputs at rest were seen in patients with or without fluid retention. Two patients studied during a phase of spontaneous diuresis showed elevated cardiac outputs.

Six patients with acute infectious hepatitis showed a normal cardiac output at rest and responded normally to exercise. Six patients with chronic alcoholism and without evidence of hepatic disease had a normal cardiac output at rest.

Increased cardiac output in cirrhosis did not appear to be related to co-existent anemia, jaundice, alcoholism, thiamine deficiency, or overt anxiety. It remains to be established whether this phenomenon is related specifically to chronic hepatic disease, to a form of malnutrition, or whether it represents the circulatory response of certain patients with hepatic disease to the laboratory situation.

*The Ability of the Human Subject to Regenerate Plasma Proteins During Prolonged Plasmapheresis.* WILLIAM S. ADAMS and WILLIAM G. FIGUEROA, Los Angeles, California. (Introduced by John S. Lawrence).

Three men have been the subjects of metabolic balance observations before, during and after plasmapheresis. Removal of plasma during the experimental periods was carried out daily and the patients' own red blood cells were returned to his circulation. Plasma protein fractionation was carried out both by chemical and electrophoretic means. Despite the removal of as much as 30 liters of plasma over a period of 85 days, these patients demonstrated an excellent capacity for regeneration of plasma proteins, and with adequate intake of protein and calories

they were able to compensate almost completely for losses due to plasmapheresis. At lower dietary levels of protein, compensation was incomplete and over a prolonged interval, serious protein deficits would be expected. The plasma proteins suffering the greatest from plasmapheresis were the gamma globulins in the case of the normal subject, and the abnormal plasma proteins in the cases of myeloma. The nitrogen balances show that upon institution of plasmapheresis there is a prompt diminution in urinary nitrogen excretion, so that in a matter of 5 days the normal subject was in nitrogen equilibrium. A somewhat longer period of time (15 days) was required to reach nitrogen equilibrium in the cases of myeloma. Following reinstatement of plasmapheresis at lower levels of dietary nitrogen, equilibrium was not established and no reduction in urinary nitrogen was noted.

From these results it is to be presumed that normal subjects could contribute large amounts of plasma without detriment to the donor, provided that an adequate protein and caloric intake is maintained. It is possible that large plasma pools from individual donors could be prepared which would reduce the likelihood of the development of serum hepatitis in the recipient. Furthermore, it might be possible to obtain gamma globulin or other plasma fractions containing high concentrations of specific antibodies by this technique.

*Extreme Elevation of Plasma Lipids in the Cholesterol-Fed Rabbit Treated with Cortisone and Hydrocortisone.* DAVID ADLERSBERG, CHUN-I WANG and LOUIS E. SCHAEFER, New York, New York. (Introduced by Ernst P. Boas).

The effect of cholesterol feeding and the injection of adrenal cortical steroids on the plasma lipids of the rabbit was studied as a continuation of our previous observations which showed an elevation of blood lipid fractions in men and animals after prolonged cortisone administration.

Daily oral ingestion of 1 gm of cholesterol, added to the standard purina chow, resulted eventually in an average total plasma cholesterol of 1188 mg per 100 ml in the rabbit. This was 27 times the control cholesterol level. The elevation affected the free and esterified fractions equally. The phospholipids rose to an average of 430 mg%, 5 times the control level; and the total lipids to 2250 mg%, 7 times the control.

In a group of animals fed the standard purina chow and injected with 5 mg of cortisone daily the total cholesterol rose to 143 mg% after six weeks, 3 times the control level. Phospholipids also increased by 3 times over the control level, rising to 285 mg%, and total lipids went up to 958 mg% (2½ times the control).



When cholesterol feeding was combined with the injection of 3.75 mg of cortisone daily for 7 weeks in another group of rabbits total cholesterol rose to 2704 mg per 100 ml from a control of 33 mg% (an increase of 82 times). This marked elevation affected the free and esterified fractions equally. Phospholipids rose to 906 mg% from a control level of 120 mg% ( $7\frac{1}{2}$  times) and total lipids rose to 5995 mg% from a control of 280 mg% (21 times).

In a 4th group of rabbits the injection of 5 mg of hydrocortisone plus cholesterol feeding for 5 weeks resulted in a rise of plasma total cholesterol to 2010 mg% from an average control level of 29 mg% (an increase of 70 times). Phospholipids in these animals rose from a control of 94 mg% to 818 mg% (8 times); and total lipids rose from 205 mg% to 4680 mg% (22 times).

It is evident that the combination of cholesterol feeding with parenteral administration of cortisone or hydrocortisone resulted in extreme elevations of plasma lipids in the rabbit. The plasma of these animals had the appearance of cream and its turbidity was much greater than in the other groups, treated with cortisone alone or cholesterol feeding alone. These observations support the concept that the adrenal cortex plays an important role in lipid metabolism.

*Iodine-Containing Amino Acids in Plasma: Failure to Demonstrate 3:5:3'-L-Tri-iodothyronine.* EDWIN C. ALBRIGHT, FRANK C. LARSON and WILLIAM P. DEISS, Madison, Wis. (Introduced by Ovid O. Meyer).

Gross et al recently reported the presence of 3:5:3'-L-Tri-iodothyronine in the plasma of euthyroid and hyperthyroid individuals. They separated thyroxin and tri-iodothyronine by two solvent pairs using two-dimensional chromatography. In their report it was inferred that thyroxin and tri-iodothyronine did not separate in single dimension chromatography with a collidine ammonia developer.

In the present study excellent separation of these two amino acids was effected by one-dimensional chromatography in both collidine-water and butanol-dioxane-ammonia systems.

Therapeutic quantities of  $I^{131}$  were administered to three euthyroid and two hyperthyroid individuals. Plasma samples were studied at intervals from two hours to ten days following the administration of  $I^{131}$ . For each determination both untreated and acidified plasma was extracted with butanol. The butanol extract was evaporated to a small volume. Colorimetrically identifiable quantities of thyroxin and tri-iodothyronine were added and the mixtures chromatographed in each solvent system. Following the development of the chromatogram, the amino acids were identified by spraying with a diazo reagent. Coincidence of the radioactivity with the known amino acid was interpreted as evidence that the body had incorporated the  $I^{131}$  into that particular amino acid.

In the butanol extracts of the acidified plasma there was a definite and consistent correlation of the radioactivity

with thyroxin throughout the period of study. There was no detectable radioactivity in the location of tri-iodothyronine. In the butanol extract of neutral plasma there was no exact correlation with either amino acid. In one patient the alpha-2-globulin was separated by starch electrophoresis at pH 8.6. A neutral butanol extract of this material was found to contain radioactivity, all of which chromatographed as thyroxine.

*Dual Hemostatic Defect in Pseudothrombophilia.* BENJAMIN ALEXANDER \* and ROBERT GOLDSTEIN, Boston, Massachusetts.

Despite advances in our knowledge of coagulation elucidating many formerly obscure hemorrhagic disorders, certain types remain unexplained. A study is presented of two subjects with a bleeding tendency diagnosed as pseudothrombophilia, wherein two distinct hemostatic defects were demonstrable. The salient clinicolaboratory features follow:

*Case I:* A five year old female with severe bleeding tendency since birth, death of a male sibling from a hemorrhagic disorder, normal hematologic findings, therapeutically unsuccessful splenectomy. Clotting times slightly to moderately elevated, rectified by normal plasma; bleeding times elevated, unaffected by plasma; platelets numerically and functionally normal; clot retraction normal; prothrombin slightly subnormal; Ac-globulin, S<sub>PCA</sub>, antithrombin, normal; no circulating anticoagulant; prothrombin consumption occasionally retarded; plasma antihemophilic activity, 5-10% of normal; nail bed capillaries irregular and distorted; increased capillary fragility. The mother, a non-bleeder, revealed only abnormal capillaries.

*Case II:* A 30 year old male with mild hemorrhagic phenomena for 8 years, negative f.h., normal hematologic findings. Borderline clotting times; several elevated bleeding times; prothrombin, Ac-globulin, S<sub>PCA</sub> normal; prothrombin conversion occasionally retarded; antihemophilic activity, 10-20% of normal; abnormal capillaries; normal capillary fragility.

The data indicate that a hemorrhagic syndrome properly termed "pseudothrombophilia" may arise from two coexistent abnormalities: (a) reduced plasma antihemophilic factor and (b) abnormal capillaries. The occasional clotting time elevations and retarded prothrombin consumption are explicable by the variable degree to which antihemophilic factor is reduced. The critical level of this factor seems to be approximately 10% of normal. Retarded thrombin elaboration at such low concentrations together with the capillary abnormality could account for the elevated bleeding time.

The syndrome of pseudothrombophilia, arising from a dual defect, is apparently heredo-familial in origin. Beside their intriguing genetic implications, these defects indicate the importance of both vascular and coagulation function in normal hemostasis. Precise classification of the hemorrhagic diseases on the basis of pathogenetic mechanisms has thus been extended.

*The Comparison of Albumin and Gamma Globulin Turn-over Times in the Normal and Severe Cardiac Failure of Varied Cause.* S. H. ARMSTRONG, JR.,\* KATHARINE McLEOD, JANET WOLTER and JOHN KUKRAL, Chicago, Ill.

In hope of finding gross abnormalities in turnover times of plasma protein fractions, serial sera are fractionated by paper electrophoresis after feeding 500 to 1,000 microcuries of S<sup>35</sup> labeled yeast. Paper areas containing major electrophoretic components are cut apart, the protein quantitatively extracted, precipitated and dried in a salt-free film suitable for both weighing and radioactivity measurements. Reproducibility demands meticulousness in techniques of extraction and film preparation.

Well-known heterogeneity of electrophoretic components is emphasized by this finding: When some preparations of electrophoretically homogeneous I<sup>125</sup> tagged albumin (Abbott) or S<sup>35</sup> tagged albumin (our laboratory) have been added to normal serum, as much as 20 per cent of the radioactivity has been found in the  $\alpha_1$  and  $\alpha_2$  positions. Recent separations show less 'tailing.'

Counterbalancing crudeness, simplicity and rapidity permit extensive clinical studies. Significance of results will depend on the finding of *gross* abnormalities in turnover times of the crude components.

The magnitude of abnormalities in our initial studies has surprised us.

Initial normal turnover time measurements confirm existing published results by other methods; namely, albumins and  $\gamma$ -globulins: between 15 and 20 days; components of intermediate mobility: a shorter time.

In initial studies in severe cardiac failure of varying cause and concomitants, the longest turnover times (days) have been:

| Albumins | Intermediate components | Gamma globulins |
|----------|-------------------------|-----------------|
| 250      | 25                      | 52              |

Despite prolongations, serum *levels* of these components deviated slightly from normal.

Uniform prolongations for all components have not been found. Thus in a patient with adhesive pericarditis and severe hypoalbuminemia, far from finding prolongation for albumins, we found thus:

| Albumins | Intermediate components | Gamma globulins |
|----------|-------------------------|-----------------|
| 24       | 8                       | 85              |

Problems in data interpretation, which indicate radical shifts in the protein dynamic equilibria in the presence of cardiac decompensation, will be discussed.

*The Metabolic Activity of 3,5,3'-I-Triiodothyronine in Myxedema.* SAMUEL P. ASPER, JR., HERBERT A. SELENKOW and CHARLES A. PLAMONDON, Baltimore, Md. (Introduced by Palmer H. Futcher).

Since *l*-thyroxine alleviates myxedema and occurs in plasma, it is generally accepted as the thyroid hormone. However, several paradoxes exist: (a) desiccated thyroid has greater calorogenic activity than accounted for by its *l*-thyroxine content, (b) unlike most hormones, delay in

specific effects is observed following exhibition, and (c) in some athyreotic patients receiving *l*-thyroxine the serum protein-bound iodine level is higher than anticipated by their clinical and metabolic status. Preliminary studies with the newly discovered *l*-triiodothyronine in three myxedematous patients indicate that this compound produces an immediate metabolic effect five to ten times that of equivalent amounts of *l*-thyroxine. The remarkable activity of *l*-triiodothyronine may afford explanation of these paradoxes.

Within six hours following exhibition of *l*-triiodothyronine in a single subcutaneous dose (0.5 to 1.0 mg.) in these patients, progressive increases in pulse rate and body temperature were observed, reaching maximum on the third day. Weight loss and diuresis also occurred. Basal metabolic rate increased promptly. *L*-triiodothyronine accelerated urinary creatine excretion; moreover nitrogen and phosphorus diuresis developed, resulting in negative nitrogen and phosphorus balances.

Following administration of equivalent amounts of *l*-thyroxine, significant but minimal metabolic changes occurred.

Serum protein-bound iodine levels increased after administration of *l*-triiodothyronine, although frequently remained in ranges found in hypothyroidism when the patients were metabolically euthyroid; yet, *l*-thyroxine increased the value to levels found in euthyroidism, although the patients remained hypothyroid. Serum cholesterol levels decreased following administration of either *l*-thyroxine or *l*-triiodothyronine; this decrement did not bear quantitative relationship to the degree of metabolic change observed. Electrocardiograms revealed more rapid reversion to normal after *l*-triiodothyronine than after *l*-thyroxine. Following *l*-triiodothyronine, ballistocardiograms first showed acute deterioration in form but subsequently improved.

*L*-triiodothyronine alleviates myxedema more rapidly and in smaller doses than *l*-thyroxine and may represent the functional constituent of the thyroid hormone.

*Minor Respiratory Diseases: Studies with Four Agents in Human Volunteers.* LEON T. ATLAS, Boston, Massachusetts. (Introduced by Kendall Emerson, Jr.).

Four respiratory disease entities were characterized in human volunteers by the instillation of nasal washings from donors. *MR-1*, *MR-2*, and *MR-3* viruses were cultivated in embryonated eggs; each reproduced in volunteers the disease suffered by the donor and studied in volunteers receiving the donor's washings. Transmission of *MR-1* and *MR-3* infections from inoculated to uninoculated contacts was demonstrated. A statistical evaluation of clinical findings for each disease will be presented.

Fourth to thirteenth egg passage *MR-1* virus produced disease in 168 volunteers. This illness was characterized by a 24 to 72 hours' incubation, pharyngitis, laryngitis, and postnasal discharge. A biphasic course with recrudescence of signs and symptoms 3 to 7 days after onset and peritonsillar lymphadenitis often developed. Twenty-four

volunteers reinfected 2 and 4 weeks later with *MR-1* developed colds as originally.

*MR-2* disease appeared 24 to 96 hours post-inoculation with rhinitis, headache and coughing. Fourth to seventh egg passage virus produced disease in 29 of 61 volunteers. No resistance to *MR-2* reinfection was demonstrable.

*MR-3* nasal washings produced illnesses in 98 of 133 volunteers. This disease was characterized by a 72 to 120 hours' incubation, running nose, cough, and chest pains. Leucopenia, migratory polyarthralgia, and pericarditis developed on occasion. Resistance to *MR-3* reinfection persisted over eight months.

*MR-4* nasal washings produced disease in 32 of 64 men. A 72 to 120 hours' incubation, rhinitis, sneezing, and resistance to reinfection over one month characterized *MR-4* disease.

In each experiment an equal or larger group of volunteers receiving control inocula developed no disease. The incidence, severity, and duration of disease produced by each agent were not modified by pretreatment of volunteers and continued therapy with antihistaminics, antibiotics, and placebos. Lyophilized skim milk added to *MR-3* or *MR-4* inocula of known infectivity enhanced the incidence and severity of disease.

*Phosphorus Turnover in Normal and Leukemic Leukocytes.* C. J. BARDAWILL, A. BRITTON and K. J. R. WIGHTMAN, Toronto, Canada. (Introduced by James A. Dauphinee).

The turnover of radioactive phosphorus in four fractions of human leukocytes has been studied to elucidate the metabolic pathway of phosphorus in these cells in both normals and leukaemics, with a study of the effect of treatment on the latter.

Sixteen studies are reported, including normals, and patients with polycythaemia vera, acute monocytic and lymphatic leukaemia, subacute monocytic and chronic granulocytic and lymphocytic leukaemia. A tracer dose of 500 to 800  $\mu$ c was given intravenously and blood samples were taken at intervals for eight to fourteen days. The leukocytes were isolated and their phosphorus content partitioned into acid soluble, phospholipid, ribonucleic and desoxyribonucleic acid fractions. The different fractions were then analyzed for  $P^{32}$  and  $P^{33}$  content, and the specific activity calculated.

The DNA phosphorus turnover was most rapid in cases of acute monocytic and granulocytic leukaemia, and extremely slow in chronic lymphocytic leukaemia. The chronic granulocytic and acute lymphocytic leukaemias occupied an intermediate position. In normal patients the curve is biphasic, reaching a maximum in eight days, with a peak of specific activity which is generally higher than that attained in leukaemia.

The specific activity curve of the acid soluble fraction is a descending one which cuts the rising curve of the phospholipid curve at its peak suggesting that it is an immediate precursor of the phospholipid.

The total desoxyribonucleic acid content of normal and leukemic leukocytes is the same. The ribonucleic

acid content is increased in the acute leukaemias and varies in the others. Acid soluble and phospholipid phosphorus are least in amount in the lymphocytic leukaemias.

Urethane therapy has been associated with a decrease in turnover of all the phosphorus containing fractions of two cases of chronic granulocytic leukaemia. ACTH has produced an increase in DNA phosphorus turnover in one normal subject.

The constituent nucleotides of the DNA fraction have been isolated and all the phosphorus in the total appears to be accounted for.

*Steroid Induced Gout in Rheumatoid Patients with Prolonged Cortisone Therapy.* ROBERT C. BATTERMAN\* and CORNELIUS H. TRAEGER, New York, N. Y.

Since the association of rheumatoid arthritis and gout is uncommon, it was surprising to find high blood uric acid levels in patients with rheumatoid arthritis treated with cortisone for many months. The effect of chronic administration of cortisone upon blood uric acid, urinary excretion of uric acid, creatinine, creatine and allantoin was studied in 5 subjects with advanced rheumatoid arthritis who had no previous experience with steroid therapy. Daily administration of 100 mg. cortisone orally from 30 to 60 weeks resulted in an increase in the blood uric acid in 4 of the 5 subjects. In 3 subjects the blood uric acid attained a level of over 6.0 mg. percent. The elevation of blood uric acid was associated in each patient with a clinical exacerbation of the rheumatoid arthritis and in one patient, after 45 weeks of continuous cortisone administration, an acute attack of gout occurred associated with a blood uric acid of 7.3 mg. percent. There was no significant change in the urinary excretion of uric acid, creatinine or creatine. The allantoin excretion increased in every subject. Sodium gentisate 1-2 gm. q.i.d. lowered and prevented the elevation of blood uric acid with alleviation of the rheumatoid process in 2 out of 3 additional subjects. Phenylbutazone administration in 2 subjects resulted in a marked lowering of the blood uric acid from the high steroid induced levels to normal. This was associated with an increase in urinary uric acid excretion contrary to effects of phenylbutazone in subjects not receiving cortisone.

*Ascitic Fluid and Plasma Protein Exchange. Studies with Radioiodinated Human Serum Albumin.* F. K. BAUER, W. H. BLAHD and M. FIELDS, Los Angeles, California. (Introduced by Samuel H. Bassett).

Radioiodinated human serum albumin (IHSA) in doses of 500 microcuries was injected in separate experiments, both intraperitoneally and intravenously, into patients with ascites due to portal cirrhosis of the liver (2 patients) and hemochromatosis with cirrhosis (1 patient). Their thyroid glands had been blocked by Lugol's solution. Samples of ascitic fluid and plasma were counted at intervals up to 28 days with a thallium activated sodium iodide ( $NaI$ ) scintillation tube. In order to prove that the integrity of the radioiodinated human serum albumin molecule had been maintained during the exchange be-

tween plasma and ascitic fluid, aliquots of each sample counted were subjected to dialysis, trichloroacetic acid (TCA) precipitation and paper chromatography. By these techniques, it was established that all the radioactivity was in the protein component. Using salt fractionation and specific antisera, it was possible to demonstrate that the radioactivity was actually confined to the albumin fraction. This would indicate that the iodine atoms remain firmly bound and that the radioactive albumin recovered represents the originally injected material.

The results of these studies indicate the establishment of a constant rate of exchange between the ascitic fluid and the plasma on or about the 5th day following the intraperitoneal or intravenous injection of radioactive I<sup>131</sup>S. Although the specific activity of the plasma and ascitic fluid closely approximated one another, the latter remained at a slightly higher level. Subsequently, there was a proportionate diminution in the radioactivity of both the ascitic fluid and plasma.

It is concluded that there is free exchange of albumin between the plasma and ascitic fluid of patients with cirrhosis of the liver.

*Value of Pulmonary "Capillary" and Left Atrial Pressure Pulse Waves in Assessing the Degree of Mitral Regurgitation in Patients with Mitral Stenosis.* GEORGE N. BEDELL, JAMES W. CULBERTSON, JOHANN L. EHRENHAF, WILLIAM H. AMES and ERNEST O. THEILEN, Iowa City, Iowa. (Introduced by Willis M. Fowler).

In 16 patients with mitral stenosis pulmonary "capillary" pressure pulse waves recorded during cardiac catheterization preoperatively have been compared with left atrial pressure pulse waves recorded during thoracotomy prior to mitral valvuloplasty to determine whether (1) mean left atrial pressure can be predicted from mean pulmonary "capillary" pressure, (2) left atrial pulse pressure can be predicted from pulmonary "capillary" pulse pressure, and (3) the character of the pressure pulse wave in either or both locations is helpful in assessing the degree of mitral regurgitation.

Mean pulmonary "capillary" pressure ranged from 10.5 to 31 mm Hg (average 21.0) in closed chests and alert patients, whereas mean left atrial pressure ranged from 13 to 40 (average 27.3) in open chests and anesthetized patients. Pulmonary "capillary" pulse pressure ranged from 3 to 15 mm Hg (average 7.3) and left atrial pulse pressure from 7 to 20 (average 10.0). In both comparisons it was impossible to establish a constant relationship or a reliable prediction formula.

Pulmonary "capillary" pressure pulse waves, which are not transmitted with undamped fidelity from the left atrium, have not been routinely helpful in assessing the degree of mitral regurgitation, failing in one instance to warn that valvuloplasty would be precluded. However, left atrial pressure pulse waves do constitute a reliable guide to significance of the inevitable mitral regurgitation. Atrial pulse pressure is an easily observed and apparently faithful index, and clinical experience suggests caution in

attacking a mitral valve when this measurement is greater than 12 mm Hg.

Direct registration of left atrial pressure pulse waves through a needle inserted via the auricular appendage is urged as a routine procedure during thoracotomy in mitral stenosis. Immediate decision can be made to proceed with valvuloplasty when favorable or to withdraw from an intact left atrium if mitral regurgitation is excessive.

*The Metabolism of Glycine-2-C<sup>14</sup> in Man: Hippuric Acid Synthesis.* NATHANIEL I. BERLIN, Berkeley, Calif. (Introduced by John H. Lawrence).

By feeding sodium benzoate and hydrolyzing the excreted hippuric acid, one can obtain a sample of the glycine available in the body for hippuric acid synthesis. This is of interest since it concerns the over-all problem of the retention of C<sup>14</sup> when administered as glycine-2-C<sup>14</sup> and the interpretation of the changes in the concentration of C<sup>14</sup> in hemoglobin. In the interpretation of the latter part of the curve—after 150 days—the finding of a level of C<sup>14</sup> activity approximately 15% of the plateau value poses the question: Does this represent a population of long-lived cells or the formation of hemoglobin at that time from a pool of C<sup>14</sup>-containing precursors? Two patients were given orally at weekly intervals 1.5 gm. of sodium benzoate, and urine was collected during the subsequent four hours. The excreted hippuric acid was hydrolyzed. As expected, the benzoate fraction did not contain C<sup>14</sup>. The specific activity of the hippuric acid falls rapidly, then, after an increase at 120 days, falls again, remaining at a plateau for the next 100 days. At 200 days, the specific activity of the glycine would be sufficient to account for approximately ¼ of the specific activity of the hemoglobin. It thus appears that a significant fraction of the specific activity of the hemoglobin observed after 150 days is due to synthesis from a pool of labeled glycine. If the other hemoglobin precursors also contain appreciable amounts of C<sup>14</sup>, then more than ¼ of the C<sup>14</sup> present could be present as the result of synthesis from C<sup>14</sup>-containing precursors.

The results reported will be related to (A) the nature of the anemia of leukemia and (B) the problem of the safe use of C<sup>14</sup> labeled compounds in patients.

*The Distribution and Fate of Intravenously Administered I<sup>131</sup> Labeled Human Serum Albumin.* SOLOMON A. BERSON, ROSALYN S. YALOW, SIDNEY S. SCHREIBER and JOSEPH POST,\* New York, N. Y.

Tracer quantities of pooled albumin labeled with an average of 1-2 iodine atoms per molecule were given intravenously to 46 subjects in 53 studies. Lugol's solution was administered to inhibit thyroid uptake. Plasma and urine radioactivity were followed for periods up to 6 weeks. All plasma radioactivity was accounted for in the electrophoretically separated albumin fraction and virtually all losses from the body were through renal excretion. There was no evidence of storage or reutilization of the radioactive label. In non-edematous subjects the iodoalbumin was distributed at two grossly different

rates into an apparent space approximately 2.5 times the plasma volume over a period of 4-7 days. The presence of ascites usually lengthened this period. Serial sampling of ascitic fluid revealed a half time for equilibration of over 40 hours in a subject with a 19 liter accumulation.

Individual lots of iodoalbumin prepared by two methods in this laboratory as well as those obtained from Abbott Laboratories differed considerably with respect to apparent rates of degradation owing to the presence of variable quantities of rapidly degraded components. Ultracentrifugal and electrophoretic analysis failed to reveal differences between the lots. Independent determinations from plasma and urine data in non-cirrhotic subjects receiving single component iodoalbumin gave excellent agreement for degradation rates of 3.3%-4.5%/day. When experimental periods were greater than 3 weeks the final rates observed in multicomponent systems were generally much slower than those obtained in shorter experiments and were usually less than 5%/day. These rates are considerably lower than those previously reported by this method but agree well with the values obtained by biosynthetic techniques utilizing  $N^{15}$  and  $S^{35}$ . The validity of the iodoalbumin tracer method is discussed and caution is urged against the complete acceptance of the applicability of these results to endogenous albumin metabolism.

*Relation of Changes in Colloidal Osmotic Pressure to Body Weight in Patients with Hepatic Cirrhosis with Ascites.* MAURICE M. BEST, JOAN D. WATSEN and WILLIAM A. BRODSKY, Louisville, Kentucky. (Introduced by J. Murray Kinsman).

Periods of large fluctuations of body weight in nine edematous patients with hepatic cirrhosis provided a situation for testing the hypothesis holding that ascites and edema are related inversely to changes of serum oncotic pressure. Patients, male and female, from 35 to 66 years of age, were selected on the basis of appropriate hepatic function tests and histologically established portal cirrhosis. They were receiving a cation exchange resin throughout the period of study. Short term observations were made at 1-2 week intervals, and long term observation covered periods as long as one year. Results were evaluated for each individual patient as well as for the group as a whole. Little, if any, relationship was noted between change in edema and colloidal osmotic pressure. Thus, the calculated regression for body weight change versus oncotic pressure was indistinguishable from that expected on the basis of change alone ( $P = < 0.50 > 0.10$ ). This regression line represented 101 observations on all patients during short term periods. In individual patients, weight increased as much as 10 lbs. with but little, and even unrelated, change in serum albumin level. Conversely, in one patient, weight decreased by 18 lbs. in one week without significant changes in serum proteins or estimated oncotic pressure. Of interest was the fact that in all patients, estimated values of oncotic pressure increased by an average of 31 percent over pre-treatment level after 6-12 months. It would appear that factors other than serum protein changes alone governed ac-

cumulation and dissipation of edema fluid. Improvement on resin therapy suggests that changes in electrolyte equilibrium may be at least as important as those of capillary oncotic pressure in the genesis of ascites and edema. The role of improved nutritional status, at least in part, must be invoked to explain some of the long-term clinical changes.

*The Behavior of Leukocytes in Man Following the Introduction of Heterologous Blood Group Substances.*

HOWARD R. BIERMAN,\* FAUNO L. CORDES, KEITH H. KELLY, ALINE M. LITTMAN, JESSE L. STEINFELD and PAUL K. YAMAUCHI, San Francisco, California.

The administration of heterologous blood group substances on fifty-six occasions to twenty-six patients was followed by a prompt and profound leukopenia, which was obtained with as little as 1 cc. of incompatible blood intravenously. Greater and more protracted decreases in circulating leukocyte levels were obtained with larger amounts of hetero-specified blood. Mature granulocytes were primarily involved in the non-leukemic recipients.

In ten patients with various leukemias similar falls in leukocyte level were observed. Patients with lymphocytic leukemia with elevated leukocyte counts showed prompt falls in leukocyte number to 28 to 30,000 per cmm. level but increases in amounts and rates of administration failed to cause any further drop. In patients with monoblastic or granulocytic leukemia, the leukopenia corresponded more nearly to that observed in the non-leukemic patients. Repeated frequent administrations of incompatible blood failed to elevate the circulating anti A or anti B titer except in 2 non-leukemic patients.

Purified group specific A & B substance caused similar although not as profound leukopenias and did not prove as potent an antigen as hetero-specific fresh whole blood.

The intramuscular, subcutaneous and prolonged continuous intravenous infusion of either incompatible whole blood or purified group specific substances was associated with more protracted falls in circulating leukocyte levels.

By simultaneous arterial and venous catheterization and portal venipuncture isolating the lung, liver, and spleen it has been possible to demonstrate that no single organ is exclusively involved and to estimate the rate of withdrawal and return of leukocytes from the major reticulo-endothelial sites.

Erythrophagocytosis in the circulating blood by polymorphonuclear leukocytes occurred within 30 to 60 seconds in patients receiving more than 5 cc. of heterologous blood. Erythrophagocytosis in the leukemic patients by immature granulocytes and monocytes but not by lymphocytes was observed. Alterations in cellular morphology and circulation dynamics will be presented.

*Measurements of Platelet-Derived Serum Vasoconstrictor (Serotonin) in Normal Subjects and in Patients with Hemorrhagic Disease.* FREDERICK S. BIGELOW, Boston, Mass. (Introduced by William B. Castle).

Vasoconstrictor in serum was measured with a kymograph actuated by a segment of ox carotid artery in an

oxygenated balanced saline bath at 37.5°C. Prior to coagulation, normal blood was almost devoid of vasoconstrictor activity, but gained this activity when coagulation occurred. Normal serum was active in dilution up to 1:8 and when undiluted equalled the vasoconstrictor potency of a solution of synthetic serotonic creatinine sulfate, 0.5 µg per ml in balanced saline. Serum of normal blood artificially deprived of platelets prior to coagulation was almost inert. Serum vasoconstrictor was found to be equal when obtained either by prothrombin conversion in whole blood, or by addition of thrombin to decalcified blood. The vasoconstrictor principle was stable at -20°C, readily dialyzable and was liberated into the supernatant when platelets were fragmented by coagulation, freezing or supersonic energy.

Vasoconstrictor was diminished in the sera of five thrombocytopenic patients. Artificial *in vitro* concentration of the patients' platelets prior to coagulation failed, in two of these subjects, to yield sera of normal vasoconstrictor potency. Remission of thrombocytopenia following splenectomy was associated with restoration of serum vasoconstrictor to normal. The evolution of serum vasoconstrictor was delayed in two hemophiliacs with normal platelet counts, but was not decreased in three subjects with pseudo-hemophilia. Decreased serum vasoconstrictor despite normal platelet concentrations occurred in two instances of severe hypoprothrombinemia and in two patients with vascular purpura.

Four patients with prolonged bleeding time, splenomegaly and marked thrombocytosis exhibited decreased serum vasoconstrictor. This defect was due neither to unusual platelet stability nor to the presence of an anti-vasoconstrictor. The deficient serum vasoconstrictor of these patients despite their elevated platelet counts may partially explain the hemorrhagic state.

*Metabolism of the Human Heart in Vivo.* R. J. BING,\* A. SIEGEL, A. VITALE, F. BALBONI and E. SPARKS, Birmingham, Ala.

The myocardial extraction and usage of glucose, pyruvate, lactate, ketones, amino acids and fatty acids were studied in 70 patients with and without cardiac failure. Coronary arteriovenous differences of sodium and potassium were ascertained. Coronary venous blood was obtained by coronary sinus catheterization. The contribution of the aerobic catabolism of these substances to the total myocardial oxygen extraction, and the relative efficiency with which the oxidative energy derived from these metabolites was converted into cardiac work was estimated. At low arterial carbohydrate concentrations, the myocardial extraction of these metabolites was low; here the aerobic energy was probably derived from the breakdown of cardiac glycogen and from non-carbohydrate sources. The ingestion of carbohydrates resulted in increased myocardial extraction and usage. The myocardial glucose extraction was directly proportional to the logarithm of the arterial glucose concentration. A slight increase in myocardial glucose usage was observed at arterial glucose concentrations of more than 110 mg.%. Pyruvate was utilized by the heart, but its contribution

to cardiac metabolism was small. Intravenous infusions of glucose resulted in a rise in myocardial glucose extraction at any arterial glucose concentration. The percentage of the total aerobic cardiac metabolism accounted for by oxidation of amino acids and ketones combined amounted to less than 10%. No extraction of sodium or potassium was found. In patients with low output failure, the myocardial oxygen extractions and consumptions were normal; the myocardial glucose and lactate extractions were normal or increased. In these patients, the conversion of energy derived from carbohydrates into mechanical work was defective.

*Osmotic Equilibria of Tissues.* WILLIAM A. BRODSKY, WARREN S. REHM and B. J. MCINTOSH, Louisville, Ky. (Introduced by A. C. Corcoran).

Recent observations on swelling of mammalian tissue slices immersed in isotonic solutions have posed a challenge to the validity of the classic concept of osmotic equality between cellular and extracellular fluids. Since swelling was greater under anaerobic than under aerobic conditions, it was inferred that in the intact animal intracellular fluid was maintained hypertonic to the surrounding environment by a process of active water extrusion. Objections to such an inference were: 1) that an *in vitro* process of protein degradation might yield increased amounts of osmotically active metabolites and 2) that cell membranes may change after removal from the body so that they become freely permeable to all water and solutes except the proteins. Therefore, experiments, designed to provide direct measurements of tissue osmotic activity, were performed on nembutalized dogs.

Aliquots of various tissues were removed surgically, immersed in liquid nitrogen, and crushed in a Carver press. Cell-free homogenates thus obtained were analyzed for osmotic activity by the cryoscopic technique. Values of freezing point depression, performed on RBC mass, spleen, brain, muscle, liver, kidney, and gut, were compared to those of simultaneous plasma. Osmotic activity of RBC mass was consistently the same as that of plasma. Spleen was iso-osmotic or slightly hypertonic, while brain and skeletal muscle were 5-10 percent higher in osmolarity than plasma. Osmotic activity of liver and kidney was consistently high, reaching values of 438 mosm/L, while that of simultaneous plasma was 295 mosm/L. Values from homogenates of renal cortex and medulla were identical. Results were not entirely in accord with recent contentions on internal hypertonicity. A tentative hypothesis holds that internal hypertonicity is restricted to those organs concerned with the production of a secretory fluid (kidney, stomach, liver, etc.), while internal isotonicity is maintained in organs which do not produce a secretion (RBC mass, muscle).

*The Four Year Course of 103 Patients with Benign Essential Hypertension and the Influence of Doctor-Patient Relationship Upon It.* ALBERT A. BRUST,\* JAMES A. HAGANS and MORTON F. REISER, Atlanta, Ga.

From a larger group of hypertensive patients, 103 who were observed during a four year period fulfilled the

criteria for chronic benign essential hypertension in that they had less than Grade III ocular fundi and had no evidence of primary renal disease or other causally related diseases. None of the patients were given blood pressure lowering therapy, but 54 were followed as frequently as necessary in a supportive doctor-patient relationship type of therapy (treated patients). Forty-nine were not followed closely but were contacted again for a four year follow-up evaluation (untreated patients).

Deaths related to hypertension in untreated and treated groups were similar (9 and 10 respectively), but the survival time was doubled in the latter (9 and 18 months respectively). Initial severity of illness was comparable in both the untreated and treated patients who died (mean blood pressure 191/111 and 183/117 mm. Hg respectively; severity index, relating degrees of renal, fundal and cardiac involvement .52 and .43 respectively).

Of the 84 patients who survived after four years, 65 were subjected to detailed follow-up studies—28 in the untreated and 37 in the treated group. The mean blood pressure of all survivors changed from 169/108 to 178/109 mm. Hg and the severity index from .19 to .28 after four years. Although the untreated survivors were initially less ill (mean blood pressure 163/103 vs 174/113 mm. Hg; severity index .14 vs .25), the treated showed a strikingly greater sustained improvement in symptomatology (65% vs 22%) and functional capacity (62% vs 18%).

The data illustrate the relative progress of hypertensive cardiovascular disease in terms of the blood pressure and target organs in these patients with relatively severe benign essential hypertension and demonstrate that the impact of the doctor-patient relationship may have rather profound effects upon symptoms, functional capacity and perhaps on survival time.

*Use of Magnamycin and Erythromycin in the Treatment of Pneumococcal Pneumonia.* PAUL A. BUNN,\* Syracuse, N. Y.

Erythromycin or magnamycin was used as initial therapy in twenty adults, with similar bacteriologic, roentgenographic and clinical evidences of pneumococcal pneumonia. Eight received magnamycin and twelve received erythromycin. Six patients had initial bacteremia; average age was 50 years (range 24 to 86); five were critically and 8 severely ill; seven had two lobe involvement; five were alcoholic. Purulent complications were not noted prior to treatment.

Average daily oral dose of erythromycin was 1.5 grams. For magnamycin 1.6 grams were administered orally and/or parenterally. Therapy extended for 7 days in most who responded well.

Only five of the 20 had febrile crises within 24 hours of instituting therapy. Eight others became afebrile within 48 hours. Defervescence by lysis in 96 hours occurred in 3 more. The fever in four was unaffected.

Clinical results in 9 were satisfactory. Slow but complete resolution of pneumonia was observed in 5 others; two of these developed pleural effusions during treat-

ment. Six of the 20 patients failed to respond satisfactorily. Three were in the erythromycin group. Clinical course of one was unaffected and her sputum contained pneumococci after 72 hours. Empyema developed in two, one had subsequent lung necrosis, and both died after 8 and 20 days. In one of these, sputum and blood cultures remained positive for 48 hours. Of the three magnamycin failures, among the 8 treated, one developed pleural effusion and extension of pneumonia after 4 days of treatment. In another blood cultures were positive at 24 hours and an empyema developed 4 days later. The initial critical condition of the third patient was unchanged for 48 hours, and her blood cultures remained positive until penicillin was administered.

No untoward side effects from either drug were observed. The results were so unsatisfactory that extension of the study was precluded.

*Clinical Evaluation of a New Antimetabolite in the Treatment of Leukemia.* JOSEPH H. BURCHENAL,\* M. LOIS MURPHY, ROSE RUTH ELLISON and DAVID A. KARNOFSKY,\* New York, N. Y.

A compound never hitherto evaluated clinically, 6-mercaptopurine (6MP), has been temporarily useful therapeutically, but not curative, in a substantial proportion of children and adults with leukemia, including cases refractory to antifolic therapy.

The mechanism of action of 6MP seems to differ from that of substances hitherto proved beneficial in leukemia. For example the antifolics block the uptake of formate in the *de novo* synthesis of purines, whereas 6MP presumably interferes with the incorporation, in nucleic acid, of preformed purines, their nucleosides or nucleotides.

Of 31 children with acute leukemia, 10 (5 antifolic resistant) had temporary clinical and hematologic remissions, 8 (6 antifolic resistant) had partial remissions and 13 (9 antifolic resistant) were considered failures. Of 11 adults, 3 (aged 15, 26 and 52) had temporary remissions with clinical and hematological improvement. Eight were considered failures.

Remissions were achieved in all of 5 patients with chronic myelocytic leukemia, of whom 2 were in the early stage, one in the late stage with generalized lymphadenopathy, and 2 in the terminal acute stage of the disease. Relapses occurred approximately 4 weeks after therapy was stopped but further remissions were often obtained with resumption of the drug.

In children daily oral administration of 2.5 mg/kg rarely caused toxic manifestations, but prolonged therapy at this dose in adults or at higher levels in children occasionally produced bone marrow depression or gastrointestinal symptoms.

In the acute leukemias resistance to 6MP may develop somewhat more rapidly than to amethopterin, but in neither the leukemias nor in bacterial systems does there appear to be cross resistance between these two antimetabolites.

An agent therapeutically active in several types of leukemia, including those refractory to antifolic therapy,

and operating as an antimetabolite of a new type, with minimal toxicity, is of fundamental as well as practical interest.

*The Influence of Hypocapnia on the Circulation: Clinical Significance in Orthostatic Hypotension.* J. F. BURNUM, J. B. HICKAM\* and H. D. MCINTOSH, Durham, N. Carolina.

The clinical observation that spontaneous hyperventilation regularly preceded collapse in a patient with orthostatic hypotension has prompted an investigation of general circulatory changes associated with hyperventilation-induced hypocapnia.

Recumbent patients were studied during one minute periods of maximal hyperventilation, and arterial blood samples were taken for CO<sub>2</sub> analysis. Intra-arterial pressures, forearm plethysmographic blood flows, and blue dye cardiac outputs were measured simultaneously.

It is not generally appreciated that hyperventilation lowers the blood pressure in normal persons. 27 of 29 control subjects showed an average maximal mean pressure fall of 17 mm. Hg during overbreathing with a rapid recovery and occasional overshoot at the end of hyperventilation. Three patients with orthostatic hypotension associated with autonomic nervous system disease exhibited much greater falls in pressure (33 to 50 mm. Hg) during hyperventilation with delayed recovery without overshoot after overbreathing. In all cases maintenance of arterial CO<sub>2</sub> tension by inhalation of 5% CO<sub>2</sub> during hyperventilation prevented a fall in arterial pressure.

The cardiac output was unchanged or increased during hyperventilation in four normal persons and three patients with orthostatic hypotension. Peripheral vascular resistance was decreased during hyperventilation in both groups, but more markedly so in those with orthostatic hypotension.

Forearm blood flows increased strikingly in eight normal subjects during hyperventilation and O<sub>2</sub> content of forearm venous blood increased. Vascular resistance in the forearm was greatly lowered.

It is difficult to account for all the results in terms of conventional physiologic concepts. Reduction of central vasomotor tone by hyperventilation-induced hypocapnia could account for the fall in overall peripheral resistance which occurs in normal subjects. However, this mechanism could not easily explain the more profound fall in peripheral resistance exhibited by the patients with orthostatic hypotension in whom reflex vasomotor control is impaired.

*The Production of L.E. Cells in Normal Individuals.* C. E. BUTTERWORTH, JR., Birmingham, Alabama. (Introduced by T. R. Harrison).

There is evidence that Systemic Lupus Erythematosus may be due to a disturbance in the normally occurring enzyme system involving desoxyribonuclease and its corresponding anti-enzyme. For this reason blood from twenty normal individuals was tested with (experimental) and without (controls) crystalline bovine desoxyribonuclease

(DNA-ase), using a modification of the clot technique of Zimmer and Hargraves. In experimental tubes 2.0 cc blood were added to 0.5 cc of physiologic sodium chloride solution containing DNA-ase and arranged so that final enzyme concentrations of 0.01%, 0.005%, and 0.002% were achieved. Controls consisted of 2.0 cc blood added to 0.5 cc physiologic sodium chloride solution.

Fourteen of the controls revealed manifestations of nucleolysis generally regarded as suggestive of Lupus, namely, rosettes, "pre-L.E. cells," and purple staining amorphous masses. Seven controls showed morphologically typical L.E. Cells in small numbers (less than 1 per 10,000).

Nineteen of the experimental tubes revealed manifestations of nucleolysis and fifteen (75%) revealed typical L.E. cells. The frequency of L.E. cells in this group varied from 1 per 1,000 to 1 per 10,000.

Although the series is too small to make definite statistical conclusions, the occurrence of even a few L.E. cells in normal individuals emphasizes the need for quantitative evaluation of the L.E. test. The apparent enhancement of L.E. phenomena by DNA-ase in this small series tends to support the idea that DNA-ase may be one of the factors involved in the production of a positive L.E. test.

*The Relationship of Thyroid Hormone Utilization to Its Serum Level.* BELTON BURROWS, Boston, Massachusetts. (Introduced by James M. Faulkner).

The rates of production and utilization of thyroid hormone, estimated from thyroid uptakes of radioactive and stable iodine, have been related to the state of thyroid function, shown by serum protein-bound iodine concentration (PBI), in human subjects. There is a significant correlation between the radioiodine uptake and the PBI in untreated patients ( $r = .78$ ,  $n = 85$ ) and patients who have received prior therapy for hyperthyroidism ( $r = .53$ ,  $n = 112$ ). In the latter group individual radioiodine uptake values for each PBI level show a greater variation, perhaps due to decreased thyroid iodine storage, which limits the diagnostic usefulness of their radioiodine uptakes.

The stable iodine uptake, however, showed a good correlation with the PBI in patients who had radioiodine uptakes above the average values ( $r = .82$ ,  $n = 25$ ,  $p$  less than .01). The relationship between the two determinations is indicated by the formal  $y = 1.2 e^{0.82x}$ , when  $y$  = stable iodine uptake (micrograms per hour) and  $x$  = serum protein-bound iodine concentration (micrograms percent). In other words, an increase of 3.5 micrograms percent in the PBI indicates on the average a three-fold increase in the amount of thyroid hormone which is being formed in the thyroid gland and utilized in the tissues, not only with the onset of hyperthyroidism, but also within the normal range.

Figures for normal hormone utilization, derived from the stable iodine uptakes in the normal range, or from the average values for radioiodine uptakes assuming a daily dietary iodine intake of 150-200 micrograms, cor-



respond to the dosage of synthetic thyroxine required to treat myxedematous patients or to suppress the radioiodine uptake in normal subjects. The average three-fold increase in hormone utilization with an increment of 3.5 micrograms percent in the PBI agrees with other studies on the extent to which thyroid hormone utilization may be augmented in hyperthyroidism.

*Changes in Cardiac Output During the Cold Pressor Test: Comparison of Low-Frequency Ballistocardiograph and Direct Fick Methods.* PHILIPPE V. CARDON, JR. and DANIEL S. LUKAS, New York, N.Y. (Introduced by Thomas P. Almy).

Cardiac output was determined simultaneously by the low-frequency (Nickerson) ballistocardiograph, and the direct Fick method, in 5 normotensive and 3 hypertensive subjects. In each subject measurements were made before, during, and after the cold-pressor test. Initial resting values agreed closely in 3 cases. In one case there was a disparity of 1 liter/minute, and in 4 cases the disparity was greater than 2 liters/minute. In 2 hypertensive and 3 normotensive subjects changes in cardiac output of more than 1 liter/minute by the Fick method occurred during or after the cold-pressor test. In 3 of these, the pressor response was caused by increased cardiac output and accompanied by a drop in peripheral resistance. In the other 2, the opposite occurred. There was fair relative correlation between the 2 methods in 5 of the 15 possible comparisons. Among the remaining 10 comparisons, 6 types of deviation were noted; namely, changes in BCG in opposite direction when Fick rose or fell; no change in BCG when Fick rose or fell; and rise or fall in BCG with Fick constant. The paired BCG estimates, within each period, in general agreed closely with each other. The 3 subjects who showed no significant changes by the Fick method were those who had the smallest pressor responses. In these subjects the BCG also indicated a steady state.

*Differences in Susceptibility of Polymorphonuclear Leucocytes from Several Species to Alteration by Systemic Lupus Erythematosus Serum.* ANA CARRERA, VIRGINIA REID and N. B. KURNICK, New Orleans, Louisiana. (Introduced by C. Thorpe Ray).

As recently reported from this laboratory, in addition to the specific factor present in Fraction II of some lupus erythematosus (L.E.) sera, other participants in the L.E. cell phenomenon are (a) desoxyribonuclease, (b) a specific intracellular inhibitor of desoxyribonuclease, and (c) a factor(s) in Fraction III of normal and L.E. sera which reacts with the intracellular factors. Since (a) and (b) are intracellular components, and since cells vary in their content of these constituents, leucocytes from different species might be expected to vary in their susceptibility to the L.E. phenomenon. Leucocytes suspended in their own heparinized plasma from each species studied were centrifuged, resuspended in the serum of a

single lupus patient and normal control, incubated, and stained smears examined for the L.E. cell phenomenon. The results were quantitated on the basis of L.E. alterations per thousand polymorphonuclear leucocytes (PMN). Marked differences were detected among the individuals examined, varying from over 700 modified PMN per thousand in the chicken to a negative result in the racoon. The leucocytes from normal man fell far down in the scale of susceptibilities. A serum from a second patient with systemic lupus erythematosus, which had been found to give a negative L.E. phenomenon when tested against washed human leucocytes, gave a strongly positive phenomenon when tested against horse leucocytes (over 500 per 1000 neutrophils).

*The Relative Effects of Strict Bed Rest and Dietary Components in the Treatment of Acute Infectious Hepatitis.* THOMAS C. CHALMERS, RICHARD D. ECKHARDT, WILLIAM E. REYNOLDS, JOAQUIN G. CIGARROA, NORMAN DEANE, ROBERT W. REIFENSTEIN and CLIFFORD W. SMITH, Boston, Massachusetts. (Introduced by Clark W. Heath).

The relative effects of strict bed rest and dietary components in the treatment of infectious hepatitis were studied in 452 American soldiers admitted to the Army Hepatitis Center in Kyoto, Japan. Patients were assigned to the treatment groups at random and were followed by carefully standardized clinical and laboratory observations. Criteria for treatment effect were the duration from admission until the total serum bilirubin and bromsulphalein retention were normal, the incidence of convalescent relapses, and the occurrence of disability shown by a follow-up study still in progress.

In the first of two studies, 65 patients were assigned to each of four treatment groups: 1) strict bed rest and forced diet high in protein and fat with choline and vitamin supplements; 2) strict rest and regular hospital diet eaten ad lib; 3) ad lib bed rest and forced diet; 4) ad lib rest and ad lib diet. No differences between the rest regimes were demonstrated. Patients on the forced diet convalesced 7 days (S. E.  $\pm$  2 days) sooner than those fed the regular diet ad lib. Convalescent relapses in the bromsulphalein test occurred in 5% of each group.

In the second study, 48 patients were assigned to each of the following strictly enforced dietary regimes: 1) 4000 calories, 19% protein calories; 2) 4000 calories, 11% protein calories; 3) 3000 calories, 19% protein calories; 4) 3000 calories, 11% protein calories. Half of each group received supplements of choline and vitamins. Patients on the 19% protein diets convalesced in 29 days, 6 days (S. E.  $\pm$  2 days) sooner than those on the 11% protein diets. There were no significant differences among the effects of varying caloric intake or supplements.

Differences in the rates of decline of the serum bilirubin indicate that the dietary effect begins during the first week of treatment.

*Cardiovascular Effects of Aramine.* DON W. CHAPMAN and W. R. LIVESAY, Houston, Texas. (Introduced by James A. Greene).

Aramine (a monohydroxyphenylalkylamine) is a synthetic sympathomimetic amine. Its cardiovascular effects were observed in 8 normal patients during continuous venous infusion.

Right heart catheterization, oxygen uptakes, arterial gas samplings and pressure recordings, and electrocardiographic tracings were used to study these patients in the control state, at the height of the pressor response, and following atropine administration.

Cardiac indices determined by the Fick principle revealed little change following administration of aramine. In one patient following the giving of intravenous atropine a doubling of cardiac output was noted. Uniformly, pulmonary arterial pressures were markedly increased and systolic right ventricular pressures were elevated as with epinephrine and norepinephrine. Pulmonary capillary pressures were somewhat elevated. Systemic arterial pressure elevations to levels of 200 to 220 mm of mercury systolic and 110 to 120 diastolic were uniformly produced. Total peripheral resistance showed marked increase which in part paralleled the hypertensive response. Left and right ventricular work were markedly increased. Pulmonary arterial resistance was increased. Electrocardiographic changes were minimal although transient ST segment depressions have been noted in 2 of 8 patients. All patients showed a bradycardia which was blocked by atropine indicating that it is mediated through the vagus nerve. Little change was noted in any of the observed hemodynamics following atropine administration despite the change in heart rate.

Aramine has been found to be a potent vasopressor agent quite effective in the control of marked hypotension. Cardiovascular effects have been presented.

*The Mechanism of Sodium Retention in Cirrhosis of the Liver.* J. J. CHART and E. S. SHIPLEY, Madison, Wisconsin. (Introduced by E. S. Gordon).

The importance of metabolic as well as mechanical factors in the production of fluid retention and ascites in cirrhosis of the liver has now been convincingly demonstrated. One of the important factors in the mechanism of this anomalous fluid balance has been shown to be the presence of a circulating anti-diuretic substance believed to be of posterior pituitary origin, but the explanation of marked sodium retention in this disease has not been clarified (but alterations in adrenal cortical function have been suspected on the basis of general physiological considerations).

24-hour urine specimens from cirrhotic patients with ascites have been extracted with chloroform by a standard method designed to remove all steroid compounds. These have been quantitatively studied for their content of 17-ketosteroid and formaldehydogenic steroid fractions, and also have been assayed for "sodium retaining factor" (SRF) using a biological assay procedure already published, employing adrenalectomized rats under standard-

ized conditions of salt loading. The sodium retention induced by pure desoxycorticosterone is used as a base of reference.

Normal adult human subjects excrete from 40 to 80 microgram equivalents of desoxycorticosterone for 24 hours. The ten patients in this study have regularly excreted amounts of this sodium active fraction up to a maximum of 3162 microgram equivalents. Cirrhotic patients without ascites fall within the normal range. An elevated urinary titer is invariably associated with a striking reduction in sodium excretion down to a minimum of 13.25 milligrams in 24 hours. Benzene-water partition study of this unknown fraction demonstrates that it is not desoxycorticosterone since the latter moves quantitatively into the benzene phase while the former is strongly water soluble. Present evidence suggests that SRF is associated with the "amorphous fraction" of Kendall.

*An Evaluation of the Pathologic Physiology of the Anemia of Cancer.* ROBERT B. CHODOS and JOHN MOSES, Boston, Massachusetts. (Introduced by Dr. Franz J. Ingelfinger).

In an effort to determine the basic pathologic physiology of the anemia of neoplastic diseases, we have evaluated blood formation and destruction in a group of normal subjects and in patients with the anemia of cancer. Blood loss, infection, hepatic, and renal disease were excluded. Serum iron levels and an estimation of red cell and plasma volumes by radiophosphorus tagged red cells were determined for all patients. Protein-bound iron 59 of high specific activity was then administered intravenously to each subject, and the rate of removal of iron from plasma over a period of hours and its per cent utilization by red cells over a period of weeks was determined. This data revealed striking differences between the groups. The plasma iron turnover rate constant, representing the fraction of total plasma iron entering or leaving the plasma per unit time, was more than doubled in the cancer group, compared with that for normal subjects. Similarly, the per cent of red cell iron renewed per day in the cancer group was twice that of normal subjects. Finally, utilization of iron in red cell formation approximated 100 per cent. These data indicate active and probably increased erythropoiesis. Nevertheless, the total circulating hemoglobin was moderately reduced in the cancer patients. This reduction may be due to increased blood destruction as suggested by our observation of an elevated hemolytic index from fecal urobilinogen determinations in a number of patients with cancer. We therefore conclude that in the anemia of cancer, as in leukemia and lymphoma, blood formation is not depressed but actually proceeds at an increased rate, though at lower levels of total circulating hemoglobin. This lowered total hemoglobin may be the result of an increased rate of red cell destruction, suggesting a common basic pathogenesis for the anemia of cancer, leukemia, and lymphoma.

*The Metabolic and Hemodynamic Response of the Human Hypertensive Kidney to a Standard Intravenous Dose of 1-Hydrazinophthalazine (Apresoline).* ARCHER P. CROSLY, JR., GEORGE G. ROWE, ROGER MCKENNA and CHARLES W. CRUMPTON, Madison, Wisconsin. (Introduced by Edgar S. Gordon).

1-Hydrazinophthalazine is capable of producing a reduction in the mean arterial blood pressure of hypertensive patients. This reduction is associated with renal vasodilatation and the disappearance of various circulating pressor principles. Speculation as to the possible interplay of these two factors led to renal hemodynamic and metabolic studies in moderate to severe hypertensive patients before and after the administration of 0.25 mg./Kg. of Apresoline.

In the cases studied, renal blood flow (RBF) increased significantly in the face of a significant reduction of mean arterial blood pressure with a resultant reduction in renal peripheral resistance. Total RBF was higher than the clearance of PAH by virtue of a significant and real reduction in PAH extraction. Simultaneously the renal-arterio venous oxygen difference ( $A-R_{O_2}$ ) decreased significantly. Oxygen consumption, calculated as the product of  $RBF \times (A-R_{O_2})$ , showed no significant change. One case simultaneously showed no significant change in  $T_m$  PAH.

These studies suggest: (1) that the metabolic response of the human hypertensive kidney to an increase in renal blood flow is similar to that of the normotensive kidney; (2) that the disappearance of pressor substance following Apresoline is not associated with a change in renal oxygen consumption; and (3) that if renal vascular shunts are active in producing the increase in RBF they act in addition to the normal circulation rather than to the exclusion of the same.

*Evidence for Hepatocellular Dysfunction in Patients with Arterial Hypertension without Signs of Intrinsic Hepatic or Renal Disease or Congestive Myocardial De-compensation.* JAMES W. CULBERTSON, JAMES G. EASTON and TED L. WELTON, Iowa City, Iowa. (Introduced by Horace M. Kornis).

Hepatocellular function was studied in 6 hypertensive patients (2 malignant) *with* and 9 (3 malignant) *without* clinical evidence of renal disease (Groups I and II, respectively). Arterial pressure elevation was similar in these groups: range 154/105-244/148 (mean average 168) mm Hg. Minute percentage disappearance rate (PDR) of plasma Bromsulfalein was determined according to Ingelfinger and associates, who had reported ranges of 9.1-16.0 in healthy subjects and 8.5-23.0 in hospital normal patients.

All Group I patients were normal: range 11.9-21.5 (mean 14.1). Four Group II patients were *low normal*: 10.8, 8.9, 8.7 and 8.7, respectively; while one was well below normal (5.5). The remaining 4 showed severe hepatocellular dysfunction, indicated by the BSP satura-

tion phenomenon in mild (1), moderate (1), and marked (2) degrees.

One patient with malignant hypertension and renal disease showed no change in his normal PDR while under treatment with hexamethonium and 1-hydrazinophthalazine, despite a substantial reduction of arterial pressure. In one patient with malignant hypertension without clinical renal disease the BSP disappearance curve changed from one of marked saturation to one of mild saturation, while the arterial pressure declined only slightly under hexamethonium therapy. In one benign hypertensive without clinical renal disease the BSP curve changed from marked saturation to regular logarithmic contour with a normal PDR of 13.4, along with an arterial pressure fall from 230/130 to 150/105 mm Hg during hexamethonium treatment.

By this method hepatocellular function appears to be normal in arterial hypertensives with clinical renal disease but impaired in a significant number of those without it. This dysfunction is not correlated with the degree of hypertension. The abnormality may be susceptible to therapeutic reversal. It is impossible to state now whether the hepatocellular dysfunction bears a primary or secondary relationship to the arterial hypertension.

*The Effect of Intravenous Protoveratrine on Digital Pulse-Volume and Skin Temperature.* J. H. CURRENS, R. B. KHAMBATTA, J. J. CRANLEY, and IDA GORDON, Boston, Mass. (Introduced by M. E. Cohen).

Simultaneous digital pulse-volume and skin temperature determinations of a finger and toe have been made on twenty-five patients (14 females and 11 males) before and after intravenous protoveratrine. Observations were continued for 30 minutes after injection of the drug. The patients all had hypertensive vascular disease with good arterial pulsations in the extremities. The amount of drug was sufficient to produce a thirty per cent average fall in both systolic and diastolic blood pressure at 10 and 20 minutes after injection.

Statistically significant correlation was found between the decrease in pulse rate and the systolic and diastolic blood pressure 5 and 10 minutes after protoveratrine. The change in the finger pulse-volume as well as the change in finger skin temperature bore a statistically significant relationship with the fall in diastolic blood pressure. The correlation of these two factors with the fall in systolic blood pressure was significant but less consistent. No significant correlation was observed between the change in the pulse-volume of the toe and the skin temperature of the toe with the systolic or diastolic blood pressure fall. Correlation was also significant at 5, 10, and 20 minutes after the drug between the increase in pulse-volume and skin temperature in the finger but not in the toe.

These findings are consistent with the clinical observations in regard to the degree of sweating and subjective sensation of warmth in the upper extremity as compared with the lower extremity after protoveratrine administration.

*Orthostatic Factors in Pulsus Alternans.* WILLIAM M. DAILY and ROY S. SHEFFIELD, McKinney, Texas. (Introduced by Ben Friedman).

We have observed a striking relationship between pulsus alternans and posture in three patients with organic heart disease. Ventricular alternans was present only in the erect or semi-erect position disappearing with recumbency. The phenomenon which was regularly reproduced in the standing position failed to appear if the subject were standing in water to the level of the nipple line. Preliminary digitalization as well as application of abdominal and leg binders also prevented the appearance of alternation in the erect position. When ventricular alternation appeared with the patient erect, it could be reversed by mild leg-raising exercises and by infusion of nor-epinephrine or of blood. In the recumbent position pulsus alternans was observed for short periods immediately following premature beats. Prolonged ventricular alternation was induced in the supine patient only after venous occlusion and Valsalva maneuvers combined with phlebotomy.

These observations together with the electrokymographic tracings of the cardiac borders suggest that there are two factors concerned in the mechanism of this type of ventricular alternation: (1) Weakened or injured heart muscle that does not contract maximally except under conditions of increased stretch, (2) A precipitating peripheral hemodynamic factor which exerts its effect by changes in ventricular inflow and peripheral resistance.

*Fructose in the Treatment of Diabetic Ketosis.* J. H. DARRAGH, R. A. WOMERSLEY and W. H. MERONEY, New Haven, Connecticut. (Introduced by John P. Peters).

Twenty-five grams of fructose were administered intravenously to four patients in severe diabetic ketosis shortly after the institution of treatment with insulin and saline. The fructose disappeared rapidly from the blood, and at the same rate as in two normal subjects to whom the same amount of fructose had been given. Two patients received twenty-five grams of glucose intravenously, one before, and one after the infusion of fructose. The increase in the concentration of glucose in the blood was greater and more prolonged with glucose than with fructose.

Six patients in diabetic ketosis of varying severity were treated with three to six liters of a primary repair solution consisting of 2.5 per cent fructose and 0.45 per cent saline. Insulin was given in the usual manner and potassium was added to the infusion when indicated. In all six patients there was a rapid reduction of the concentration of ketones in the blood. The maximal concentration of fructose in the blood observed during the period of infusion of fructose was 60 mgm. per cent. There was a steady decline in the concentration of total hexose in the blood. The results were compared with similar groups of patients who had received glucose early in the treatment of diabetic ketosis. The concentrations

of hexose in the blood were lower in the patients receiving fructose. Water and electrolyte balance studies showed that saline was retained as a hypotonic solution, indicating that water had entered the cells.

It is suggested that the dehydration associated with diabetic ketosis can be corrected rapidly by infusing a hypotonic electrolyte solution made up to normality with fructose. By using fructose instead of glucose, carbohydrate can be administered early in the course of treatment without increasing the effective osmotic pressure of the extracellular fluid. Balance studies of electrolytes, water, and carbohydrate will be reported.

*Experimental Fibrinoid Necrosis of the Coronary Arteries in Streptococcus-infected Rabbits.* FLOYD W. DENNY, JR., ROBERT A. GOOD and LEWIS THOMAS,\* Minneapolis, Minn.

The generalized Shwartzman reaction, with bilateral cortical necrosis of the kidneys, was produced in a majority of rabbits given an intravenous injection of group A hemolytic streptococci followed by an intravenous injection of gram-negative bacterial endotoxin.

With large doses of streptococci, cardiac lesions consisting of extensive myofiber necrosis occurred; these lesions did not differ from those encountered when the generalized Shwartzman reaction was produced by two intravenous injections of meningococcal toxin without streptococci.

With smaller doses of streptococci, the incidence of typical renal and cardiac lesions of the generalized Shwartzman reaction was reduced, and a new lesion involving the coronary arteries appeared in over 50 per cent of rabbits. The lesion consisted of necrosis of the walls of the arteries, and deposition of large quantities of fibrinoid material within the walls, in the periadventitial tissues and in the valves. Photographs to illustrate these lesions will be shown.

The administration of anticoagulant doses of heparin has been shown to prevent the occurrence of bilateral renal cortical necrosis following two intravenous injections of meningococcal toxin. In rabbits given streptococci followed by meningococcal toxin, the renal lesion was similarly prevented by heparin. However, heparin did not alter the incidence of fibrinoid necrosis of the coronary arteries.

The coronary artery lesion is considered to be due to selective preparation of this tissue for the Shwartzman reaction in the course of a systemic streptococcal infection. Data to support this concept will be presented.

*Intravenous Fructose in Diabetes Mellitus: Advantages Over Glucose in the Treatment of Diabetic Emergencies.* HENRY DOLGER, SHERMAN KUPFER, JOHN J. BOOKMAN and JULIUS CARR, New York, N. Y. (Introduced by Ephraim Shorr).

Previous workers have shown that insulin is unnecessary for the metabolism of intravenously administered fructose in the normal and diabetic subject. Fructose enters directly into the glycolytic cycle. It is available

for glycogen formation in the absence of insulin. Fructose offers a unique advantage in the diabetic by decreasing ketone production and protein catabolism without aggravating hyperglycemia or glycosuria.

Blood and urine fructose and glucose values were determined periodically during the intravenous infusion of 100 gm of either fructose or glucose in 10% solution in four hour periods. Normal and stabilized diabetic controls, pre- and post-operative diabetics and patients admitted in acute diabetic ketoacidosis were studied.

Fructose administered to normal individuals produced an average rise of 22 mg % in blood glucose. The maximum blood fructose averaged 16 mg %. Urinary losses of glucose and fructose averaged 1.7 gm and 0.9 gm respectively.

When stabilized diabetic patients were given glucose marked hyperglycemia and glycosuria, averaging 27 grams, were observed. When they received fructose there was a minimal rise in blood glucose, and glycosuria was less than half that observed during glucose administration. Blood fructose and fructosuria were similar to that obtained in non-diabetics. In the pre- and post-operative management of diabetic patients with fructose pre-existing hyperglycemia and glycosuria were not aggravated.

Fructose along with fluid and saline could surmount the objections to the initial use of glucose in the treatment of diabetic acidosis and ketosis. In mild ketosis fructose was sufficient to effect clinical improvement and abolish ketonuria. Fluid balance was maintained, hyperglycemia was not appreciably increased, and glycosuria decreased. In severe ketosis insulin was also administered. The use of fructose seemed to overcome the objections to the early use of glucose. There was neither aggravation of hyperglycemia, nor increased glycosuria, nor further dehydration, nor resistance to insulin.

*Hemodynamic Effects of Lanatoside C in Patients with No Heart Disease and in Patients with Heart Disease but Not in Heart Failure.* DAVID T. DRESDALE, ROBERT J. MICHOM and MARTIN SCHULTZ, New York, N. Y. (Introduced by William Dock).

Utilizing the technique of right heart catheterization, seven patients without heart disease and four patients with heart disease but not in heart failure were studied before and after the administration of 1.2 to 1.6 mg. of Lanatoside C through the catheter into the pulmonary artery. Six of the eleven patients had two control determinations. Cardiac output determinations and pressure measurements were made from 48 to 121 minutes after the drug was administered.

The following observations were made: 1) The systemic pressure did not change in six cases; in one case it fell slightly and in four it rose slightly. 2) In the seven cases in which the pulmonary artery pressure was measured before and after Lanatoside C, it rose slightly in one of the normal cases, and remained unchanged in the others. 3) The right ventricular end-diastolic pres-

sure fell in one normal case in which it was elevated in the control period, but remained unchanged in the others. 4) The mean right atrial pressure fell more than two mm. Hg in only two cases; in four it fell less than two mm. Hg, including the case mentioned in (3). 5) The peripheral venous pressure was unchanged in the seven cases in which it was measured. 6) The cardiac output varied from plus 13% to minus 13% of selected control values, eight cases varying within the range of plus or minus 10%. 7) Electrocardiographic changes were noted in all cases, some as early as 16 minutes after Lanatoside C. There were no significant changes in the pulse rate.

The findings are discussed with especial reference to a critique of the method and the importance of determining two control cardiac outputs to prevent ascribing changes to drug action erroneously.

*The Use of Chromium 51 as an Erythrocyte Tagging Agent for Determining the Survival of Transfused and Autotransfused Erythrocytes.* F. G. EBAUGH, JR., J. F. ROSS\* and C. P. EMERSON,\* Boston, Massachusetts.

The differential agglutination technic is the most useful method presently available for estimating red cell survival. Among its disadvantages is necessity for injecting large volumes of donor blood and limited number of donor-recipient combinations that are suitable for this type of study. In search of a more accurate method, and one permitting the use of autotransfusion in survival studies, the authors have investigated  $\text{Na}_2\text{Cr}^{51}\text{O}_4$  as an erythrocyte label. Survival of chromium tagged donor cells, previously stored from six hours to 26 days, was followed simultaneously by means of radioactivity and selective agglutination counts. "100 per cent survival value" of transfused erythrocytes was calculated from the quotient of the total radioactivity of injected erythrocytes and recipient red cell mass as determined by the T-1824 dye dilution method. Comparison of data based on simultaneous  $\text{Cr}^{51}$  and unagglutinable cell counts in individual transfusion recipients indicated that chromation *per se*, whether accomplished prior to storage or immediately before transfusion, did not affect viability of donor cells. There was a gradual elution of chromium from tagged cells which continued throughout the period of their survival at a constant exponential rate with a half life of 75 days. Chromium released from donor cells as a result of elution or hemolysis was cleared rapidly from circulating blood and excreted in urine and feces; none reappeared in the recipient's erythrocytes. Cell-bound chromium was detectable throughout the life span of transfused tagged erythrocytes and measurable with an accuracy of from one to three per cent. The results indicate that a reliable estimation of red cell survival is possible following the transfusion, or autotransfusion, of as little as 25 ml. of  $\text{Cr}^{51}$  tagged erythrocytes. Clinical application of this method as a measure of blood destruction in hemolytic disorders will be illustrated and discussed.

*Plasma Protein-lipid Relationships in Acute Hepatitis.*

HOWARD A. EDER and ELLA M. RUSS, New York, N. Y. (Introduced by David P. Barr).

When normal human plasma is fractionated by Cohn's method 10, all of the lipid is recovered in two fractions: one, IV + V + VI, contains alpha lipoprotein; and the other, I + III, contains beta lipoprotein.

Fractionation by this method of plasma from individual patients with early, active acute hepatitis has revealed that although all the lipid is present in the same two fractions, the composition of lipid in each is characteristically altered.

In hepatitis none of the cholesterol in fraction IV + V + VI is esterified, whereas in the normal approximately 77% is esterified. In fraction I + III 27% of the cholesterol is esterified in hepatitis, whereas in the normal 68% is esterified. Another striking change is in the ratio of cholesterol to phospholipid in fraction I + III. Whereas in the normal this ratio (weight) is 1.31, in hepatitis it is .53, due usually to an absolute increase in the amount of phospholipid in this fraction. In fraction IV + V + VI the cholesterol to phospholipid ratio is .49 in normals and .43 in hepatitis.

The abnormalities in lipid composition of the fractions begin to revert to normal early in recovery from hepatitis. The rates at which they disappear are similar in the two fractions.

These alterations in lipid composition occur not only in hepatitis but also in biliary cirrhosis and in other types of biliary obstruction both intra- and extra-hepatic. In diseases such as atherosclerosis, nephrosis, and idiopathic hypercholesterolemia the distribution of lipids between the two fractions is altered, but changes in per cent of esterified cholesterol or in cholesterol to phospholipid ratio described above have not been found.

The evidence suggests that in biliary obstruction there are formed characteristic lipoproteins which differ from the normal and which have not been found in other disease states.

*Nature and Localization of In Vivo Reactions between Simple Chemical Allergens and Tissue Protein.* HERMAN N. EISEN, SIDNEY BELMAN and MARY E. CARSTEN, New York, N. Y. (Introduced by Ludwig W. Eichna).

The delayed type of allergic skin response considered characteristic of "tuberculin" hypersensitivity is reproducible with many drugs which, presumably, combine *in vivo* with proteins. Despite wide acceptance of this concept, the reactions involved have remained conjectural. The experiments described below characterize *in vivo* reactions of some simple allergens and demonstrate their tissue localization.

Previously we found that when 2,4-dinitrophenyl (D) compounds, differing only in the substituents on carbon-one, were applied to skins of guinea pigs and humans previously sensitized with 2,4-dinitrofluorobenzene, four compounds produced allergic lesions (elicitors) and four did not (non-elicitors). Elicitors derivatized proteins

*in vitro* and *in vivo*; non-elicitors did not. No significant configurational differences existed between elicitors and non-elicitors.

These experiments are now extended with two more elicitors, differing again only in their carbon-one substituents. *In vivo* reactions were investigated as previously by determining amino acid residues with which the compounds combined. From skins treated *in vivo* for 20 hours with elicitors, dinitrophenyl-amino acids were isolated from epidermis, but not from dermis. Of the six elicitors, three previously studied yielded N<sup>5</sup>-D-lysine and three others, including one examined earlier, yielded S-D-cysteine, indicating reactions with  $\epsilon$ -NH<sub>2</sub> and S of protein-incorporated lysine and cysteine, or cystine, respectively.

Reactions *in vitro* with epidermis, hair, and some serum proteins were studied at several pH values spectroscopically, chromatographically, and by equilibrium dialysis. At pH 7, all elicitors duplicated with keratins, their respective *in vivo* reactions. Non-elicitors formed reversible complexes with serum albumins, but did not derivatize protein.

The results demonstrate that for allergenic activity, simple chemicals must form covalent bonds with protein, and suggest that this reaction occurs within epidermis. The latter inference was found to be valid by histologic study of skin biopsies with a staining technique developed specifically for aromatic nitro groups.

*Distribution and Decay of I<sup>125</sup> Tagged Albumin and Gamma Globulin in Patients with Cirrhosis.* WILLIAM J. EISENMENGER and ROBERT J. SLATER, New York, N. Y. (Introduction by Henry G. Kunkel).

Labelled albumin and gamma globulin were alternately administered intravenously or intraperitoneally to 25 patients with cirrhosis of the liver and 8 normal controls. The value and limitations of I<sup>125</sup> and the dye T-1824 as protein labels were studied by employing zone electrophoresis and specific immunological precipitation. T-1824 was reliable in determining rates of transfer of albumin in the rapid phase of equilibration, but gave highly variable results as an indicator of the decay rate of albumin, the half life range being 3.5-11.0 days.

Despite the difference in molecular weights, the rates of transfer of both tagged proteins across the peritoneal membrane were similar, so that transfer of albumin and gamma globulin occurred in a ratio approximating that found in plasma. The day on which equilibrium occurred in each patient was approximately the same for both proteins (2.5-6.0 days). Following intraperitoneal administration in certain patients, equilibrium did not occur for over two weeks.

In patients with cirrhosis without ascites, the half life of albumin I<sup>125</sup> was normal (9.5-11.5 days). Therefore, the plasma concentration of albumin directly parallels hepatic synthesis of albumin. Patients with ascites had subnormal rates of metabolic decay of albumin. Despite the rapid transperitoneal transfer of protein, the albumin, while contained in the peritoneal cavity, is

probably not catabolized. By correcting for the albumin in the ascites, the rate of metabolic breakdown of albumin was found to be normal.

Gamma globulin I<sup>235</sup> in normal subjects had a half life of 11.5-19 days. Patients with cirrhosis both with and without ascites had half life values from 6 to 13 days, thus indicating a more rapid metabolic decay of gamma globulin I<sup>235</sup>. Thus, hypergammaglobulinemia occurs in cirrhosis despite an increased metabolic breakdown of gamma globulin.

*The Influence of Estrogens on the Protein Bound Iodine of Serum.* WILLIAM W. ENGSTROM, BLANCH MARKARDT and ALBERT LIEBMAN, Milwaukee, Wisconsin. (Introduced by Randall G. Sprague).

It is recognized that the protein-bound iodine (PBI) of serum, which is thought to be a measure of the level of circulating thyroid hormone, increases during normal pregnancy. The cause and significance of this phenomenon are not known. Since estrogens depress the rate of respiration of tissue homogenates and since increased elaboration of estrogen is characteristic of normal pregnancy and administration of estrogens or thyroid has been advocated in threatened abortion, the effect of estrogen on the PBI was studied.

From 20 to 100 mgm. of diethylstilbesterol were administered to 14 patients and 5 mgm. of Premarin (conjugated estrogens equine) to 2 patients. The PBI rose in all instances; usually the maximum rise was reached by about the third week of administration. The resting pulse rate was unaltered and no clinical signs of hyperthyroidism appeared. No significant change in the BMR was detected in the few cases in which a satisfactory BMR could be obtained. Estrogen therapy was discontinued in 10 individuals and the PBI slowly fell toward the normal control range.

To evaluate whether the rise in PBI was brought about by increased thyroid activity, the effect of estrogen on the PBI was studied in six instances in patients with myxedema or anterior pituitary insufficiency who were on maintenance doses of exogenous desiccated thyroid. In no instance did the PBI rise significantly. In one instance of anterior pituitary insufficiency there was a slight, but transitory and probably not significant rise in PBI. The data suggest that the rise of the PBI, under the influence of estrogen, and possibly also during normal pregnancy, is due to an increase in activity of the thyroid gland. Also, the tolerance to thyroid hormone is probably increased by estrogen.

*Glucose Tolerance and Glycogen Deposition in Experimental Fatty Liver.* RICHARD H. ETTINGER, BURTON GIGES and SHELDON S. WALDSTEIN, Chicago, Ill. (Introduced by Richard B. Capps).

Patients with fatty infiltration of the liver frequently have diabetic-type glucose tolerance curves which may return to normal when the fat disappears. The common explanation for this failure of glucose utilization, that glycogen deposition in the liver is impaired, lacks ade-

quate experimental confirmation. To study the mechanism of this defect, an attempt was made to reproduce the abnormality in dogs by feeding a choline-free, low methionine diet.

The experimental animals remained in good condition during five weeks on the diet. Fatty infiltration of the liver, up to 20% of the wet weight, and typical diabetic-type glucose tolerance curves were regularly produced within this period. The liver glycogen concentrations of the animals with fatty liver averaged about 7% of the wet weight, compared with 3% in control animals. This increase was particularly significant in view of the marked decrease in total liver nitrogen which occurred with the infiltration of fat.

Immediately before and after standard two-hour intravenous glucose tolerance tests (0.5 gm. glucose/kg. body weight in the first 30 minutes), portions of the liver were removed for analysis of glycogen, fat and nitrogen. Both the test and control animals showed small decreases in glycogen and fat during this two-hour experimental interval.

The explanation that failure of glycogenesis is responsible for the diabetic-type glucose tolerance tests is not consistent with the findings of a) high glycogen content, and b) failure of even normal animals to deposit glycogen with the small amount of glucose infused. Studies of the changes in concentration of glucose, phosphate, pyruvate, lactate, alpha-ketoglutarate, and citrate in the arterial and venous blood or peripheral muscle and of liver during glucose infusion have been made to help define this metabolic defect.

*Metabolic and Hematologic Studies in Patients Receiving Chloramphenicol.* WILLIAM W. FALLOON and JOAN W. NOLL, Syracuse, N. Y. (Introduced by Charles W. Lloyd).

Untoward effects of aureomycin upon nitrogen metabolism and of chloramphenicol upon the hematopoietic system in man have been described. In view of these results nitrogen balance, stool fat and peripheral blood studies were carried out during the administration of chloramphenicol to three males, two with resolving pneumonia and one convalescent from acute alcoholism. While receiving constant diets containing 80, 77, and 58 gm. of protein per day respectively, each patient was studied during three periods: initial control (4 to 6 days), chloramphenicol (2 gm. orally, daily for 6 to 7 days) and final control (4 to 6 days). In two patients repeated eosinophil counts, liver biopsies and bone marrow aspirations were carried out.

Nitrogen balance was essentially unchanged or became slightly more positive in all patients. Positive balance was maintained in one subject and slightly negative balance in the initial control period was altered to slightly positive balance in another patient. Stool nitrogen and fat were unaltered by chloramphenicol.

Liver biopsies revealed no change during chloramphenicol and bone marrow aspirations showed only increased eosinophilia in one patient with no change in another.

Direct eosinophil counts rose from 1100 per cu. mm. before to 1800 on the 6th day of chloramphenicol and fell toward normal after it was stopped. In a second patient eosinophil counts rose during chloramphenicol and continued to rise for 6 days after it was withdrawn. No significant changes in leukocytes, hematocrit, hemoglobin or erythrocytes occurred in any of the three patients.

The results suggest that chloramphenicol does not alter nitrogen metabolism, fat absorption or hepatic histology.

*The Relative Amounts of Body Sodium and Water in Patients with Heart, Renal or Hepatic Disease.* SAUL J. FARBER\* and ROBERT J. SOBERMAN, New York, N. Y.

The purpose of this study was to characterize the sodium and water retention in heart disease as compared to fluid accumulation due to other causes. The data indicate that there is associated with heart disease a derangement causing more sodium than water retention, a derangement which may persist despite the patient having been rendered edema free with therapy.

Total 24 hour exchangeable sodium and total body water were determined by radioactive sodium and antipyrine dilution techniques in 22 subjects without and 95 subjects with heart, renal or liver disease. The sodium to water ratio was calculated by dividing total body sodium by total body water.

In 22 subjects without renal, hepatic or cardiac disease the average ratio was 81. For twenty-one patients with edema due to causes other than heart disease (renal or hepatic) the average ratio was 87 indicating equivalent sodium and water or excess water retention. In contrast, 43 patients with less or equivalent degrees of edema due to heart disease, the average ratio was 109. Moreover, in a group of 31 patients with heart disease, who were previously edematous, 17 had high ratios increasing the group average to 97.

In one-third of the patients, total body chloride was determined by bromide dilution; the chloride to water ratio was not typical for any group.

The high sodium to water ratio in edematous patients with heart disease signifies more sodium than water retention. This excessive amount of sodium persists in the majority of patients with heart disease who have been rendered edema free. This abnormal retention may be due to a metabolic defect involving either an increased cellular permeability to sodium and/or an augmented deposition of this ion in some unidentified tissue reservoir (bone, cartilage, connective tissue).

*Myocardial and Mechanical Factors in Mitral Stenosis of Rheumatic Origin.* M. IRENE FERRER,\* REJANE M. HARVEY, PHILIP SAMET and ANDRE Cournand, New York, New York.

During a study of a number of patients with rheumatic mitral stenosis who were being evaluated for commissurotomy, a group of nine was isolated and characterized by a history of cardiac insufficiency, including bouts of congestive failure, and considerable enlargement of the

heart coupled with normal or almost normal pulmonary artery pressures at rest once heart failure was relieved. In some of these individuals hemodynamic measurements (using the cardiac catheterization technique) were made during exercise and during acute digitalization. It appears that these patients are suffering from some form of myocardial insufficiency even when they are no longer in congestive heart failure, and that this factor represents their primary difficulty rather than a block at the mitral valve. Since this could not be proved on medical grounds alone, three of them had mitral commissurotomies. The symptoms and the dynamics were unchanged after surgery. This group of patients will be contrasted to a group with normal dynamics and no symptoms, as well as to a group in whom mechanical block due to mitral stenosis constituted the major disability. There is nothing in the level of cardiac output which distinguishes the group with myocardial insufficiency from the patients with mitral block alone. The importance of this group with primarily myocardial insufficiency is obvious when surgery, which apparently cannot alleviate their dysfunction, is being urged on so many patients with mitral stenosis. A number of questions raised by the isolation of this group of patients will be discussed.

*Effect of Heparin and a Synthetic Heparinoid on the Physical State of Serum Lipids.* JOHN B. FIELD, WILLARD J. ZINN, and GEORGE C. GRIFFITH, Los Angeles, Calif. (Introduced by Paul Starr).

Atherosclerosis and the serum lipoproteins may be etiologically related. The present study was designed to investigate the relationship between ultracentrifuge lipoprotein fractions, alterations in chylomicronemia produced by a fat meal, and fasting serum fat emulsification as measured by the chylomicron index (the per cent of dark-field visible fat droplets measuring 0.3 to 0.5 microns or greater) especially as they are influenced by heparin and a synthetic heparinoid, Treburon.

Fifty-three hospitalized patients with a variety of disorders were studied. First a comparison was made of the immediate response of all the Svedberg flotation fractions (12-20, 20-35, 35-100) and lipomicronemia as counted by dark-field illumination. This was done in a prolonged fasting state, 3 hours following a 20 gm. fat meal, and finally 30 minutes after the second sampling when heparin or Treburon were given parenterally. The patients alternately received the fat meal and one drug on one day, the same meal and the other drug the next day. Secondly, observations on the changes in the same serum fractions were made following repeated administration on the two anticoagulants over one week.

It was found that both heparin and Treburon significantly reduced the Svedberg flotation fractions as well as the chylomicron index following a fat meal. Whereas Sf 35-100 fractions were equally and sharply depressed by both agents, a more profound suppression of lipomicronemia and Sf 12-35 particles followed heparin than occurred with Treburon. When given repeatedly to patients from whom only fasting specimens were obtained,



Treburon significantly reduced all the Svedberg aggregates as well as the chylomicron index while heparin had little effect. It appeared that the chylomicron index most closely paralleled the alterations in the Sf 12-35 fractions. The significance of these findings will be discussed.

*Patterns of Erythropoiesis in Hemolytic Anemia.* CLEMENT A. FINCH\* and DANIEL H. COLEMAN, Seattle, Washington.

Erythropoiesis in hemolytic anemia has been evaluated by (1) the erythroidmyeloid ratio of the marrow which permits an estimation of relative fixed erythron mass; (2) "hemoglobinization curve" or rate of appearance of radioiron in the circulating mass which reflects the rate of hemoglobin synthesis and (3) morphologic studies of the circulating erythrocytes. These data have been correlated with quantitative measurements of erythrocyte turnover in normal subjects and in patients with the following hemolytic disorders: congenital spherocytic anemia, sickle cell anemia, acquired hemolytic anemia, splenic anemia and myeloid metaplasia.

Three patterns of erythropoiesis may be recognized in the peripheral blood smear. The altered erythropoiesis of each will be explained from marrow and radioiron studies.

I. *Compensated erythropoiesis* in which increased blood production is accomplished by an increased marrow mass with little or no acceleration in maturation. There is slight anemia and normal erythrocyte morphology (except for changes characteristic of the specific hemolytic disease), and the reticulocytosis is proportional to the rate of erythropoiesis.

II. *Decompensated erythropoiesis* in which marrow mass is increased, but there is also accelerated hemoglobin synthesis. Anemia is severe. Marked basophilia, siderocytes, poikilocytosis and anisocytosis are found in the peripheral blood. Reticulocytosis exceeds that expected for the rate of blood production.

III. *Dyserythropoiesis* in which accelerated hemoglobin synthesis and associated peripheral blood changes are found, but associated with low blood production rates. Here the damaged marrow shows a maximal response, which however falls far short of the production capacity of the intact marrow.

*Immunologic Mechanisms of Leukocyte Abnormalities.* STUART C. FINCH, JOSEPH F. ROSS\* and FRANKLIN G. EBAUGH, JR., Boston, Mass.

Immunologic mechanisms now are recognized as responsible for certain disorders of erythrocytes (e.g. acquired hemolytic anemia) and of blood platelets (e.g. idiopathic thrombocytopenic purpura). It is probable that in a similar fashion immunologic mechanisms are responsible for abnormalities and diseases of leukocytes. The purpose of this study was to observe the effects on human leukocytes of the action of leukocyte iso-antibody, and to compare these changes with those observed in certain human diseases.

An anti-human granulocyte serum was produced in rabbits which, upon incubation with normal human granulocytes, produced the following phenomena:

1. Extensive phagocytosis of granulocytes by neutrophilic polymorphonuclear leukocytes and monocytes. Many of the cells observed appeared identical to the L.E. cell of Hargraves. Occasionally a polymorphonuclear leukocyte was seen in the process of entering the vacuolated cytoplasm of a monocyte.

2. Clumping of neutrophilic granulocytes was demonstrated by agglutination, sedimentation and stained smear techniques. Frequently these clusters of neutrophilic granulocytes resembled closely the "rosettes" of the L.E. test.

3. Marked autolytic cellular changes consisting of cytoplasmic vacuolization occurred in neutrophilic granulocytes and monocytes, but no true *in vitro* leukolysis was demonstrated.

The factor responsible for these changes appears to be a specific leukocyte antibody which combines with or coats human granulocytes, rendering them susceptible to agglutination, phagocytosis and autolysis. The striking similarities between the action of this antibody and the serum obtained from human subjects with drug-induced leukopenias and disseminated lupus erythematosus would suggest that an autoimmune leukocyte antibody may be responsible for the leukopenia and morphologic abnormalities observed in these disorders.

*Renal Function after Recovery from Acute Renal Failure.*

JOHN T. FINKENSTAEDT, MAURICE P. O'MEARA, JOHN M. WELLER and JOHN P. MERRILL,\* Boston, Massachusetts.

The evolution of renal function following acute renal failure ("lower nephron nephrosis") has been studied in ten patients. None had a previous history of cardiovascular or renal disease. The etiologies of their acute renal failure were: transfusion reaction—4 cases, carbon tetrachloride poisoning—2 cases, premature separation of the placenta with shock—2 cases, intravascular hemolysis with septic abortion—1 case, and mercuric chloride poisoning—1 case. The average age was 33 years (22-47 years). These patients have been followed by repeated studies for as long as 4½ years following the episode of acute renal failure.

Three months after the onset of acute renal failure, the following show varying degrees of abnormality: urinalysis, modified Mosenthal concentration test, PSP test, blood urea nitrogen, and intravenous pyelogram. These return to normal between 6 and 12 months.

Clearance techniques utilizing accepted methods for inulin, PAH, and urea revealed subnormal or low normal values in all instances. After 3 months following recovery from acute renal failure, these clearance values showed no significant improvement. Persistent impairment of renal function has been found as long as 4½ years following acute renal failure.

In these 10 patients C inulin ranged from 42 to 101 ml/min.;  $C_{PAH}$ , using low plasma levels, from 226 to 606

ml/min., and C urea from 24 to 59 ml/min. Filtration fractions were between .142 and .259.

Clearance values obtained indicate persistent diminution of renal function following acute renal failure in contrast to the apparent complete clinical recovery shown by these patients.

*Cerebral Hemodynamics in Acute Induced Postural Hypotension.* FRANK A. FINNERTY, JR., LLOYD WITKIN and JOSEPH F. FAZEKAS, Washington, D. C. (Introduced by Harold Jeghers).

A recent study indicates that the production of severe hypotension using methoniums plus upright tilting has resulted in a higher than normal incidence of cerebral complications. The increasing application of this technique in anesthesia makes it important to study the cerebral hemodynamic changes in such induced hypotension.

With the patient in a 30 degree head-up tilt, cerebral blood flow and oxygen consumption, cardiac output, and electro-encephalographic and electrocardiographic tracings were determined. Hexamethonium was administered intravenously to induce hypotension to the point of early syncope or collapse.

In the 5 patients with malignant hypertension, manifestations of syncope or collapse became evident when the mean arterial pressure was reduced to 100 to 110 mm. Hg. In milder hypertensives and normotensive subjects, syncope symptoms developed at mean arterial pressure levels of 30 to 60 mm. Hg. Convulsions developed in 3 normotensive patients when the mean carotid arterial pressure fell below 32 mm. Hg.

Significant reduction of cerebral blood flow occurred in all of the 13 cases studied. In these subjects, there was a relatively critical rate of cerebral blood flow at which signs and symptoms of syncope became manifest. This level ranged between 24 and 46 cc. blood/min./100 gm. of brain with a mean of  $34.5 \pm 11.8$ . There were no significant changes in cerebral vascular resistance. Due to a marked increase in A-V oxygen difference, no significant change occurred in cerebral metabolic rate.

The electroencephalograms showed occasional spiked patterns and slowed activity. Electrocardiograms showed no change.

With the onset of collapse, cardiac outputs and right ventricular pressures decreased sharply. These latter observations are consistent with the concept that the mechanism of cerebral ischemia was due to loss of vasoconstrictor tone which in these tilted subjects promoted failure of venous return and hence of cardiac output.

*Serum Complement as an Indication of the Presence and Degree of Inflammatory Reaction in Various Diseases.* EDWARD E. FISCHEL, New York, N. Y. (Introduced by Charles Ragan).

The presence of inflammation gives rise to many changes in the plasma which are reflected in tests such as the erythrocyte sedimentation rate and determination of C-reactive protein. Complement (C'), a complex of

serum proteins, has been found to increase markedly in inflammatory conditions. The increase represents another of the biological phenomena associated with tissue damage.

Determination of C' by the spectrophotometric method for 50 per cent hemolysis in the presence of optimal [Mg<sup>++</sup>] and [Ca<sup>++</sup>] is relatively simple and may be of value for the study of the process of inflammation as well as for a screening test for the presence of many diseases of an inflammatory nature.

An increased C' was found in a variety of acute and chronic disorders including tonsillitis, pneumonia, and bacteremia. In sterile inflammations there is also a rise in C' as observed in patients with myocardial infarction, various neoplasias, thyrotoxicosis, drug allergies, and a variety of other conditions. Therefore, there is not necessarily a bacterial or allergic factor related to changes in complement, although C' is traditionally associated with such factors. In previous studies it was found that an increased C' content may be used as a sensitive indication of the presence of rheumatic activity. The increased C' in various diseases is in marked contrast to the decided diminution of C' observed in acute glomerulonephritis, lupus erythematosus disseminatus, some instances of serum sickness and terminal states. The content of C' is not apparently affected by certain virus infections.

The correlation of an increased C' content with other manifestations of inflammatory reaction such as fever, leukocytosis, increased sedimentation rate, changes in serum globulins, C-reactive protein and other substances will be discussed.

*Magnesium Deficiency in Delirium Tremens.* EDMUND B. FLINK, FRANCIS L. STUTZMAN, ARTHUR R. ANDERSON and THEODORE J. KONIG, Minneapolis, Minnesota. (Introduced by Wendell H. Hall).

Low serum magnesium levels have often been reported in a wide variety of conditions, but specific symptoms related to magnesium deficiency have not been clearly defined. Our attention was first drawn to a possible causal relationship between a low serum magnesium level and tremors, by observation of the following case:

A thirty-three year old female patient had a severe hypochloremic hypopotassemic alkalosis and hypophosphatemia as a result of prolonged parenteral fluid administration. As this condition improved, she developed fibrillary twitchings, gross muscle tremors and choreiform movements of the extremities, jaws, tongue and facial muscles. She was unable to talk or eat. At this time magnesium deficiency was recognized (serum magnesium 1.19 mEq./L.). Magnesium sulfate was given intramuscularly and the muscular twitchings promptly diminished; within twenty-four hours she was able to speak clearly and to eat. This experience induced us to study the role of magnesium in delirium tremens.

Nineteen patients with delirium tremens have been studied. Serum magnesium levels were found to be uniformly low. (Mean 1.40 mEq./L.  $\pm$  .27 compared with a normal mean of  $1.91 \pm .20$  mEq./L.) This difference is statistically significant. ( $t = 9.8$ ;  $p < .01$ .) Fifteen of

seventeen patients improved when given magnesium sulfate intramuscularly. Some improved dramatically. One patient responded on three occasions. Two of these patients died of hepatic coma shortly after admission.

The lowest serum magnesium levels were roughly correlated with the most severe symptoms. This was particularly true of tremors. Delirium occurred at times when the level was only moderately decreased.

These observations confirm and strengthen the sparse available information on the relation of magnesium to tremors in humans. It is interesting to note that such a relationship has been clearly recognized in veterinary medicine, the so-called "grass staggers" in cattle being a specific magnesium deficiency.

#### *The Cardiac and Hepatic Intracellular Fate of Digitoxin.*

MEYER FRIEDMAN\* and SHIRLEY ST. GEORGE, San Francisco, California.

The intracellular fate of digitoxin in the heart and liver of the intact animal was studied using a combination of two techniques: (1) the separation of various cell components by ultracentrifugal separation and (2) the assay of these same components for their digitoxin content by means of the micro-sensitive embryonic duck heart preparation.

It was found that nuclear, mitochondrial, microsomal and residue-soluble component could be isolated from the heart of the rat (given digitoxin, 1 mcg./gram of body weight) and assayed for its digitoxin content. The nuclear, mitochondrial and microsomal components were found to contain negligible quantities of digitoxin (0.05, 0.03, 0.10 mcg., respectively). The residue-soluble component, however, contained 1 mcg. of digitoxin or about 50 per cent of the total amount of digitoxin known to be present in the whole organ. Almost identical results were found when the intracellular components of the liver were separated and assayed for their digitoxin content.

The absence of digitoxin from the mitochondrial component strongly suggests that digitoxin does not exert its action by influencing the enzymes concerned with the glycolytic processes of Krebs's cycle which are concentrated in this cellular element. The abundance of digitoxin, however, in that component of the cell containing, among other things, actin, myosin and ATP is consonant with the concept presently held by many investigators that digitoxin may exert its effect directly upon the contractile elements of the cardiac cell.

#### *Some Effects of Alterations in Body Electrolytes on Saliva Electrolyte Composition.* THOMAS F. FRAWLEY, Albany, New York. (Introduced by R. T. Beebe).

Previous studies have shown that the salivary Na/K ratio of human mixed saliva is related inversely to adrenal cortical activity. Information has been sought relative to the degree to which the saliva composition varies with serum electrolytes and the effect of other alterations in body electrolytes on saliva.

Steroid therapy was withheld in two patients with adrenal cortical insufficiency receiving a constant salt

intake. There was a gradual reduction in serum sodium from 146 mEq/L to 127 mEq/L in one patient and from 143 mEq/L to 120 mEq/L in the other. In both patients there was a decrease in the secretion rate of saliva and a reduction in saliva sodium concentration from 39 mEq/L (normal 23 mEq/L) to 11-13 mEq/L at the lowest serum concentrations. At low levels of serum sodium (below 130 mEq/L) the saliva sodium concentration varies in the same direction as the serum. Under the conditions studied the reduction in saliva sodium could not be attributed to increased adrenal cortical activity. The concentration of saliva potassium remained quite constant throughout the study and does not vary with the rate of secretion.

The administration of sodium-ion exchange resins to patients with edema produced an initial loss of salivary sodium which decreased gradually as the serum sodium concentration was reduced below 140 mEq/L. The early development of a decrease in saliva sodium with only a slightly reduced serum level is believed to be related to increased adrenal cortical activity since there was a slight increase in salivary potassium and no change in the rate of saliva secretion. Potassium chloride 3.0 Gms daily given to normal patients caused no appreciable increase in saliva potassium concentration. The administration of mercurial diuretics in edematous patients daily for three successive days caused no increase in salivary sodium or chloride. Factors other than the adrenal steroids must be considered in evaluating changes in saliva electrolytes.

#### *A Comparative Study of the Hemodynamic Alterations Produced by Hexamethonium and l-hydrazinophthalazine (Apresoline) in Hypertensive Patients.* EDWARD D. FREIS,\* JOHN C. ROSE, FRANK A. FINNERTY, JR. and EDWARD A. PARTENOPE, Washington, D. C.

Following l-hydrazinophthalazine there was a marked increase in cardiac output (Fick) and in estimated hepatic-portal blood flow. This agent also reduces blood pressure and increases renal blood flow in hypertensive patients. Its hemodynamic effects, therefore, are similar to those which Bradley has shown to be induced by pyrogens. There were no significant changes in the plethysmographic determinations of muscle or foot blood flow.

Following hexamethonium there usually was a decrease in cardiac output in patients with compensated hearts. By contrast patients with cardiac failure exhibited an increased output. In the former the total peripheral resistance changed only slightly whereas in the latter there was a significant decrease. Right heart pressures decreased markedly in both instances. It is suggested that the beneficial effects of hexamethonium observed in cardiac decompensation are due to both an "unloading" of the congested right side of the heart and to a decreased work load on the left side secondary to the decrease in peripheral resistance.

In contrast to the marked increase in foot blood flow observed after hexamethonium there was a decrease in

renal and hepatic-portal blood flow and insignificant changes in muscle blood flow. It is suggested that these opposing hemodynamic effects of hexamethonium and Apresoline may explain our previous clinical observations of an additive effect when these agents are combined.

Finally, as judged by the observed changes in blood flow in different vascular areas it seems apparent that both agents induce a selective rather than generalized vasodilation in the various organ systems of the body.

*The Mechanics of Pulmonary Ventilation in Emphysema.*

DONALD L. FRY and RICHARD V. EBERT,\* Minneapolis, Minn.

The purpose of this study was to measure the pressure required to ventilate the lung in pulmonary emphysema. This pressure can be evaluated by simultaneous measurement of intrathoracic pressure and respiratory flow. The difference between intraesophageal pressure and intraoral pressure was used as a measure of intrathoracic pressure and the pneumotachygraph served to measure respiratory flow.

The intrathoracic pressure ( $P_T$ ) is composed of essentially two pressures. The first, ( $P_L$ ), is caused by the elastic force of the lung which varies with the degree of distention of the lung. The second, ( $P_K$ ), is caused by the force required to overcome resistance to air flow and tissue viscosity. These pressures are related as follows:

$$P_T = P_L + P_K$$

$P_K$  will be negative during inspiration and positive during expiration.  $P_L$  will always be negative.

$P_K$  was measured by suddenly closing a valve while the subject was breathing. The difference in intraesophageal pressure before and after closure of the valve represents  $P_K$ . Curves were then constructed relating  $P_K$  to respiratory flow.

The pressure,  $P_K$ , required to produce a given respiratory flow was increased in subjects with pulmonary emphysema as compared with normal subjects. Studies comparing the effect of breathing air, argon, neon and helium suggested that the increased  $P_K$  in emphysema was required to overcome resistance caused by turbulent gas flow rather than tissue viscosity.

The pressure flow curve in emphysema during expiration is asymptomatic. Beyond a certain point, further increases in pressure produce no further increase in flow. The maximum flow achieved is related to the degree of distention of the lung. This is because collapse of the small bronchioles occurs when the pressure gradient between the alveoli and small bronchioles exceeds the elastic force of the lung and the elastic force of the bronchiolar walls.

*Metabolic Effects of Antibiotics Administered to Undernourished Men.* GEORGE J. GABUZDA, JR., THOMAS M. GOCKE and BEN D. LOVE, JR., Boston, Mass. (Introduced by Maxwell Finland).

It was previously reported that undernourished men given aureomycin (2.5 gm. orally daily for 9 days)

demonstrated losses of body weight and increased excretion of urinary nitrogen and riboflavin. Further studies in two patients given terramycin in comparable doses yielded qualitatively similar results. No such changes were noted in two subjects given bacitracin and polymyxin (150,000 units, and 500 mg. daily, respectively) orally, although these essentially non-absorbable agents produced profound alterations in the fecal flora.

One patient given "alkaline rearranged" (microbiologically inactive) aureomycin orally showed neither the metabolic changes nor any alteration in the fecal flora until active aureomycin was subsequently given; he then showed increases in urinary nitrogen excretion and in serum nonprotein nitrogen concentration which did not revert to control levels following cessation of aureomycin but did when riboflavin was supplemented orally. Likewise, this patient's rate of gain in body weight was less than that noted during the initial control period until after riboflavin was given. However, in subsequent periods the administration of 50 mg. of riboflavin orally daily before and during aureomycin administration did not prevent the metabolic effects of the latter.

The fecal flora changed in all patients given active antibiotics, but the alterations differed in each patient. Bacitracin and polymyxin produced the most striking reductions in bacterial flora. One patient given 150 mg. aureomycin orally daily for 15 days demonstrated alterations in the fecal flora comparable to those previously noted when he received 2.5 gm. orally daily for 9 days, yet significant metabolic alterations did not occur with the smaller dose. There was no discernible qualitative or quantitative correlation between the changes in fecal flora and the metabolic effects of the antibiotics studied.

*The Excretion of Solutes by Patients with Edema.* A. V. N. GOODYER and D. W. SELDIN,\* New Haven, Conn.

In patients with edema due to congestive heart failure or to hepatic cirrhosis, the administration of hypertonic glucose or saline fails to elicit the natriuresis which occurs in the normal subject. In the present study other solutes were tested with respect to their diuretic potency in edematous patients, in a further effort to analyse the retention of sodium which is characteristic of the state of edema.

Infusions of urea, mannitol, sodium bicarbonate and sodium sulfate were administered to edematous patients in sixteen experiments. Changes in the concentrations of the loading anion and of pertinent electrolytes in the serum and urine were compared with data obtained in similar experiments on normal subjects or on patients free of edema.

The results with urea and mannitol were similar to those which have been reported for glucose. Sodium bicarbonate was well excreted by some patients, but very poorly by others who had particularly low initial rates of excretion of sodium, and subnormal values for the clearance of inulin. In these latter patients, bicarbonate was retained despite an increased concentration of the

serum bicarbonate to as much as 35 meq./l. Sodium sulfate was well excreted in spite of low rates of glomerular filtration and initial sodium excretion in some patients. The excretion of potassium was much increased and the excretion of ammonia only minimally affected by the loads of sodium sulfate and sodium bicarbonate in both the normal subjects and in most of the patients.

These experiments reemphasize the priority of internal regulatory factors over intrarenal osmotic effects of administered solutes on the excretion of sodium. The results with sodium sulfate and bicarbonate indicate that the retention of administered sodium by patients with edema is strongly conditioned by the anion with which it is administered.

*The Relationship Between Gastric Acidity and the Absorption of Iron from the Upper Gastrointestinal Tract.*

WILLIAM J. GRACE and RONALD K. DOIG, New York, N. Y. (Introduced by Paul Reznikoff).

The absorption of iron was studied by the iron tolerance curve in a fistulous subject in whom simultaneous measurements of gastric acidity could be made at half hourly intervals following the administration of a test dose of ferrous or ferric iron. The results of 27 experiments show that, although there was considerable variation in the absorption of iron from day to day in this subject, the absorption bore no direct relationship to the level of gastric acidity. The absorption was either great or small at high levels of acidity (pH 1-2) or at low levels of acidity (pH 5-7).

Ten iron absorption studies were performed on 9 patients who exhibited histamine refractory achlorhydria. The results indicate that the absorption of iron is as great as in those with adequate free hydrochloric acid. Seven "normal" persons were studied and later restudied after taking large doses of sodium bicarbonate with the test dose of iron. The absorption of iron in each of these individuals was the same, whether or not sodium bicarbonate was taken.

The results of these studies indicate that iron absorption from the upper gastrointestinal tract is not dependent upon the presence of hydrochloric acid or the degree of gastric acidity, but is probably related to some as yet undefined function of the upper small intestine.

*The Selective Tagging of Red Blood Cells and Plasma Proteins with Radioactive Sodium Chromate and Chromic Chloride: Its Use in the Simultaneous Determination of Red Cell Mass and Plasma Volume in Man.* SEYMOUR J. GRAY\* and HEDDY FRANK, Boston, Mass.

A new biological tracer, radioactive chromium with a half-life of 26.5 days, was found to tag red blood cells and plasma proteins selectively and has been utilized to determine the circulating red cell mass and plasma volume simultaneously in man.

The anionic form, sodium chromate ( $\text{Na}_2\text{Cr}^{210}\text{O}_4$ ), labels the red cells selectively *in vitro* and *in vivo* while the

cationic hexavalent chromic chloride tags the plasma proteins without labelling the red cells.

The addition of radioactive sodium chromate to red blood cells *in vitro* (or *in vivo*) results in an instantaneous tagging of the cells. The red blood cells retain their radioactivity without significant loss to the plasma for periods of one day or longer when reinjected into humans or animals.

When radioactive chromic chloride is injected intravenously, 98% of the radioactive chromium is bound immediately and firmly to the plasma proteins. No significant counts are taken up by the red cells. All the plasma proteins appear to be tagged uniformly.

This selective tagging of the erythrocytes and plasma proteins by two forms of the same isotope was utilized in the simultaneous measurement of the circulating red cell mass and plasma volume in humans.

The plasma volume and red cell mass were determined simultaneously in humans by reinjecting an aliquot of the patient's red cells, which had been tagged previously with sodium chromate, and injecting radioactive chromic chloride intravenously at the same time.

The accuracy of the method has been confirmed by the withdrawal or transfusion of known volumes of red cells and plasma. The red cell mass and plasma volume may be measured by this technique with an error approximating 3%.

*The Effects of Platelet and Thrombin Concentration on Fibrin Strand Width.* RICHARD W. GREEN, Syracuse, New York. (Introduced by Eugene L. Lozner).

The relationship of the average radius of fibrin strands to changes in platelet and thrombin concentration was measured using the light scattering technique. Washed human platelets were added to bovine fraction I solutions at pH 7.2, ionic strength of 0.15, and measurements taken one hour after adding thrombin to a final concentration of 0.3 units per cc. The average fibrin strand radius increased with increasing platelet concentrations up to approximately 250,000 platelets per cu. mm. at which point further increases in platelet concentration had little effect on the radius. This effect on fibrin strand width was not seen until the platelet concentration was greater than approximately 11,000 per cu. mm. Retracted clots had the same fibrin strand width as unretracted clots.

The radius of the fibrin strand varied inversely to the thrombin concentration when the latter was between 0.5 and 0.02 units per cc. Thus, at the lowest thrombin concentrations the widest fibrin strands were observed.

A hypothesis is presented on the basis of these observations concerning polymerization of fibrinogen and the structure of the clot during the early stages of retraction.

*Spirography in the Evaluation of Pulmonary Function Prior to Surgery.* DAVID G. GREENE, Buffalo, New York. (Introduced by John H. Talbott).

A good spirographic tracing with measurement of the vital capacity and maximal breathing capacity has been in our experience the single most useful laboratory test

of pulmonary function in evaluating patients for surgery. The refinement of criteria for operation is a long process, because so much of the accumulated experience is of negative value only. The good-risk patient usually has nothing to suggest pulmonary dysfunction clinically, so that the finding of good function by spiographic methods and a subsequent successful pulmonary resection add little to the critical evaluation of the method. Similarly, the poor-risk patient usually has many clinical danger signals. When these are confirmed by the spiogram, which shows a poor performance, and surgery is not elected, mistakes in the direction of over-caution are not discovered. It is only when a patient's function proves inadequate at the operating table or when a patient with poor functional performance surprises by doing well, that significant information accrues. This report is concerned with such instances gleaned from an experience with spiograms in over 300 patients, over 100 of whom were studied with the question of surgery specifically in mind. One patient with excellent maximal breathing capacity succumbed of right heart failure within 24 hours of left pneumonectomy. Two patients with poor function clinically and by spiogram died in the post-operative period, following thoracic surgery. Four patients with clinical and spiographic evidence of severely limited pulmonary function did well following extra-thoracic surgery. The addition of further studies, such as arterial blood gases, may help to forestall accidents such as the first case. The spiogram was a good guide, unfortunately disregarded, in the second and third cases. In cases of extra-thoracic surgery poor pulmonary function may indicate caution, but does not absolutely contraindicate surgery.

*Prevention of Cardiac Abnormalities in Acute Rheumatic Carditis.* LAWRENCE GREENMAN, F. A. WEIGAND and T. S. DANOWSKI,\* Pittsburgh, Pa.

Forty-four children hospitalized during an initial attack of rheumatic carditis received oral cortisone in divided doses for eight weeks; 300 mgm per day for six weeks and then gradually decreasing amounts until therapy was discontinued two weeks later. Treatment was further prolonged if rheumatic activity persisted. It must be emphasized that the regimen included rigid sodium restriction, a high potassium intake, bed rest, and penicillin. Post-treatment observation periods extend from three to thirty-five months.

Of the ten patients treated within two weeks of onset, nine now have apparently normal hearts, insofar as can be determined by all available criteria, despite the fact that all ten had apical systolic murmurs of grade II intensity, or greater, one had a presystolic murmur, five had cardiac enlargement, three were in congestive failure, and two others had hepatomegaly.

Twenty patients were treated two to six weeks after onset. Again all had apical systolic murmurs; three had apical diastolic and three aortic diastolic bruits. Cardiac enlargement was present in seven, pericarditis in one and four were in congestive failure. Fourteen of these now have apparently normal hearts.

Therapy in the remaining fourteen was started six or more weeks after onset. At present cardiac findings are within normal limits in only one. The group consisted as a whole of more seriously ill patients since, in addition to the uniformly present systolic murmurs, three had apical diastolic and two had aortic diastolic bruits; the heart was enlarged in twelve, six were in failure and three others had hepatomegaly.

Under the regimen employed, which included cortisone, the incidence of persistent cardiac abnormalities was strikingly low following initial attacks if therapy was begun within six weeks, and especially within two weeks of onset.

*Cardiovascular and Renal Effects of Hexamethonium and 1-Hydrazinophthalazine in Hypertensive Patients.* DAVID GROB\* and VICTOR A. MCKUSICK, Baltimore, Md.

Intravenous administration of hexamethonium to hypertensive patients in doses which lowered the blood pressure to normotensive or near normotensive levels resulted in reduction in stroke volume, cardiac output and left ventricular work, and either no change or an increase in peripheral resistance. Estimated coronary blood flow was unchanged or slightly diminished, and coronary resistance was decreased. Renal blood flow, glomerular filtration rate and potassium clearance were moderately reduced, while sodium clearance and urine flow fell more markedly. Renal blood flow returned to the original level in approximately 90 minutes, even though the blood pressure increased but slightly during this time. Filtration rate and potassium clearance returned slightly more slowly than renal blood flow, while sodium clearance and urine flow returned much more slowly. The mechanism of this antidiuresis and antinatriuresis, which was accompanied by an increase in plasma and extracellular fluid volume, was investigated by transfusion studies. The effect of prolonged oral administration of hexamethonium and 1-hydrazinophthalazine on renal blood flow and function and on plasma and extracellular fluid volume was also investigated.

During postural hypotension induced by quiet sitting or standing following intravenous or oral hexamethonium there was further reduction in cardiac output. The reduction in renal blood flow, filtration rate and potassium clearance that occurred was only slightly greater than the alterations that followed quiet sitting or standing alone, while the reduction in sodium clearance and urine flow was considerably greater.

Observations on the mechanism of tolerance to these drugs included determination of the effect of their prolonged oral administration on peripheral vascular reactivity to adrenaline and nor-adrenaline.

*The Failure of Antistreptolysin to Protect Against Scarlet Fever or Streptococcal Pharyngitis.* MORTON HAMBURGER\* and HENRY M. LEMON, Cincinnati, Ohio.

During the Spring of 1945 an epidemic of Type 19 streptococcal infections swept through an ASTP unit

housed in a large field house at the University of Utah. On April 19, shortly after the epidemic had reached its peak, nose and throat cultures were made on each of the 203 students, and blood drawn for antistreptolysin determinations. During the next 5 weeks a small secondary wave of Type 17 infections occurred. Among those attacked were 5 men who had recovered from Type 19 infections and who, prior to the Type 17 infection, exhibited antistreptolysin titres of 166, 166, 250, 500 and 615 respectively; one man infected with Type 3 who had acquired an antistreptolysin titre of 2500, and 2 men with negative nose and throat cultures whose antistreptolysin titres were 333 and 625 respectively. Six of the 8 Type 17 attacks consisted of sore throat and fever and required hospitalization. Two men acquired strongly positive cultures but were not hospitalized and did not report to sick call; no record of their symptoms was available. It was of particular interest that two of the Type 17 cases developed the typical rash of scarlet fever even though the Type 19 strain, which had given rise to scarlet fever in other students, did not produce rash in these two men when it caused their sore throats.

These observations lend support to the widely accepted belief that immunity to streptococcal infections is type specific. They demonstrate that acquisition of a non-type specific antibody, namely antistreptolysin, does not confer immunity against another streptococcal infection.

*The Effect of Acute Hemorrhage on the Hepatic Blood Flow and Splanchnic Oxygen Consumption of the Dog.*

LADD W. HAMRICK, JR. and J. D. MYERS,\* Durham, N. C.

Certain metabolic as well as hemodynamic alterations occurring in the shock syndrome have been attributed to deficient hepatic blood and oxygen supplies. Therefore, hepatic blood flow (HBF) and splanchnic oxygen consumption ( $SpO_2$ ) were determined by the BSP extraction method in 13 dogs under chloralose anesthesia. Measurements were made before and 30 to 60 minutes following a single massive hemorrhage equivalent to 33 to 63% of the estimated total blood volume.

Mean arterial blood pressure fell from a control average of 147 mm. Hg to 67 mm. Hg post-hemorrhage. Although BSP excretion rate following hemorrhage remained essentially unchanged, a pronounced increase in arterial BSP concentration and arterial-hepatic venous BSP difference occurred. HBF fell from a control mean of 47 ml./min./Kg. to 18 ml./min./Kg. after bleeding ( $p < 0.01$ ). In contrast, the arterial-hepatic venous oxygen difference rose from a mean of 4.6 Vol. % to 11.3 Vol. % ( $p < 0.01$ ). Hence, no change in  $SpO_2$  was observed, the mean value being 1.9 ml./min./Kg. before and after hemorrhage.

To determine the limit of this ability of the splanchnic area to maintain its oxygen consumption, four dogs were studied before and during sustained hypotension with mean arterial pressure of 20 to 40 mm. Hg, produced by bleeding into an inverted bottle. In each instance there was a dramatic fall in HBF, total oxygen

consumption and  $SpO_2$ , the decrease in the latter two being proportional. A major factor limiting maintenance of  $SpO_2$  was reduction in total oxygen delivered to the splanchnic area, for often all but 1 Vol. % of the total arterial oxygen content was extracted.

It is concluded that the splanchnic area, including the liver, has a remarkable ability to maintain its oxygen consumption when HBF is reduced. In fact, oxygen consumption does not fail until oxygen delivery and removal approach one another.

*The Half-Life of Normal Human Gamma Globulin in Patients with Hepatic Cirrhosis.* W. PAUL HAVENS, JR.\*, JAMES DICKENSHEETS, JAMES N. BIERLY and THEODORE P. EBERHARD, Philadelphia, Pennsylvania.

Previous studies of the capacity of patients with chronic hepatic disease to make antibody revealed unusually large amounts of circulating antitoxin following the injection of purified diphtheria toxoid in Schick-negative patients with hepatic cirrhosis. It was not certain whether this represented an increased potential for the production of antibody or a diminished capacity to metabolize it.

As a step in the elucidation of this question, normal human gamma globulin labeled with  $I^{131}$  was injected intravenously into six patients with hepatic cirrhosis and nine controls (normal subjects and patients convalescent from other diseases). In three experiments, the half-life of injected gamma globulin measured from 5.2 to 10.8 days (av. 7 days) in the patients with hepatic cirrhosis, and from 10.6 to 16.6 days (av. 13.5 days) in the controls. The rates of disappearance of radioactivity from the blood were rapid and similar in both groups during the first 24 hours; 16-25% disappeared during the first 4 hours after injection, and 25-50% had disappeared at the end of 24 hours. Quantitative evaluation of the radioactivity in the urine was made in three patients (two controls and one cirrhotic) during the first 4 days after injection, and 40-60% of the amount administered was found.

*The Major Pathways of Steroid Metabolism in Man.*

LEON HELLMAN, ROBERT ROSENFELD, DAVID FUKUSHIMA, MAXWELL EDINOFF, ALEXANDER BRUNSCHWIG and THOMAS GALLAGHER, New York, New York. (Introduced by David Karnofsky).

Cholesterol, coprosterol, the bile acids, and the steroid hormones are the principal cellular constituents characterized by a similar steroid framework. In order to define biochemical interrelationships in synthesis and degradation of these compounds, radioactive hydrogen ( $H^3$ ) labeled acetate, deuterium ( $H^2$ ) tagged testosterone, and cholesterol containing radiocarbon ( $C^{14}$ ) in the ring were simultaneously administered to a patient with a complete bile fistula, in good clinical condition.

This procedure permitted study of the endogenous synthesis of the steroid nucleus and its derivatives from 2-carbon fragments ( $H^3$ -acetate), the conversion of exogenous dietary sterol ( $4-C^{14}$ -cholesterol) to the other

steroid compounds, and comparison of the fate of a preformed steroid hormone ( $H^2$ -testosterone) with the metabolites of that hormone derived in vivo from  $H^2$ -acetate and  $C^{14}$ -cholesterol.

The following metabolic interrelationships have been demonstrated:

1. The steroid hormones, as characterized by metabolites isolated from urine, are derived from dietary cholesterol and from acetate. An enterohepatic circulation for steroid hormone metabolites is not an obligatory step preceding urinary excretion.

2. Biliary cholesterol is derived from both acetate and dietary cholesterol.

3. Biliary cholic acid is derived from dietary cholesterol and from acetate. Serially identical specific activities indicate that cholic acid, biliary cholesterol, and plasma cholesterol are derived from a common precursor.

4. Plasma free and ester cholesterol originate from both dietary cholesterol and acetate. Cholesterol in the red cell stroma is in equilibrium with plasma free cholesterol.

5. Failure to detect radioactivity in the respiratory  $CO_2$  indicates that rupture of the steroid nucleus to small molecules is not an important metabolic pathway.

These results constitute a firm experimental basis for the view that the steroids of biological interest have a multiple origin and converge during their metabolic transformations. The pathways examined account for the major portion of the metabolism of the steroid nucleus.

#### *The Effect of Intravenously Administered Human Serum Albumin on Calcium Metabolism in Osteoporosis.*

PHILIP H. HENNEMAN, FREDERIC C. BARTTER, ANNE P. FORBES and ELEANOR F. DEMPSEY, Boston, Mass. (Introduced by Fuller Albright).

Five studies are presented in which 50 grams of human serum albumin were administered daily for 9 to 30 days to four patients with osteoporosis under complete metabolic balance conditions. The albumin produced a rise in serum albumin and a fall in urinary calcium excretion virtually to zero when albumin administration was continued for 20 days or more. Fecal calcium excretion was unaltered. The limit to albumin-induced calcium retention, therefore, appeared to be the height of the urinary calcium excretion during the control period. In one patient exactly the same amount of albumin was given by mouth as had been given by vein; this was followed by no rise in serum albumin and no fall in urinary calcium.

The albumin-induced calcium retention is explained in small part only by the increased calcium content of plasma and extracellular fluid. It is believed that a major portion of the calcium retained represents new bone formation. This premise rests upon the fact that 99% of body calcium occurs in bone, the observation of concomitant phosphorus retention, the step-like fall in urinary calcium during albumin administration, and the similar gradual rise in urinary calcium to levels not exceeding those of

the control period when albumin was stopped. Maximum depressions of urinary calcium excretion ranging from 41 to 199 mg per day were noted in these five studies, values comparable to the calcium retention obtained with estrogen therapy in postmenopausal women. These considerations suggest that intravenous administration of albumin is a potent stimulant to bone formation and that serum albumin may actually be a transport form of bone matrix precursor.

#### *The Cerebral Circulation and Metabolism in Hypertensive and Arteriosclerotic Cerebral Vascular Disease, with Observations on the Effects of Oxygen Inhalation.* ALBERT HEYMAN\* and JOHN L. PATTERSON, JR., Atlanta, Ga.

Cerebral blood flow (CBF) and metabolism ( $CMRO_2$ ) were measured by the nitrous oxide method in 39 patients with acute cerebral vascular accidents (CVA) or with encephalomalacia caused by recurrent episodes of CVA or progressive arteriosclerosis. The results are compared with those in 48 control subjects of varying ages. Because oxygen is commonly employed in the treatment of CVA, 17 patients were studied with 100% and 50%  $O_2$ .

A graded reduction in cerebral blood flow was found among the four groups as follows: (I) Control subjects under 45 years—52 cc./100 Gm. brain/min.; (II) control subjects over 45 years—46 cc.; (III) patients with initial episodes of CVA—40 cc.; (IV) patients with recurrent episodes of CVA—35 cc. The  $CMRO_2$  in these groups was, respectively: 3.1, 2.9, 2.7, and 2.1 cc./100 Gm. brain/min. These values reflect the relative state of the structural and functional cerebral vascular disease and the resultant damage to the brain in Groups I, II, III and IV. The much greater derangement in Group IV is clearly evident.

Repeat studies on 8 patients within four weeks after onset of acute CVA showed no improvement in mean  $CMRO_2$ . Group III patients showed CBF increases which often paralleled clinical improvement.

Inhalation of 100%  $O_2$  produced moderate reduction in CBF, whereas 50%  $O_2$  caused no significant changes. Neither concentration of  $O_2$  produced an increase in  $CMRO_2$ . One hundred per cent  $O_2$  may be contraindicated in patients with CVA in whom the cerebral circulation is critically impaired.

#### *Redistribution of Water and Electrolytes in Acute Adrenal Cortical Insufficiency in Man.* A. GORMAN HILLS, THEODORE N. CHALMERS and GEORGE D. WEBSTER, Philadelphia, Pa. (Introduced by William A. Jeffers).

The effect of acute adrenal insufficiency upon the distribution of water and electrolytes in the human body was studied in adrenal-deficient subjects by comparing simultaneous observations of water and electrolyte balance and of changes in the volumes of distribution of inulin (measured) and of chloride (calculated) during the induction of acute adrenal insufficiency. In six patients who had



previously had therapeutic subtotal adrenalectomy performed for severe hypertension, some evidence of mild to moderate adrenal insufficiency was obtained following withdrawal of replacement steroid therapy with feeding of a constant diet of rather low sodium content. All these patients showed little or no response to an 8 hour intravenous corticotrophin test.

The inulin space was measured prior to and two days following hormone withdrawal. A large decrease was observed in all six instances, averaging 2.6 liters ( $26 \pm 3.7\%$ ). During the same period a smaller but still significant calculated decrease of the chloride space occurred, averaging  $0.78 \pm .15$  liter. In no instance could the decrease of the inulin space, nor the calculated decrease of sodium and chloride in the inulin space, be attributed to net external loss; the balances accounted, on the average, for only about a quarter of the calculated water and electrolyte losses. Large internal shifts of fluid and electrolytes therefore occurred.

Two other patients who, following subtotal adrenalectomy, showed a striking response to corticotrophin, and who on the standard low-salt, no hormone regimen developed no evidence of acute adrenal insufficiency, exhibited no significant changes in the inulin space. No change in the inulin space was observed in a preoperative hypertensive individual following two days of drastic dietary salt restriction.

The interpretation of the data will be discussed.

*Metabolic Aspects of Rheumatoid Arthritis as Influenced by Nitrogen Mustard.* ROBERT E. HODGES, WILLIAM D. PAUL, JOSEPH I. ROUTH, KATE DAUM and JAMES T. BRADBURY, Iowa City, Iowa. (Introduced by Elmer L. DeGowin).

In a preliminary report we have described the ameliorating effects of nitrogen mustard on the process of rheumatoid arthritis. Ten patients were observed over an eight-month period on the Metabolic Ward, and studies of their endocrine and metabolic functions were made.

During the four-month control period it was found that they exhibited a negative nitrogen and calcium balance, a positive phosphorus balance, low normal 17-ketosteroid excretion, an abnormal glucose tolerance curve, and an abnormal plasma electrophoretic pattern.

Nitrogen mustard therapy had the immediate effect of inducing a markedly negative nitrogen, calcium and phosphorus balance for the first week after treatment. These balances gradually returned to normal after seven days. Leukocytes fell transiently for the first ten days, but recovered thereafter. Eosinophiles did not fall. Daily determinations of 17-ketosteroid excretion failed to indicate that the adrenals had been stimulated. Protein bound iodine determinations remained normal throughout the study. Liver function as estimated by the bromsulphalein test remained normal. The slightly elevated glucose tolerance curve was unaffected by treatment, and the electrophoretic pattern changed slightly toward normal.

The rationale of nitrogen mustard therapy lies in its

ability to depress the antigen-antibody phenomena, and in the theory that this disease is somehow related to these phenomena. Our preliminary results suggest that nitrogen mustard does have a prompt effect upon the acute arthritic process and that this effect is not mediated through the pituitary-adrenal system.

*Factors Controlling Urinary Cation Excretion in Chloride Deficiency.* MALCOLM A. HOLLIDAY, Indianapolis, Ind. (Introduced by William M. Wallace).

Cellular potassium depletion in metabolic alkalosis implies a urinary excretion of endogenous potassium. Factors governing this excretion are the subject of this study.

Adult rats, adjusted to an electrolyte-free diet, were divided into five groups. Group I (control) was subjected to peritoneal lavage with Ringer's solution. Groups II-IV were subjected to lavage with isotonic sodium bicarbonate solution, and Group V to lavage with sodium nitrate solution, each producing a deficit of chloride, replaced by bicarbonate and nitrate respectively. All groups were subsequently maintained on the electrolyte-free diet for three days; daily urine collections were made; and the animals were sacrificed to obtain serum and muscle samples. Group III was given sodium bicarbonate following lavage, and Group IV was given sodium acid phosphate.

In the control group, following lavage, an increase in anion load was covered by an increase in both ammonia and hydrogen excretion, and conservation of body potassium and sodium was continued.

In Group II, depletion of chloride was followed by an increase in anion excretion, accompanied by an increase in hydrogen, but a sharp reduction in ammonia excretion. The remaining urinary cation was supplied principally by endogenous potassium.

Groups III and IV, given sodium bicarbonate and phosphate respectively, sustained a greater loss of potassium than Group II, indicating that a sodium load, irrespective of urine pH, enhanced potassium excretion. Group IV showed a disassociation of hydrogen and ammonia excretion similar to Group II.

Group V also sustained a loss of body potassium but urinary excretion of ammonia and hydrogen increased.

The uniform response to chloride depletion was the excretion of endogenous potassium. Bicarbonate replacement of chloride regularly reduced ammonia excretion while nitrate replacement increased it.

The serum and muscle changes characteristic of potassium deficiency varied in proportion to the potassium lost and were independent of other variables.

*Clinical Experience with a New Method for Estimating Serum Acid Phosphatase of Prostatic Origin.* F. HOMBURGER and W. H. FISHMAN, Boston, Massachusetts. (Introduced by J. H. Pratt).

The inhibition of prostatic acid phosphatase by 1-tartrate, as described in tissue studies by Abdul Fadl and King and demonstrated cytochemically by Seligman, has been applied to the measurement of serum acid phos-

phatase (Fishman and Lerner, *J. Biol. Chem.* 200:89, 1953).

The range of "prostatic" serum acid phosphatase in 155 determinations done in aged men without demonstrable carcinoma of the prostate was 0 to 0.5 unit, with the majority of the values falling between 0.05 and 0.25 unit. In 66 patients with various non-prostatic diseases there were only three with "prostatic" acid phosphatase values from 0.53 to 0.7 unit in cases with ulcerative colitis, cirrhosis and bowel obstruction.

The only other conditions causing elevated serum levels of prostatic acid phosphatase were prostatic massage, the administration of testosterone and cancer of the prostate. Chronic prostatitis and benign prostatic hypertrophy (71 patients) did not cause an increase in prostatic serum acid phosphatase.

In 21 cases of proven prostatic cancer (3 of them without demonstrable metastases) 4 showed normal King Armstrong serum acid phosphatase values, with 3 of these having definitely increased prostatic "serum phosphatase."

In more than 100 instances of high serum acid phosphatase (King Armstrong) the enzyme consisted largely of "prostatic" acid phosphatase.

Studies were made in selected patients during 12 to 24 months to investigate prostatic activity induced by sex hormone administration or due to the presence of cancer. Detailed clinical observations have been made on the effects of orchietomy and bilateral adrenalectomy upon prostatic serum acid phosphatase.

From these studies it may be concluded that the "prostatic" serum acid phosphatase reflects more accurately than the conventional methods fluctuation of prostatic activity.

*Viremia in Human Poliomyelitis.* DOROTHY M. HORSTMANN\* and ROBERT W. MCCOLLUM, New Haven, Conn.

One year ago, experiments were reported indicating that viremia occurs during the incubation period in *experimental* poliomyelitis with some regularity. On the basis of these findings, it was decided to reinvestigate the occurrence of viremia in *human* poliomyelitis, concentrating efforts not on the clinical case, but on contacts who might have the minor illness or might be in the incubation period. Specimens were therefore collected during an epidemic in Ohio in June and July of 1952 from 120 contacts of cases. Blood, rectal swabs, and throat swabs, were obtained. These have all been tested for the presence of poliomyelitis virus using tissue culture methods, and in some instances, monkey (*M. mulatta*) inoculation. Fifteen individuals out of 120 were found to be infected with Type 1 virus as evidenced by the detection of virus in rectal or throat swabs, or both. Of these, 3 were found to have viremia, while 6 already had antibodies to the infecting strain of virus, and 6 blood specimens were negative. The three positive results were obtained with blood from 3 children, aged 10,

4, and 3, in one family, all of whom had the typical findings of a "minor illness," and all of whom had positive throat swabs and rectal swabs. All three children were entirely well within 24-48 hours. Virus was not found in any of 105 blood specimens from contacts whose rectal and throat swabs were negative. The results indicate that viremia may occur in human poliomyelitis in association with the minor illness. Its significance in terms of the pathogenesis has not yet been clearly delineated, but the presence of virus in the blood early in the course of infection supports earlier hypotheses of rapid primary multiplication outside the CNS whether it be in neural or non-neural tissues.

*Studies in Survival and Separation of Transfused Red Cells by Differential Agglutination Compared to a Method of Differential Hemolysis.* THOMAS H. HURLEY and RUSSELL WEISMAN, JR., Cleveland, Ohio. (Introduced by Thomas Hale Ham).

The survival of group O erythrocytes transfused into three normal subjects and into three patients with anemia was studied in parallel by two immunohematologic methods, namely, the Ashby technique of differential agglutination which was compared with a newly devised method of differential hemolysis, employing Anti A or Anti B isohemolysins. Recipients were of blood group A, B or AB.

Commercially produced, dried, immune rabbit sera were used in a standard manner in the agglutination method. Differential hemolysis was produced by potent isohemolysins in immune human sera which were stored in the frozen state in sealed tubes, each containing sufficient serum for one determination. Freshly drawn venous blood, containing no anticoagulant, was diluted immediately to 1/51 with physiological saline. Exactly equal volumes (approximately 0.1 ml) of the red-cell suspension and of hemolytic serum containing complement were mixed. For sera which required complement, one part of fresh serum from the recipient was added. Hemolysis of the recipient's red cells in the mixture occurred on incubation for 15 to 90 minutes at 37° C with "blank" counts of unhemolyzed recipient's cells of less than 30,000 red cells/mm<sup>3</sup> in each subject. There was no significant variation in these blank counts using sera which were frozen for three months.

There was close agreement of the results of the two immunohematologic methods. Differential hemolysis facilitated the counting of the unhemolyzed donor's cells by eliminating the large clumps of agglutinated recipient cells which are a source of confusion and of possible error in counting unagglutinated cells in the Ashby method.

It is anticipated that the method of differential hemolysis will be of value in the separation and study of detailed changes in donor's red cells following transfusion. For example, it was shown that donor red cells did not acquire siderotic granules when transfused into a splenectomized patient who had numerous siderocytes.

*A New Method for the Study of Thyroidal Function in Man, Using Radioactive Iodine.* SIDNEY H. INGBAR, Washington, D. C. (Introduced by Edward H. Kass).

The rates of organic binding of iodide and of synthesis of thyroid hormone have not previously been measured in man. A new method of study makes possible in man the simultaneous measurement of the thyroidal iodide space (total thyroidal iodide/plasma iodide concentration) and the fractional rate of binding of thyroidal iodide to protein. The thyroidal rate of clearance of plasma iodide is calculated as the product of these two measurements, and, unlike the clearances measured by previous methods, indicates the rate of transfer of plasma iodide to hormonal iodine.

In 28 euthyroid subjects, the mean thyroidal clearance rate was  $0.95 \pm .06$  liter/hr., while the thyroidal iodide space and binding-rate averaged  $1.0 \pm .05$  liters and  $90.7 \pm 3.3\%/hr.$ , respectively. In 21 patients with untreated Graves' disease, the mean thyroidal clearances were increased ( $25.3 \pm 5.7$  liters/hr.) as a result of increases in both thyroidal iodide space ( $6.1 \pm 0.3$  liters) and protein-binding rate ( $392 \pm 53\%/hr.$ ).

In 14 patients with nontoxic goiter, the thyroidal iodide space was increased ( $1.9 \pm 0.2$  liters) and the binding-rate significantly decreased ( $60.1 \pm 7.1\%/hr.$ ). Clearance rates were normal ( $1.2 \pm 0.2$  liters/hr.). In these patients, thyroidal enlargement and the consequent increase in thyroidal iodide space may result from and compensate for a diminished ability to convert iodide to hormonal iodine.

This method permits quantitative assay of antithyroid agents in man. The proportional reduction of the rate of protein-binding of thyroidal iodide in response to a single dose of propylthiouracil was no greater in hyperthyroid than in normal subjects. Thus, rather than limiting the absolute rate of protein-binding of iodide, standard doses of propylthiouracil produced a proportionate reduction in this function. Studies carried out at three dosage levels revealed that approximately 40 mg. is the average single dose required to reduce the binding-rate by 50%.

*A Study of Methods for the Prediction of Plasma Volume in Fifty Normal Subjects.* SCOTT R. INKLEY, HARVEY KRIEGER and LAURA BROOKS, Cleveland, Ohio. (Introduced by Walter Pritchard).

In order to evaluate methods for predicting plasma volume, fifty normal subjects of both sexes and varied body types were studied. Plasma volumes were determined by both T1824 and iodinated albumin methods. Total body water by antipyrine dilution and specific gravity were also determined and values for lean body mass were estimated from these figures.

This study permitted a critical comparison between two methods for determining plasma volume as well as a comparison between blood volume using plasma volume and hematocrit values and blood volume from gamma counting of whole blood. This latter method eliminated the error of hematocrit correction.

Plasma volumes were compared with body weight, height, surface area, total body water and lean body mass.

There was good agreement between mean values for Evans blue and  $I^{125}$  plasma volumes, the values being 41.2 cc/kg for  $I^{125}$  and 40.7 cc/kg for Evans blue. Blood volume determined by counting the radioactivity of whole blood gave a value of 73.3 cc/kg while hematocrit and plasma volume gave a value of 71.4 cc/kg.

When plasma volume was plotted against body weight, height, total body water, lean body weight, and surface area, the smallest standard deviation from the line of regression was found when either weight or surface area was used. The difference between these two was slight. It would seem, then, that surface area and weight are the most practical methods for predicting plasma volume.

The standard deviation of plasma volume plotted against surface area is 264 cc. This figure reflects the wide variability of plasma volumes in normal subjects and emphasizes the difficulties encountered in predicting plasma or blood volumes in individual patients.

*Metabolic Studies in Diabetes Mellitus. Blood Sugar Level and Urinary Excretion of Sugar and Nitrogen in Relation to Exogenous Insulin in Patients with Unstable and Stable Diabetes.* JOSEPH L. IZZO, JOANNE HOFFMASTER and ALFREDA M. GABIGA, Rochester, New York. (Introduced by William S. McCann).

Three patients with unstable and three with stable diabetes were studied on the metabolic division for periods of four to six weeks. The two groups were similar in respect to age and insulin requirements (30-60 units daily). Diet was constant for each patient. Insulin was administered as a single daily dose of NPH. Fasting blood sugar and 24-hour urinary excretion of sugar and nitrogen were determined each day. Nitrogen in diet and feces was also determined. Despite constancy of diet, insulin and environment, the unstable group displayed wide unpredictable swings in blood and/or urinary sugar. There was a strong tendency for positive nitrogen balance to be associated with low sugar levels and vice versa. Reduction in insulin dose to 55-60 per cent of optimal resulted in progressive rises in sugar levels, negative nitrogen balance and ketosis. Correlation coefficients between a) blood and urinary sugar, b) blood sugar and urinary nitrogen, c) urinary sugar and nitrogen were: a) .778, .584, .612; b) .605, .428, .637; c) .586, .752, .921 in the three patients respectively. Each value is based on at least 24 observations and is significant at the 1% level. In striking contrast the blood and urinary sugar could be maintained constant at almost any desired level in the stable group by adjusting the insulin dose. Discontinuing insulin for periods up to two weeks resulted in a delayed, gradual, leveling-off rise in blood sugar. Glycosuria was absent in one patient, moderate and constant in the other two. Negative nitrogen balance and ketosis were absent. A significant correlation between sugar and nitrogen was seen in one patient, only on stopping insulin. The nitrogen balance shifted from slightly positive to equilibrium. Correlation coefficients were: a) .036, .877, .779; b) .080, .262,

.492; c) .100, .207, .507 in the three patients respectively. Some theoretic and practical implications of the findings are discussed.

*The Effect of Cortisone upon the Urinary 17-Ketosteroid Excretion in Various Adrenal States.* JOSEPH W. JAILER,\* JAY J. GOLD and SEYMOUR LIEBERMAN, New York, N. Y.

The administration of cortisone to 12 patients with Addison's disease resulted in an increased urinary excretion of the total neutral 17-ketosteroids. The steroids are separated by column chromatography and identified by infra-red spectrophotometry. There is an approximate 4 per cent recovery of the administered steroid as measured as 17-ketosteroids. This is due primarily to an increase in the 11-keto-etiocholanolone fraction, which is a degradation product of cortisone.

When adequate amounts of cortisone are given to patients with virilism due to adrenal hyperplasia, a fall in the 17-ketosteroids results. This is due to the disappearance of dehydroisoandrosterone, androsterone, and etiocholanolone. However, there is a concomitant rise in the 11-keto-etiocholanolone fraction. On the other hand, in four patients with virilism due to an adrenal tumor, cortisone had but little effect upon the value of the 17-ketosteroids and steroidal pattern.

Five patients with Cushing's syndrome due to bilateral adrenal hyperplasia responded with an approximate 30 to 50 per cent fall in the 17-ketosteroids after cortisone had been administered for four to five days. However, in one patient with Cushing's syndrome due to an adrenal carcinoma and with metastases, a rise in 17-ketosteroids resulted when 100 mg. per day of cortisone was administered intramuscularly.

These results would indicate that in those patients with adrenal hyperplasia with both the virilizing and Cushing's syndromes, the administration of cortisone results in a suppression of the endogenous adrenal cortical secretion. Whereas, in the five patients with adrenal tumors, giving rise to both syndromes, cortisone had no effect upon the secretions of the tumor.

*Metabolic Fate of N<sup>15</sup>-Labelled Erythrocytes Transfused into Normal Man.* G. WATSON JAMES, III, LYNN D. ABBOT, JR., BO NORBERG, STEFFEN BIRKELAND and EVERETT I. EVANS, Richmond, Virginia. (Introduced by Robert E. Johnson).

Erythrocytes were labelled with heavy nitrogen (N<sup>15</sup>) in the heme of hemoglobin by oral feeding of isotopically tagged glycine to a normal adult man (Type 0). After a maximum constant tag was observed, approximately 500 milliliters of his washed erythrocytes were infused into a second normal adult man (Type A), who had had a 750 milliliter phlebotomy immediately before the infusion. At weekly intervals hemin was isolated from the recipient's venous blood and analyzed for N<sup>15</sup>. His feces were collected in four day periods continuously for 160 days. From each collection stercobilin was isolated and

the N<sup>15</sup> content determined. Complete hemograms, differential agglutination, blood volume and quantitative fecal urobilinogen studies were also performed.

The only source of N<sup>15</sup> in the recipient was the infused erythrocytes and the greatest tag was found in the pigment fraction excreted 120-124 days after erythrocyte labelling. Detectable amounts of N<sup>15</sup> did not appear either in the plasma proteins or urinary nitrogen during the period of breakdown of the labelled transfused cells in this study. Appearances of stercobilin N<sup>15</sup> had striking correlation with disappearance of hemin N<sup>15</sup>. The latter was not as accurate an index of red cell disintegration as was the appearance of N<sup>15</sup> in fecal stercobilin. Blood destruction immediately after the transfusion was insignificant. Beginning on the 70th day, stercobilin N<sup>15</sup> content increased steadily until the 122nd day, then decreased along a similar curve.

From the various data the average life span of the infused red cells was: hemin, 113 days; stercobilin, 115 days; differential agglutination, measuring total survival, 110 days. Average life span of the cells in the donor from stercobilin data was 119 days. Study of stercobilin tag clearly demonstrates the metabolic fate of transfused erythrocytes containing N<sup>15</sup>-labelled hemoglobin.

*The Influence of Cortisone on the Efficacy of Antibiotic Therapy of Experimental Infections.* ERNEST JAWETZ,\* San Francisco, California.

Cortisone is well known to promote the activation and spread of microbial infection in man and experimental animals. For this reason antibiotic agents are often administered to "cover" this action of cortisone when the latter drug is used clinically in the presence of infection. The question arises, however, whether the interference of cortisone with the natural defenses of the host might significantly impair the therapeutic efficacy of antimicrobial agents.

Experiments were carried out in two subacute infections of mice to investigate this possibility. This dose of cortisone employed (0.2 mg injected subcutaneously daily for 5 days) permitted normal weight gain and did not result in any mortality in uninfected animals. Twenty-four hours after the first dose of cortisone animals were infected intramuscularly with 100 LD50 of either a beta-hemolytic *Streptococcus* or a *Klebsiella*. All untreated animals succumbed in from 2 to 6 days. Treatment with either penicillin or aureomycin was administered, according to various time-dose schedules throughout the period of cortisone injections, to groups of 25 or more animals, observed for 3 weeks.

The results clearly indicated that cortisone interfered with the therapeutic effect of the antibiotics. Thus, for example, 2.4 milligrams of aureomycin per mouse, given in a period of 5 days, cured 88% of animals with a *Klebsiella* infection, while the addition of cortisone reduced the cure rate to 28 per cent ( $p = 0.0001$ ). This interfering effect of cortisone was more marked with the bacteriostatic aureomycin than with the bactericidal penicillin, and could, to some extent, be overcome by a large excess

of the antibiotics. The possible mechanisms and implications of these results will be considered.

*Intravenous Adrenal Cortical Steroids.* DALTON JENKINS, JOSE A. GARCIA-REYES and BRIAN H. MCCracken, Boston, Massachusetts. (Introduced by Clifford L. Derick).

Solutions satisfactory for continuous intravenous infusion of the following steroids have been prepared: cortisone, hydrocortisone, corticosterone, desoxycorticosterone, 11-desoxy-17-hydroxycorticosterone, tetrahydrocortisone and 17-epihydrocortisone. Quantitative dose-response and time-response studies of the metabolic effects elicited have been carried out and the therapeutic applicability of these solutions determined. At a dosage rate of 10-12 mg. per hour in normal subjects: (a) Tetrahydrocortisone and 17-epihydrocortisone are metabolically inert. (b) The influence of cortisone and hydrocortisone on electrolyte excretion equals that of corticosterone and desoxycorticosterone; renal sodium retention is maximal in eight hours. (c) Alterations in blood sugar level and urinary excretion of nitrogen, potassium and phosphorus are ordinarily widely divergent from theoretical ratios. (d) Following an initial lag period, urinary 17-hydroxycorticoid excretion correlates well with dosage rates. No deleterious effects have been observed in more than 300 continuous infusions. Intravenous cortisone or hydrocortisone constitute the therapy of choice in acute adrenal insufficiency and in other conditions requiring an immediate and sustained circulating level of adrenocortical hormone.

*The Intravenous Injection into Patients of Crystalline Pancreatic Desoxyribonuclease. Its Excretion in the Urine and Its Distribution in Certain Body Fluids.* ALAN J. JOHNSON and PAULINE R. GOGER, New York, N. Y. (Introduced by William S. Tillett).

In developing further investigations of streptococcal desoxyribonuclease (streptodornase), comparative observations have included intravenous injections of crystalline pancreatic desoxyribonuclease into patients. Desoxyribonuclease has been injected to determine: a) the amount that may be administered without apparent toxic effect, b) its urinary excretion, and c) its distribution in certain body fluids.

The intravenous dosage has been successively increased from 10 units to 1,000,000. Forty individuals have been studied, 6 receiving 500,000 units, and 10 receiving 1,000,000 units. Complete hematological studies, urine analysis, blood pressure, pulse, temperature, and symptomatology have revealed no abnormalities.

The following measurements have been made.

1. A maximum serum level of about 8-10 units/ml. was usually seen to occur immediately after the infusion of 500,000 or 1,000,000 units of desoxyribonuclease. The decrease below expected dilution levels may be due to inhibition, diffusion or metabolism of desoxyribonuclease.

2. When desoxyribonuclease was infused for 1-1½-2 hours, urinary excretion reached a maximum of about

30,000 units per hour in 3 hours, disappearing in 24. With dosage ranges of 10,000-1,000,000 units of desoxyribonuclease, 5-10% was recovered in the urine. The molecular weight of desoxyribonuclease being approximately 60,000, its presence in urine without demonstrable albuminuria or hematuria suggests special features of the molecular construction of desoxyribonuclease in relation to renal function.

3. The diffusion of desoxyribonuclease into inflammatory pleural effusions, ascites, spinal fluid, bronchial secretions and wound exudates has been observed. The amount of enzyme diffusing into these areas varied with the dosage and speed of the infusion, time of sampling, and the special conditions obtaining in each local area. When the amount of pre-injection and post-injection desoxyribonuclease were compared, the increase ranged from .1 to 1.6 units, representing a maximum of 16 fold. The levels of desoxyribonuclease achieved in spinal fluid were considerably lower, ranging from .1-4 units. The diffusion of desoxyribonuclease as described represents transfer across membranes as an expression of its special permeating characteristics in relation to the blood level.

*Studies on Congestive Failure: The Effects of Exercise, Salt Loading and of Intravenous Apresoline on Cardio-renal Hemodynamics and the Excretion of Sodium and Water.* WALTER E. JUDSON, WILLIAM HOLLANDER, IRWIN H. FRIEDMAN, J. D. HATCHER and MEYER H. HALPERIN, Boston, Massachusetts. (Introduced by Robert W. Wilkins).

Alternate straight-leg raising (supine position) caused significant decreases in renal plasma flow (RPF) and glomerular filtration rate (GFR) in all, and decreases in sodium and water excretion in half, of 20 patients in congestive failure, in contrast with no change in these functions in subjects not in failure. The degree and duration of reductions in RPF, GFR, or sodium and water excretion bore no consistent relationship to control values of, or to changes in, cardiac output, A-V oxygen difference, pulmonary arterial, "pulmonary capillary," or right ventricular end-diastolic pressures as measured during cardiac catheterization. However, the reductions were most marked in two patients who developed pulmonary edema and "pulmonary capillary" pressures above 40 mm. Hg during exercise.

In 12 failure patients without marked edema (even by history) chronic salt loading (13 gm. NaCl/day for 1 to 2 weeks) did not produce symptoms, gain in weight, or urinary sodium retention. All had diminished RPF, but, with one exception, normal GFR. On administration of acute salt loads (300 cc. of 5% NaCl) 6 of 12 showed abnormally low and delayed excretions of sodium and water, while 6, including 2 with elevated right ventricular end-diastolic pressures, excreted salt and water normally.

Intravenous Apresoline caused greater increases in GFR and RPF and smaller increases in cardiac output in 10 failure patients (with one exception) than in subjects without failure. It caused 20% to 100% increases in sodium excretion in 6 failure patients, while in 2 with

hypertension it caused 100 fold increases. Four failure patients who had abnormally low excretion of sodium under acute salt loads, had marked increases in excretion of sodium when then given Apresoline intravenously. By contrast, subjects not in failure had no increase in GFR or sodium excretion after intravenous Apresoline even when under acute salt loads.

*Distribution and Metabolism of Radioactive l-Triiodothyronine Compared with Radioactive l-Thyroxine in the Rat.* F. RAYMOND KEATING, JR.\* and A. ALBERT,\* Rochester, Minnesota.

Physiologic doses of radioactive l-triiodothyronine and radioactive l-thyroxine, prepared by exchange and purified by chromatographic separation, were injected into immature rats. Groups of 4 animals were killed at intervals for fifteen days, and radioactivity was measured in blood, various organs, residual carcass, urine and feces with a well-type gamma counter.

Both substances were distributed immediately and identically in the liver and were similarly massive in the enterohepatic circulation. The rate of gastrointestinal secretion was approximately 130 per cent per hour and the rate of fecal excretion about 3 per cent per hour for both substances. The difference between these values may indicate approximately the rate of gastrointestinal reabsorption.

Both substances rapidly disappeared from the body. After fifteen days, 1.25 per cent of triiodothyronine and 2.5 per cent of thyroxine radioactivity remained. After injection of triiodothyronine, 54.8 per cent of excreted  $I^{131}$  appeared in urine compared with 36 per cent after injection of thyroxine. A much higher rate of renal excretion for triiodothyronine (2.5 per cent per hour versus 1.4 per cent per hour), a more rapid accumulation of  $I^{131}$  in thyroid (24 per cent versus 2 per cent residual radioactivity after fifteen days) and more copious secretion of radioactive material by gastric mucosa suggest more deiodination in disposal of triiodothyronine than for thyroxine.

Following initial equilibration with the gastrointestinal tract, thyroxine was selectively retained in the liver so that after fifteen days more than 55 per cent of residual radioactivity was in liver and less than 40 per cent in carcass. Fifteen days following administration of triiodothyronine virtually all radioactivity was either in thyroid or carcass. The behavior of these substances seems compatible with the suggestion of Gross and Pitt-Rivers, that triiodothyronine, a potent calorogenic agent, is perhaps the tissue form of thyroid hormone.

*Influence of Corticosteroid Hormones on Infection in vitro.* EDWIN D. KILBOURNE and ISAO TATENO, New Orleans, Louisiana. (Introduced by George E. Burch).

The mechanisms are obscure by which cortisone influences infection with many varieties of microorganisms to the detriment of the host. A consistent observation, however, has been the presence of increased concentra-

tions of the infecting agent in the cortisone-injected host. It appeared important to establish whether increase in an infecting agent might be induced by cortisone in host tissue isolated from the influence of collateral cortisone effects on cellular infiltration, lymphoid tissue, and antibody formation. Accordingly, studies have been effected employing chick chorioallantoic membrane tissue fragments incubated in a simple glucose-salt medium (Method of Fulton and Armitage). Repeated experiments have demonstrated that the addition of small (14 mcg/cc) quantities of cortisone to tissue suspensions of this type results in significantly greater final concentrations of Influenza B virus than are observed in control cultures. Studies of viral concentrations at varying time intervals have revealed no difference in initial rates of viral increase, but rather continued increase in virus in the later time periods (36-72 hrs.) with cortisone. Similar effects have been observed with the related corticosteroid 11-dehydrocorticosterone (Compound A), and have been demonstrated with both hormones in tissue cultures of the Maitland type.

It is suggested that the sustained viral increase effected by cortisone and Compound A are related to protection of virus-synthesizing infected cells from effects of virus toxicity. Such protective action has been demonstrated in Influenza B virus infection of the chick embryo. Experiments are in progress to determine whether infections with microorganisms other than viruses may also be influenced at the tissue level by corticosteroid hormones.

*Changes of Tissue Electrolytes in Diabetic Acidosis.*

HARVEY C. KNOWLES, JR. and GEORGE M. GUEST, Cincinnati, Ohio. (Introduced by M. A. Blankenhorn).

Metabolic balance studies on diabetic subjects with ketoacidosis have yielded much indirect evidence of disturbances in cellular electrolytes. To study such derangements directly, ketoacidosis was induced in alloxan-diabetic rats. Investigations were done on three groups: a control group of normal rats, diabetic rats developing ketoacidosis shortly after receiving alloxan, and diabetic rats allowed to develop ketoacidosis after an initial period of maintenance with insulin.

In the acidotic rats conditions of hyperglycemia, ketosis, and acidosis were comparable to those of severe ketoacidosis in human subjects. Changes of water and electrolytes were similar in the tissues of both groups of acidotic rats. In relation to fat-free solids the water content decreased considerably in muscle, moderately in heart, very little in erythrocytes, and increased in liver. Chloride decreased slightly in heart, but did not change in muscle or liver. Sodium increased in the liver, decreased slightly in the heart, and remained unchanged in muscle and erythrocytes. Potassium decreased considerably in muscle, moderately in erythrocytes, and remained unchanged in heart and liver. Derived values for extra and intracellular spaces in muscle revealed no consistent change in extracellular water, a considerable decrease in intracellular water, and no change in intracellular concentrations of sodium and potassium.

These disturbances suggest that in diabetic ketoacidosis the loss of body water is predominantly intracellular. Extracellular water appears to decrease when the intracellular water falls to a critical point. The differences in changes of water content of various organs suggest that the transfer of water across cell membranes is not dependent on osmosis alone. The vital organs, liver and heart, probably do not lose potassium until the muscle potassium has decreased to a critical level.

*Electrical Energetics of the Myocardium in Man.*  
CHARLES E. KOSSMANN,\* STANLEY A. BRILLER and  
NATHAN MARCHAND, New York, N. Y.

An instrument was designed and constructed by means of which simultaneous vectorial loci in two planes at intervals of 0.0025 second could be recorded selectively during excitation or during recovery of the ventricles. The equivalent spatial vectors were calculated from the relation,  $E = \sqrt{x^2 + y^2 + z^2}$ . The reference system used was the isocles tetrahedron with the apex at the 7th dorsal spine.

The records contained the necessary quantities, voltage and time, for determining the energy involved in accordance with the equation for electrical work,  $W = E \cdot I \cdot t$ . The corporeal impedance being constant, or almost so, an expression of the relative work done in depolarization and repolarization reduces to:

$$\frac{W_1}{W_2} = \frac{E_1^2 \cdot t_1}{E_2^2 \cdot t_2}$$

As applied to the ventricular myocardium, this "D/R ratio" can also be expressed in (millivolt)<sup>2</sup> seconds as a sum,  $W_1 + W_2$ , designated as the cyclical energy consumption (CEC).

Lacking adequate integrating instrumentation the areas,  $E^2 \cdot \Delta t$ , were hand calculated in 4 patients by triangulation of each vector, regarding  $E^2$  as the altitude, and  $\Delta t$  as the base, and obtaining the total area,  $S_t$ , from the formula,

$$S_t = \sum_1^n E^2 \cdot \frac{\Delta t}{2}$$

where  $n$  is the number of triangles in the spatial loop.

In the 4 patients the D/R ratio varied from 1.07 to 24.02. The CEC varied from 15.19 to 183.03 (millivolt)<sup>2</sup> milliseconds. Three of the 4 patients had heart disease. The one patient with no clinically demonstrable disease nevertheless displayed a left bundle branch block. In this small series no correlations could be made out between the clinical features and the measurements obtained.

The significance of the research thus far lies in the methodology designed for determining the exchange of electrical energy during activity of the ventricles in man and in the correlations which may be established between this exchange and the mechanical and chemical energetics of the normal and abnormal myocardium.

*Stress and Eosinophils.* JOHN C. LAIDLAW and DALTON JENKINS, Boston, Massachusetts. (Introduced by George W. Thorn).

Eosinopenia occurring during the course of certain disease processes and exposure to stress and eosinophilia

during convalescence have long been recognized. Eosinopenia has been correlated with adrenal cortical activation. This has been demonstrated most consistently in man following the intravenous infusion of ACTH, and crystalline adrenal steroids (cortisone or hydrocortisone); reproducible dose-response curves have been established in this manner. Concomitant measurement of urinary 17-hydroxycorticoids shows: (a) an ACTH infusion rate of 2 units per hour or more produces intense adrenocortical stimulation; (b) consistent activation occurs at infusion rates as low as 0.25 unit per hour; (c) 17-hydroxycorticoid excretion consistently increases when a 50 per cent eosinopenia reflects adrenocortical stimulation (ACTH). Intravenous epinephrine produces eosinopenia without increasing 17-hydroxycorticoid excretion. Epinephrine may enhance ACTH-induced eosinopenia without affecting steroid output. Various stresses short of major trauma may also induce eosinopenia without increasing adrenocortical steroid excretion. Therefore, although eosinopenia following ACTH reliably reflects adrenocortical activation, the interpretation of eosinopenia accompanying non-specific stress must consider factors other than the adrenal cortex.

*Serum Complement Levels as a Guide for Diagnosis and Therapy of the Nephrotic Syndrome.* KURT LANGE, LAWRENCE B. SLOBODY and RUTH H. STRANG, New York, N. Y. (Introduced by Henry Lauson).

Serum complement levels are always lowered in acute and subacute glomerulonephritis and the edematous phase of the nephrotic syndrome. They are the expression of a complement binding fixed antigen-antibody reaction.

With healing of the active glomerulonephritis or prior to the disappearance of edema in the nephrotic syndrome complement levels return to normal.

They fall to subnormal levels shortly before the recurrence of the nephrotic edema.

ACTH and Cortisone depress antibody formation and clinically lead to a rise in complement shortly before diuresis. When only one course of ACTH therapy is given, clinical relapses with concomitant lowering of serum complement are common.

ACTH was therefore given to 6 patients with the nephrotic syndrome (100 mgm per day for 7 days) and followed by 6-8 weekly courses of ACTH therapy for 3 successive days (100 mgm per day). With this regime only one case has had a recurrence of edema after a severe purulent infection.

With this therapy plasma proteins rise only slowly and proteinuria and hypercholesteremia disappear gradually. When oral Cortisone (400 mgm per day) was given to 5 children and one adult for 6-10 weekly courses on 3 successive days of each week, the results appeared rapidly and prolonged hospitalization became unnecessary. Plasma proteins and cholesterol returned to normal in all cases within 4 weeks and proteinuria decreased rapidly or vanished.

During observation of 3-5 months after cessation of therapy edema recurred in one case after a highly febrile

upper respiratory infection, but spontaneous remission occurred within two weeks.

*The Effect of the Upright Position on Protein Excretion and Renal Function in Renal Disease.* WILLOUGHBY LATHEM, BETTY S. ROOF and JAMES F. NICKEL, New York City, N. Y. (Introduced by Stanley E. Bradley).

Variations in protein excretion in patients with renal disease may be ascribed to changes in the underlying disease process or to temporary functional alterations secondary to vascular adjustments. Proteinuria apparently increases on assumption of the upright position in many patients. During recovery from acute glomerulonephritis this phenomenon is particularly difficult to interpret.

Measurements of protein excretion (Kjeldahl), glomerular filtration rate (GFR-inulin clearance) and renal plasma flow (RPF-PAH clearance) have been made in patients with acute and chronic renal disease before, during and after orthostasis in order to define the mechanism of the apparent increase in proteinuria. Although the urinary protein concentration increased in the erect position, with relatively little change in plasma protein concentration (falling drop), protein excretion decreased by 46 per cent (on the average); GFR and RPF fell by 43 and 45 per cent, respectively. The urinary protein pattern (paper electrophoresis) showed no change. Glucose Tm (3 subjects) decreased in proportion to GFR. All values returned toward control levels during recovery.

These changes indicate cessation of filtration in a large number of glomeruli secondary to intrarenal vasoconstriction. Since all urinary proteins are equally affected, the glomerular (or tubular) defects which permit protein escape must be qualitatively similar. The individual variations observed in protein-inulin clearance ratios suggest that preferential elimination of more severely damaged nephrons and alterations in intraglomerular pressure may modify the change in protein output. Although proteinuria did not increase following this brief period of orthostatic vasoconstriction, more prolonged standing might lead to increased functional impairment, particularly in the more severely compromised nephrons.

*The Role of Water Intake in Producing or Abolishing Diuresis of Sodium Resulting from Pitressin Administration in Man.* ALEXANDER LEAF, FREDERIC C. BARTTER, ROBERTO F. SANTOS and OLIVER WRONG, Boston, Massachusetts. (Introduced by Walter Bauer).

Natriuresis and chloruresis have frequently been claimed and denied as effects of pitressin. Results which may reconcile these conflicting reports were obtained in studies in man.

The effects of pitressin were investigated in ten subjects including normal adults and patients with panhypopituitarism, diabetes insipidus, Addison's disease, and ovarian agenesis. Subjects received a constant diet and fluids were controlled at various levels. Pitressin Tannate in Oil, 1.0 unit every twelve hours, was administered for two to four days to insure a continuous antidiuretic ef-

fect. Measurements were made of urinary and serum electrolyte composition and total solute concentrations. In three studies renal clearances were measured.

The results clearly indicate that on a fluid intake sufficient to cause water retention during pitressin administration, there occurred a progressive and huge renal excretion of sodium and chloride. Restriction of fluid intake prevented any such loss of electrolytes during pitressin administration. The loss of electrolyte thus seems to be the result of fluid retention and expansion of body fluid compartments rather than a primary action of pitressin.

Some increase in glomerular filtration rate occurred during the water retention and natriuresis. However, the demonstration that ACTH will prevent such natriuresis indicates that tubular reabsorptive capacity for sodium had not been exceeded. The finding of huge excretions of sodium and chloride at a time when their concentrations in the serum were falling, clearly indicates that serum concentrations of sodium and chloride were no longer effective stimuli for their renal conservation. It is thought that this dumping of sodium and chloride by the kidney in the presence of obligatory retention of water is a homeostatic response to over-expansion of body fluid volume.

*Epidemiology of Penicillin and Aureomycin Resistant Staphylococci in a Hospital Community.* MARK H. LEPPER, GEORGE GEE JACKSON and MARVIN M. HIRSCH, Chicago, Ill. (Introduced by Harry F. Dowling).

A study of the epidemiology of antibiotic resistant staphylococci in a hospital revealed the following:

1. Staphylococci obtained from hospital personnel early in the study, and 3 months later had the same distribution of penicillin sensitivities, but the resistance to aureomycin had increased. Eighty-eight per cent were resistant to 1 u/ml of penicillin and 31.3% to 100 u/ml. At the end of the study 90% were resistant to 1 mcg/ml of aureomycin and 50.9% were resistant to 100 mcg/ml.

2. Among the staphylococci obtained from patients on admission, 45.1% were sensitive to 1 u/ml of penicillin and 27.4% were resistant to at least 100 u/ml. Similarly, 59.4% were sensitive to 1 mcg/ml of aureomycin and only 7.2% resistant to 100 mcg/ml.

3. Organisms obtained at discharge from patients who received penicillin were inhibited by 1 u/ml of penicillin in 26% and 50% were resistant to at least 100 u/ml. Similar figures for organisms from patients treated with aureomycin were 16% and 28.5% respectively, those from patients treated with aureomycin plus penicillin 11.11 and 61.1% respectively, and from untreated patients 28.5 and 28.5% respectively.

4. Similarly 28.6% of these organisms were sensitive to 1 mcg/ml of aureomycin and 22.5% were resistant to 100 mcg/ml of aureomycin in the penicillin group. In the aureomycin group these figures were 7.9% and 51.3% respectively, in the combination group 5.6% and 56% respectively and in the no treatment group 51.2% and 9.8% respectively.

5. These results can be explained by a simple mixing of the flora from the personnel and the patients with a limited antibiotic effect.



*Role of Bone, Connective Tissue and Muscle Electrolytes in the Maintenance of Extracellular Fluid Composition.*

MARVIN F. LEVITT and LOUIS B. TURNER, New York, N. Y. (Introduced by Louis J. Soffer).

Marrow-free cortical bone, tendon and muscle of normal and acidotic rats were analyzed for electrolyte and water content. Normal rats contained 0.28, 0.03, and 0.03 meq. of sodium, chloride, and potassium, respectively per gram of dry bone and 0.19, 0.20 and 0.04 meq. of sodium chloride, and potassium, respectively, per gram of dry tendon. The water content of bone and tendon averaged 18 and 50%, respectively. Muscle values agreed with those reported by others.

Acidosis was produced by 100 mM ammonium chloride intraperitoneally (10 cc/100 grams of rat). Four hours later the rats were killed by exsanguination. In the acidotic rats, the serum sodium was reduced by 10%, serum chloride 6% and pH to 7.0. Two-thirds of the ammonium chloride was absorbed from the peritoneum, the unabsorbed peritoneal fluid stabilizing at serum levels. 50% and 20% of the absorbed water and chloride, respectively, were excreted during the four hour period.

Muscle of the acidotic rats showed a 35% fall in both sodium and chloride content, a slight increase in potassium content, and no change in water content. These changes imply a reduction in extracellular fluid with a reciprocal expansion of the intracellular fluid of muscle. Tendon showed a 17% fall in sodium content (in part referable to the reduced serum sodium concentration) without change in other modalities. The disproportionately greater reduction in tendon sodium may reflect either the transfer of sodium out of tendon or a diminished extracellular fluid volume (as noted in muscle). In the latter circumstance, the unchanged chloride content would suggest adsorption of chloride by connective tissue. Bone showed a reduction of 8% in its "excess" sodium content.

These data suggest that "excess" electrolyte in bone and connective tissue can be rapidly mobilized to defend extracellular fluid composition. The concomitant changes in muscle are difficult to interpret.

*The Study of Thyroid Function by Means of a Single Injection of Thyrotropin.* RICHARD P. LEVY, LUTHER W. KELLY, JR. and WILLIAM MCK. JEFFERIES, Cleveland, Ohio. (Introduced by William S. Jordan, Jr.).

Current tests of thyroid function serve to reflect the activity of the gland at the time of the procedure, but give no indication of its ability to respond to stimulation, i.e., thyroid reserve. A study in normal individuals indicates that a single intramuscular injection of thyrotropin (TSH) in a dose as small as 10 mg. produces a significant increase in serum protein-bound iodine (PBI) and thyroïdal uptake of  $I^{131}$  within 24 hours. Patients with primary hypothyroidism show no such response. A patient with panhypopituitarism had a low initial uptake of  $I^{131}$ , but responded well to this dose of TSH.

The administration of thyroid extract to normals causes a decrease in the initial  $I^{131}$  uptake, but response to TSH is not inhibited. Iodide obscures the uptake of  $I^{131}$  both

before and after TSH, but an increase in PBI still occurs. Hence, neither thyroid extract nor iodide blocks TSH effect on the thyroid gland, and it is possible to determine thyroid reserve in spite of the administration of either of these substances.

After subtotal thyroidectomy or  $I^{131}$  therapy for hyperthyroidism, patients may show normal initial levels of these two indices, but poor response to stimulation by TSH, a finding consistent with decreased thyroid reserve in the residual tissue. Cases of ophthalmopathic Graves' Disease may or may not have normal responses to TSH.

These studies indicate that a single injection of a comparatively small dose of TSH can be used to study thyroid function in a manner which has not previously been possible.

*Central Nervous System Involvement in the Disorders of Collagen.* BERNARD I. LEWIS, DAVID W. SINTON and JOHN R. KNOTT, Iowa City, Iowa. (Introduced by William B. Bean).

Forty-three patients with representative members of the collagen disease group were subjected to detailed medical and neurological examinations including 3 specific procedures:

- (1) Electroencephalography
- (2) CSF analysis
- (3) The Vocabulary-Kohs Index (VKI), a special psychometric test which provides an objective measurement of impaired intellectual function.

Our preliminary findings, which this report details, revealed pretreatment evidence of CNS involvement in 34 patients (79%). Twenty-five presented abnormal EEG's with a startling number (13) of focal type records; 17 demonstrated CSF abnormalities related to the protein content and colloidal gold test; 7 VKI's were subnormal indicative of acquired mental deficit. In particular, 12 of 13 rheumatoid arthritics, 8 of 9 patients with rheumatic fever and 8 of 9 with disseminated lupus provided abnormal test results. Eighteen patients received ACTH and/or Cortisone therapy and 8 were retested post-treatment with a mild but definite improvement in their test abnormalities. Contrariwise, 1 patient with disseminated lupus and another with dermatomyositis, both with abnormal pretreatment EEG's, suffered fatal cerebrovascular accidents during hormonal therapy. Neurologic findings were minimal or absent otherwise and in no patient was there basis for the pretreatment abnormalities other than the collagen disorder present.

These data suggest that the collagen disorders are attended by a high incidence of CNS involvement. The numerous focal type EEG's probably denote more than simple disturbed physiologic function. Our findings, in the light of previous histopathologic reports, foster the concept that neurovascular lesions are an integral component of these disorders. These lesions are likely responsible for the acute neurologic accidents in syndrome like disseminated lupus whereas they may underlie the intellectual, behavioral and emotional aberrations which frequently occur in the more common rheumatic and arthritic

groups. Further observations are being made to validate these findings and substantiate this concept.

*Metabolism of Bone Disease Studied by the Calcium Tolerance Test.* ISAAC LEWIN, HERTA SPENCER and DANIEL LASZLO, Montefiore Hospital, New York. (Introduced by Bernard S. Oppenheimer).

The need for improved and simplified methods to study calcium metabolism is generally recognized. Such a method should give information similar to metabolic balances; it should be sensitive and reflect spontaneous or therapeutically induced changes of mineral metabolism. In the calcium tolerance test, the 24 hour urinary calcium excretion is determined following the slow infusion of calcium gluconate. It was performed in conjunction with metabolic balances in 105 instances in normal subjects and in patients with bone disease. This test appears to meet the above outlined criteria since a linear relationship with a high coefficient of correlation was noted between the induced calcinuria and metabolic balances.

The analysis of the experimental material disclosed three distinct groups into which the results of the test, the metabolic balances, the serum calcium levels and the clinical state of the patient fitted well. The average induced calcinuria/24 hours was  $58 \pm 7.3$  mg for patients with a tendency to mineral retention,  $200 \pm 11$  mg for normals and  $423 \pm 28$  mg for patients with demineralization. In the analysis of ten cases of the third group, the greater sensitivity of this test as compared with balance studies and other laboratory aids was noted, since the latter two were normal while the response to the test was abnormally high. This observation may be explained by the slow rate of demineralization progressing over a prolonged period of time which leaves overall mineral balances unaffected and yet results in the inability of the skeletal system to accept a sudden load of calcium.

The information obtainable with the aid of this test will be illustrated by several representative cases.

*Factors Responsible for the Variable Effectiveness of ACTH in Man.* G. W. LIDDLE, A. P. RINFRET, D. ISLAND and P. H. FORSHAM,\* San Francisco, Calif.

It is not possible to predict, on the basis of USP units alone, just how effective a given preparation of ACTH will be in stimulating steroidogenesis by the human adrenal. The initial physiological status of the subject's adrenals, as well as the duration, route, and frequency of administration of the ACTH, all influence the effectiveness of a given dose. In addition, at least three factors which depend upon the chemical nature of ACTH influence the effectiveness of ACTH. None of these is assessed in the official assay method, which is based upon depletion of ascorbic acid of the rat adrenal following the intravenous administration of ACTH. Special studies concerning the three factors have revealed the following:

First, pituitary fractions have been prepared which show no potency in the ascorbic-acid-depletion assay but which have proved highly steroidogenic when administered to man.

Second, practically all of the available preparations of ACTH are subject to fifty to ninety per cent inactivation when administered intramuscularly, as compared with their maximum effectiveness when administered intravenously under otherwise identical conditions. A polypeptide preparation (ACTH "A", Fisher et al) was found to be nearly immune to intramuscular destruction; but it was rendered subject to inactivation by partial hydrolysis (ACTH "B"), which did not impair its intravenous activity significantly.

Third, pituitary fractions having neither the ascorbic-acid-depleting action nor the steroid discharging capacity of ordinary ACTH were shown to act synergistically with ACTH in promoting steroidogenesis in man.

Since anterior pituitary extracts might contain varying proportions of non-ascorbic-acid-depleting factors, ACTH "A", ACTH "B", and synergistic factors, an assay based upon steroidogenesis in guinea pigs in response to repeated intramuscular administration of ACTH has been developed and found to be reliable in determining the overall clinical effectiveness of a given "ACTH" preparation.

*The Effects of Sodium Acetate Administration Upon Ketone Body Production in Normal and Diabetic Subjects.* S. R. LIPSKY, B. J. ALPER, M. RUBINI and M. GORDON and VAN ECK, New Haven, Connecticut. (Introduced by Philip K. Bondy).

Intravenous injections of 0.16 to 0.50 M sodium acetate were given over a two hour period to eleven subjects including six normals, one in starvation ketosis, and five diabetic subjects in ketosis. Serum was analyzed for electrolytes and citric acid, and blood for ketones, glucose, and pyruvic acid at significant intervals for six hours.

Metabolic alkalosis as evidenced by increases of serum bicarbonate of four to six mEq./L. and concomitant decreases in serum chloride occurred consistently. In normal subjects, ketone levels increased twofold to concentrations as high as 4.3 mgm. per cent. Diabetic subjects deprived of their insulin for two or more days showed an acute rise in total ketone levels of six to twelve mgm. per cent following acetate administration. Blood glucose content in the normal individuals showed small decreases as compared to the diabetic group which exhibited very striking depressions. There were no significant changes in pyruvic acid levels, whereas citric acid levels increased slightly. Control subjects given sodium bicarbonate infusions of similar magnitude and producing corresponding degrees of alkalosis developed considerably smaller degrees of ketosis than occurred after acetate infusions.

Observations were made in three diabetic patients utilizing hepatic vein catheterization technique. Using Michael's method for ketone determinations, it was possible to detect hepatic vein, arterio-venous differences during the course of these studies. A striking rise in net splanchnic ketone output was noted from two to four hours after the acetate infusion. Contrastingly, a considerable decrease in net splanchnic glucose production occurred. Hepatic blood flow remained relatively constant.

Although alkalosis *per se* increases ketosis, the exag-

gerated ketone response to acetate in these experiments suggests that this two-carbon molecule serves as an immediate precursor for ketone production. This problem is being investigated further with deuterium-labeled acetate. The response to acetate appears to be accentuated in uncontrolled diabetics.

*Physiologic and Hemodynamic Studies of Patients with CO<sub>2</sub> Narcosis.* FRANK W. LOVEJOY, JR., PAUL N. G. YU, ROBERT E. NYE, JR., HOWARD A. JOOS and JOHN H. SIMPSON, Rochester, N. Y. (Introduced by Nolan Kaltreider).

Three patients with the syndrome of pulmonary insufficiency accompanied by marked CO<sub>2</sub> retention associated with respiratory acidosis who developed coma following the administration of oxygen, have been treated by mechanical ventilation in a Drinker respirator. Their course was followed by serial arterial blood gas analysis and pH determinations.

Two cases recovered and are at present in less respiratory distress than in the past several years.

The clinical and laboratory findings of all 3 cases were similar in the following respects: (1) Clinically each patient had severe dyspnea, cyanosis and tachypnea at rest. All had a history of chronic respiratory difficulty which had recently been accentuated. In all there was evidence of right ventricular failure manifested by distended jugular veins and enlargement of the liver. Shortly after admission, oxygen, given because of severe respiratory distress, resulted in coma in every case. (2) Laboratory studies revealed severe hypercapnia, hypoxemia, elevated hematocrit, and respiratory acidosis in all instances. After treatment lung volume studies demonstrated that the pulmonary insufficiency was mainly due to fibrosis in two cases and severe emphysema in the third case. (3) Cardiac catheterization performed after therapy showed a moderate degree of pulmonary hypertension associated with a normal cardiac output. The pulmonary artery pressure in one case was determined before treatment. In this patient the mean pulmonary artery pressure after treatment fell from 60 to 29 mm. Hg. This was associated with a decline in the CO<sub>2</sub> tension of arterial blood from 77 to 48 mm. Hg and a rise in arterial blood oxygen saturation from 50 to 87%. (4) The clinical response to mechanical ventilation was slow in every instance. On the average after 9 to 10 days in the respirator chemical improvement of the blood became stabilized. Improvement was manifested by a return of mental clarity, a gradual fall in the CO<sub>2</sub> tension and a rise in the pH of arterial blood.

*The Isolation of Crystalline d-urobilinogen and Its Probable Significance.* PAUL T. LOWRY, Minneapolis, Minn. (Introduced by C. J. Watson).

Recent studies have strengthened the concept that mesobilirubinogen is intermediary in the bacterial reduction of bilirubin to stercobilinogen. These two members of the urobilinogen group, on dehydrogenation, yield,

respectively, an optically inactive urobilin plus the strongly levorotatory stercobilin. A dextrorotatory urobilin was previously isolated from infected fistula bile and from feces of patients receiving aureo or terramycin. The immediate precursor of this pigment, "d-urobilinogen," has now been crystallized for the first time, thus permitting study of its nature and relationship to other pigments of biliary derivation.

The material has been crystallized repeatedly from feces of patients who have received terramycin, also following brief sodium amalgam reduction of crystalline d-urobilin. An alcoholic extract of feces is passed through alumina from which the d-urobilinogen is eluted with water. It is extracted with petroleum ether and crystallized as a light yellow powder with properties of a dihydromesobilirubin, intermediate between mesobilirubin and mesobilirubinogen, but easily distinguished from the dihydromesobilirubin obtained by catalytic reduction of bilirubin, *in vitro*. The compound exhibits a small optical activity ( $\alpha_{20}^d = +18.6$ ) in contrast with the remarkable activity of d-urobilin ( $\alpha_{20}^d = +5000$ ). Elementary analysis agrees well with the formula C<sub>28</sub>H<sub>42</sub>N<sub>4</sub>O<sub>6</sub>; thus it is an isodihydromesobilirubin. It is readily converted to d-urobilin on mild oxidation, and with more rigorous treatment to mesobiliviolin and glaucobilin. It is readily reduced to mesobilirubinogen either with sodium amalgam or with ferrous hydroxide, as in the quantitative procedure for the urobilinogen group in urine or feces.

It is regarded as not unlikely that d-urobilinogen is normally intermediary in the bacterial reduction of mesobilirubin to mesobilirubinogen although the possibility that it represents an abnormal pathway is not wholly excluded.

*The Sodium-Retaining Corticoid of Human Urine: Its Nature and Some Physiological Factors Governing Its Secretion.* JOHN A. LUETSCHER, JR.\* and BEN B. JOHNSON, San Francisco, California.

Previous work has demonstrated increased sodium-retaining activity in the corticoids of urine from certain patients with edema and from normal men on low-sodium diets. These results are determined by bio-assay and are controlled and evaluated by the response to known doses of desoxycorticosterone acetate.

Chromatographic fractionation of actively sodium-retaining urine extracts has resulted in the quantitative recovery of biological activity in a fraction moving with a rate similar to cortisone. The active material is not cortisone, since the amounts of cortisone present in this fraction have no sodium-retaining activity. Other fractions, including those corresponding with the other known, biologically active corticosteroids, show no significant sodium-retaining activity.

The sodium-retaining activity of the fraction moving with cortisone is decreased in the urine of patients with the nephrotic syndrome when diuresis follows administration of ACTH or cortisone. This reduction of activity is not necessarily dependent on increased excretion of 11-

oxysteroids, which promote sodium excretion in the assay used.

Normal controls show insignificant sodium-retaining activity in any chromatographic fraction; but when dietary sodium is withheld, increased activity has been observed in the cortisone fraction.

The chromatographic behavior of the active substance resembles that of the unidentified mineralocorticoid reported by Grundy, Simpson, Bush, and Tait in beef adrenal extract and in mammalian adrenal vein blood. Further studies on the chemical nature of the sodium-retaining corticoid will be presented.

*Antihormone Formation in Patients Receiving Hog Pituitary FSH; Possible Therapeutic Application.* WILLIAM O. MADDOCK, ICHIRO TOKUYAMA and ROBERT B. LEACH, Detroit, Michigan. (Introduced by Gordon B. Myers).

Antihormone formation was detected in 4 of 6 patients who received a hog pituitary FSH preparation, 12.5 to 25 rat units twice daily for 52 to 90 days. Antihormones first appeared 44 to 76 days after starting treatment. The patients' plasma, administered to immature female rats, prevented the action of hog, rat and human pituitary gonadotrophin, and human chorionic gonadotrophin. The mechanism of blocking chorionic gonadotrophin action was indirect, resulting from neutralization of the assay rats' endogenous gonadotrophin. This is consistent with the established fact that presence of some pituitary gonadotrophin is necessary for chorionic gonadotrophin to exert its characteristic actions in the immature female rat. One-fiftieth the amount of plasma sufficed to demonstrate antihormone activity when chorionic gonadotrophin, instead of hog FSH, was used as the "test hormone" for detecting antihormones. Anti-hog FSH activity disappeared from the plasma within 2 months after treatment, but antihormone activity tested against chorionic gonadotrophin was still demonstrable 9 months after therapy.

A 7-year-old girl with constitutional precocious puberty and an 18-year-old patient with severe idiopathic menorrhagia received FSH for 2 and 3 months, respectively, in order to elicit antihormone formation and possibly achieve temporary cessation of ovarian function. Antihormones appeared during the second and third month of treatment. Estrogen excretion increased during the first month and then fell to less than pretreatment levels by the end of therapy. In the patient with menorrhagia, low estrogen excretion and amenorrhea have persisted for 2 months. Low estrogen levels and amenorrhea were maintained for 4 months in the precocious patient and then estrogen increased and 2 regular menses occurred. Ten days after starting a second course of FSH, antihormones increased markedly and estrogens decreased. It is concluded that antihormones, forming in patients receiving hog FSH, can produce temporary cessation of ovarian function.

*The Effect of 1-Hydrazinophthalazine on the Hepatic Circulation in Dogs.* P. A. MARKS and P. C. REYNELL, New York City, N. Y. (Introduced by I. M. London).

The fall in arterial pressure following administration of 1-hydrazinophthalazine in man and animals has been attributed to decreased peripheral vascular resistance. Cardiac output and renal blood flow are increased proportionately, whereas blood flow through the extremities is little affected. Splanchnic vasodilation therefore seems likely.

Hepatic blood flow (Bromsulfalein method), cardiac output (direct Fick) and mean arterial pressure (mercury manometer) were measured before, and after, intravenous administration of 1-hydrazinophthalazine (0.5 to 1.0 mg/kg) in 17 dogs under nembutal anesthesia.

Hepatic blood flow increased by 330 ml/min. or 65 per cent on the average within 25 to 35 minutes after giving the drug. The Bromsulfalein extraction decreased (average 40 per cent) with a change in total Bromsulfalein removal. Cardiac output always increased (average 1.7 L/min. or 90 per cent). Since mean arterial pressure fell by 40 mm. Hg or 32 per cent on the average, the calculated splanchnic vascular resistance fell 55 per cent and total peripheral vascular resistance 65 per cent. Although the total oxygen consumption (closed circuit spirometer) rose from an average of 79 to 105 ml/min., splanchnic oxygen consumption did not increase significantly. Respiratory rate and ventilation increased prior to these changes. Hepatic blood flow increased despite initial splanchnic vasoconstriction secondary to the withdrawal of the blood samples and despite the fall in arterial pressure. Splanchnic vasodilatation thus contributes significantly to the reduction in the total peripheral vascular resistance. This hemodynamic response bears a striking similarity to that observed during the pyrogenic reaction.

*Effect of Glucose, Fructose, and Insulin on the Leukocytes of Diabetics.* SAMUEL P. MARTIN,\* GORDON R. MCKINNEY and R. GREEN, Durham, No. Carolina.

Since diabetic subjects are prone to infection and the bacteriocidal capacity of their whole blood is low, a study was undertaken to compare the metabolic activities of white cells from diabetic and normal subjects. The blood was collected and handled in a non-wetting system. The red cells were separated by the dextran sedimentation method. The white cells were further concentrated by centrifugation and resuspended in a phosphate buffer for metabolic studies. Cells collected in this manner have good oxygen utilization and produce lactic acid in the presence of oxygen. In the normal leukocyte, this lactate production is increased by the addition of glucose and fructose. Glucose causes a sharp increase in lactate formation as the concentration of hexose is increased, while fructose produces a slow rise. At 100 mg percent hexose the rate of lactate production from glucose is  $50.53 \pm 15$  (S.D.) gamma per ten million cells per hour and with fructose is  $34 \pm 9$ . In diabetic leukocytes, the situation is reversed, with glucose  $38.6 \pm 13$  gamma per ten million cells per minute and fructose  $49.7 \pm 7$ . This data would indicate that leukocytes from diabetic subjects had im-

paired utilization of glucose and with this had an impaired lactic acid production. The addition of insulin (0.1 u) with glucose returned the lactate production toward normal. Since many of the early chemical changes in areas of inflammation as well as bacteriocidal activity may be correlated with lactate production in tissue, this defect in diabetic leukocytes might lead to an alteration in inflammation and susceptibility.

*The Role of the Alarm Reaction in the Pathologic Physiology of Experimental Diabetic Acidosis.* J. W. McARTHUR, G. A. SMART, D. HARTING, E. GAUTIER, A. GODLEY, K. A. SWALLOW, F. A. SIMEONE and D. HUME, Boston, Mass. (Introduced by A. M. Butler).

The extent to which the alarm reaction to stress may figure in the pathologic physiology of diabetic acidosis and the degree to which increased adrenal secretory activity may contribute to the metabolic manifestations of this particular stress have been investigated by means of insulin deprivation experiments in depancreatized and in depancreatized-adrenalectomized dogs.

It was found that eosinopenia and increased corticosteroid excretion occur comparatively late in experimental diabetic acidosis. Closely associated in time with these evidences of increased adrenal secretory activity are a number of other metabolic events. These include: (1) a sharp increase in the negativity of the nitrogen, potassium, and phosphorus balances, (2) a loss of potassium, presumably from intracellular fluid, in excess of nitrogen, (3) a decreased sensitivity to injected insulin, and (4) a sudden increase in the degree of lipemia and (in some experiments) of ketonemia.

Evidence that an increase in adrenal secretory activity is required for the manifestation of some of these features of the reaction to acute insulin deficiency was obtained by studying the course of the adrenalectomized-depancreatized animal maintained on a fixed dose of cortical hormones during the period of insulin deprivation. In such an animal the rate of protoplasmic catabolism is reduced, as compared with that of the adrenally-intact animal, there is no loss of excess potassium and sensitivity to insulin is actually increased.

It is concluded that the alarm reaction to stress, catalyzed by the presence of the adrenal cortical hormones, contributes significantly to the pathologic physiology of experimental diabetic acidosis. In the normal organism this response, one of whose purposes appears to be the conservation of carbohydrate stores and the utilization of body fat and protein as sources of energy, is believed to be homeostatic. In the diabetic organism, metabolically vulnerable to stress, it seems probable that certain effects of the alarm reaction are detrimental.

*The Behavior of Populations of Tubercle Bacilli in Vivo.* ROBERT M. McCUNE, N. Y., N. Y. (Introduced by Ralph Tompsett).

Bacterial multiplication *in vivo* is required for establishment of infection and progressive disease. Hence, the dynamics of tuberculous infection should be sensitively re-

flected by quantitative changes in the population of tubercle bacilli within the organs of infected hosts.

A study has been made of the behavior of bacterial populations in mice with a chronic infection induced with known numbers of virulent human tubercle bacilli. The influence of a number of controlled variables including antimicrobial therapy was measured. The technics employed permit enumeration of microorganisms in tissues.

It was first found that host-tubercle bacillus relationships are different in lung and spleen (confirming recent studies by others). In the lung the bacilli multiplied progressively whereas in the spleen initial multiplication was followed by a decrease and then stabilization.

It was further found that the type of response of the bacterial populations to drugs was also distinctly different in the lung and spleen.

Isoniazid markedly reduced the population of tubercle bacilli in the lungs of all animals whereas in the spleen an immediate reduction was followed by a stabilization of census which persisted despite 90 days of intensive therapy.

In contrast to isoniazid, streptomycin administered from the time of infection prevented multiplication in the lung but failed to lower the census. In the spleen, however, multiplication actually occurred under streptomycin although an eventual reduction followed by stabilization ensued.

Enumeration of drug-resistant mutants in the spleens of both treatment groups conclusively demonstrated that drug resistance per se did not explain the presence of persisters.

Concurrent administration of both drugs proved only slightly more effective than isoniazid in reducing microbial populations.

The influence of established lesions, variations in infecting dose, and the point in time when population shifts of susceptible and resistant bacilli results in drug neutralization, have also been quantitatively studied.

*Eck-Fistula.—A Cause of Episodic Stupor in Humans.*

WILLIAM McDERMOTT and RAYMOND D. ADAMS,\* Boston, Mass.

Recently, in an attempt to effect a surgical cure of carcinoma of the head of the pancreas in a 69 year old man, the portal vein was resected and an anastomosis was established between the superior mesenteric vein and the inferior vena cava. An Eck fistula was thus created for the first time in a human who had neither pre-existing portal hypertension nor an established collateral circulation. His liver appeared normal by gross examination and biopsy. Liver function was normal according to all of the standard laboratory tests. Convalescence was uneventful until the fifth post-operative week when the first of a series of episodic stupors occurred. These consisted of an impairment of consciousness, which varied from mental confusion to deep coma, a fluctuating rigidity of the limbs, reflex grasping and sucking and extensor plantar reflexes. The onset was acute and without assignable cause, the course progressive for several days, and

recovery was rapid and complete. The singular character of this neurologic disorder prompted us to undertake a prolonged metabolic study, the results of which will be reported.

During a six months period 16 episodes of stupor or coma were observed. Eighteen EEG tracings were obtained during and between these periods of coma. There was a close correlation between the severity of impairment of consciousness and the degree of EEG abnormality. One consistent biochemical abnormality was found—an elevation of blood ammonia. This was usually 50 to 75 micrograms above normal levels and rose sharply at the onset of each episode of stupor. Following this lead we discovered that it was possible on 8 separate occasions to reproduce the neurologic disorder and to raise blood ammonia levels by the administration of a high protein diet, ammonium chloride, urea, or an ammonia liberating cation exchange resin. No other metabolic disorder was found. Since the time of this experiment (5 months ago) a low protein diet and sulfathalidine, for the purpose of suppressing the growth of intestinal bacteria, have effected a reduction in blood ammonia levels and have prevented all except 2 or 3 mild periods of confusion.

This neurological syndrome is probably the same as that referred to as "meat intoxication" in Eck-fistula dogs and is affiliated to hepatic coma. Eck-fistula deserves to be ranked with hypoglycemia, uremia, acidosis, hypokalemia, porphyria and Addison's disease as a cause of episodic stupor and coma.

*Cardiac Output and Pulmonary Arteriolar Resistance in Mitral Stenosis and Mitral Incompetence.* LAWSON McDONALD and MURRAY RABINOWITZ, Boston, Mass. (Introduced by Lewis Dexter).

Cardiac catheterization was performed in 87 patients with mitral valvular disease; 56 subsequently underwent cardiac surgery. Effective mitral valve area was calculated by the hydraulic formula of Gorlin, cardiac output determined by the direct Fick method and pulmonary vascular resistance was estimated. At operation, actual valve size was estimated by the surgeon. In all, there was good correlation between the findings at catheterization and operation. Cases were divided into three groups.

I. Mitral stenosis without significant insufficiency

II. Combined mitral stenosis and insufficiency

III. Mitral insufficiency without significant stenosis

Pulmonary arteriolar resistance generally was much higher in patients with mitral stenosis than in those with mitral insufficiency. Pulmonary arteriolar resistances of 1000 to 2000 dynes seconds cm.<sup>-2</sup> were common in patients with severe mitral stenosis; they did not occur in severe mitral insufficiency. Intermediate values were found in the combined group. Cardiac index bore an inverse relation to pulmonary arteriolar resistance in all groups; in the presence of mitral insufficiency a lower cardiac index occurred than in cases of mitral stenosis with a similar pulmonary arteriolar resistance. In this study pulmonary vascular disease appeared most marked in cases with

severely narrowed valves and was accompanied by a greatly decreased cardiac index. In patients with significant mitral insufficiency, a low cardiac output was also found, but might occur with a much less degree of pulmonary vascular disease.

*Observations on the Circulatory Phenomena Produced by the Valsalva Maneuver.* H. D. McINTOSH, J. F. BURNUM, J. B. HICKAM\* and J. V. WARREN,\* Durham, N. Carolina.

The suggestion that the Valsalva maneuver may be of practical diagnostic value led us to make observations on 15 subjects with particular attention to the period immediately following release of intrathoracic pressure. The cardiac output, measured by the dye technique, was found in most instances to be diminished during the recovery period at which time the usual overshoot of arterial pressure and bradycardia occurred. The total peripheral resistance was increased. The vasoconstriction was not generalized, however, since forearm plethysmography demonstrated a disparate increase in muscle blood flow.

Studies on patients with mitral stenosis showed a similar overshoot of pressure and bradycardia in most instances. Usually the response did not appear different from the expected normal. In one patient with severe mitral stenosis, but a normal overshoot following the Valsalva, there was an increased cardiac output which was accompanied by a decrease in vascular resistance. In this situation it would appear that the rise in arterial pressure was caused by the increase in cardiac output. In a patient with diffuse impairment of the autonomic nervous system, a 45% increase in cardiac output during the recovery period failed to raise the arterial pressure promptly.

These studies demonstrated that a hypertensive and bradycardic phase may occur during recovery from the Valsalva maneuver in both normal individuals and patients with severe mitral stenosis. The data indicate that this response may be due primarily to an increase in total vascular resistance and less often to an increase in cardiac output. Since the arterial overshoot is a function of variable changes in both cardiac output and peripheral resistance it may not serve as an accurate index of cardiac function.

*Clinical Measurement of Resistive and Elastic Properties of the Lungs.* JERE MEAD and JAMES L. WHITTENBERGER,\* Boston, Massachusetts.

Although the basic principles of the mechanics of breathing and the relationship of abnormal mechanics to respiratory symptoms have been appreciated for many years, until recently there has not been an accurate and relatively convenient method for obtaining the necessary data in patients. The information required consists of instantaneous rate of flow of air into and out of the lungs, volume change of the lungs, change in pressure difference from mouth to pleural surfaces of the lungs, and

a means of separating the total pressure difference into fractions due respectively to elastic properties and to resistive properties. The changes in intrapleural pressure can be reliably measured from a small balloon in the esophagus. At the beginning and end of each respiratory half-cycle the pressure difference is due solely to non-resistive forces. A constant of proportionality between volume change and elastic forces can thus be derived and electrically subtracted from the total pressure difference. When this operation is carried out with registration of flow vs. resistive pressure drop on a cathode ray oscilloscope, the result is a continuous plot depicting the resistance of the respiratory system. Changes in resistance due to disease, and the influence of drugs, inspired gas density, and other variables have been studied. A comparison of this method of measuring resistance with the airway interruption method has shown that the latter does not measure alveolar pressure, as had previously been thought.

The ratio of volume change to non-resistive pressure difference is called compliance and is a measure of the elastic properties of the lungs. Alterations of compliance have been found in patients with mitral stenosis, pulmonary granulomatosis, emphysema, and chronic poliomyelitis.

*The Effect of Splanchnicectomy on the Renal Excretion of Electrolytes in Dogs.* EDWARD MEILMAN and BERTRAM M. WINER, Boston, Mass. (Introduced by Samuel L. Gargill).

The phenomenon of "denervation diuresis" has been considered to be temporary and elicitable only under anesthesia. We have shown that aminophylline given to pentobarbital-anesthetized unilaterally-splanchnicectomized dogs accentuates the difference in excretion of water, Na and Cl, between the denervated (D) and innervated (N) sides. It can be demonstrated for at least 42 days after denervation.

Verney found in the unanesthetized unilaterally-denervated dog that urine flow is the same on both sides during water diuresis, excitement or exercise. Surtshin *et al* studied electrolyte excretion in such dogs during water or saline diuresis and found no difference between the two sides. We have found in each of eight normally hydrated unanesthetized unilaterally splanchnicectomized dogs, that there was increased excretion of sodium on the denervated side although urine flows were essentially the same on both sides. The mean ratios of D/N were for urine flow 1.18, Na excretion 2.28, K excretion 1.63. In seven of eight of these unanesthetized dogs aminophylline produced a diuresis, greater on the denervated side. The mean ratios of D/N were for urine flow 1.79, Na excretion 1.83, K excretion 1.57.

These data indicate that "denervation diuresis" is readily reproducible in the same dog by anesthesia or by aminophylline. The finding of a consistent increase in sodium excretion by the denervated kidney in the unanesthetized normally hydrated dog, while urine flows on the two sides are the same, demonstrates that in the absence

of water or saline diuresis, the denervated kidney is less efficient in conserving sodium than the normally innervated one. This suggests a mechanism for the efficacy of sympathectomy in hypertension, as well as the reported synergistic action of sympathectomy and low-sodium diet in treating hypertension.

*Continuous ACTH Therapy of Nephrotic Syndrome in Children.* ARTHUR J. MERRILL\* and GEORGE L. MITCHELL, JR., Atlanta, Ga.

Seven children with nephrotic edema have been followed for five to twelve months after institution of a new continuous method of corticotropin GEL administration. All except one had had one to ten ten-day intermittent courses of treatment followed by continued albuminuria and relapse into the edematous state. Duration of disease varied from one to twenty months. Two had beginning nitrogen retention and all had considerable albuminuria and edema. Ages were from nine months to four years. With some initial variations each was given an appropriate dose once daily until either free of albuminuria and hematuria or until a steady state of mild albuminuria was achieved. Then they were abruptly changed to ACTH GEL every other day and the dose reduced gradually. All have been clinically well since their initial diuresis, whereas they had fluctuated between edematous and dehydrated states with intermittent courses. Six have been without albuminuria for seven to ten months except for rare relapses accompanying colds or too rapid reduction of dose. These relapses were abolished promptly by temporarily increasing the dose. The sixth patient is clinically well but has a very faint trace of albuminuria. Two additional patients are now free of albumin and edema but follow-up is inadequate. All have been normally active after a brief initial period. Treatment has been stopped in two patients for three and six months without relapse and for brief periods in three others without relapse.

*An Approach to the Measurement of Uterine Blood Flow in Pregnancy.* JAMES METCALFE, SEYMOUR L. ROMNEY, LLOYD H. RAMSEY and DUNCAN E. REID, Boston, Massachusetts. (Introduced by C. Sidney Burwell).

The concentrations of nitrous oxide in a uterine vein and a systemic artery have been measured during a thirty-minute period of nitrous oxide inhalation. This was done at term Caesarean section performed under spinal anesthesia. In four of five patients, the arterio-venous nitrous oxide difference at the end of thirty minutes of gas administration was less than 0.2 volume per cent.

The solubility of nitrous oxide in amniotic fluid, uterine and fetal tissue is identical with its solubility in blood when equilibrated with 100 per cent nitrous oxide *in vitro*. However, the *in vivo* concentration of nitrous oxide in the uterine contents at the end of thirty minutes exposure to the gas is less than its concentration in maternal blood, and the values have no constant relationship in different patients. The uterine contents can, however,

be sampled at the end of the period of nitrous oxide inhalation, at which time samples of blood from an umbilical artery and the umbilical vein and a sample of amniotic fluid are taken. The volume of amniotic fluid is calculated by dilution of Evans' blue dye. Assuming that the uterine wall has the same nitrous oxide concentration as the maternal blood, that the fetal tissues have the same concentration of nitrous oxide as blood in the umbilical artery and that the uterine myometrium at term weighs 1 kilo, then the blood flow to the pregnant uterus can be calculated under the conditions specified. From this result and the  $O_2$  and  $CO_2$  contents of arterial and uterine venous blood, the uterine oxygen consumption, carbon dioxide production and respiratory quotient can be derived.

The results of several cases studied in this manner will be reported.

*Observations on Renal Cellular Electrolyte Transport Systems in Normal and Nephrotic Children.* JACK METCOFF,\* J. A. JAMES and G. GORDILLO, Boston, Massachusetts.

In normal individuals, infusion of a carbonic anhydrase inhibitor (CAI), "6063," inhibits renal  $H^+$  excretion while the obligatory anion p-aminohippurate (PAH) facilitates it. Increased excretion of  $Na^+$ ,  $K^+$  and phosphate occurs with both substances. Demonstrable tubular secretion of  $K^+$  is not usually observed. Since excessive reabsorption of  $Na^+$  with loss of  $K^+$  in acid urines has been observed during the Nephrotic Syndrome in children without renal insufficiency, a reversible defect in  $K^+$  and  $H^+$  transport was postulated. The hypothesis was tested by infusions of CAI and CAI + PAH simultaneously in normal and nephrotic children.

In the normal children, CAI produced alkaline urines and relative excretory increments of:  $HCO_3^- > K^+ > Na^+ > (HPO_4^{2-}/H_2PO_4^-)$ .  $Na^+$  and  $HCO_3^-$  were the predominant ions. (CAI + PAH) induced further excretion of  $Na^+$  and  $(HPO_4^{2-}/H_2PO_4^-)$  with  $HCO_3^-$  reabsorption. The urine remained alkaline. Tubular  $K^+$  secretion was not demonstrated.

In contrast, in edematous nephrotic children CAI alkalinized the urine with relative excretory increments of  $HCO_3^- > Na^+ > K^+$ .  $K^+$  was the principal urine cation and was excreted at greater than filtration levels. (CAI + PAH) remarkably enhanced  $Na^+$  excretion despite further tubular  $K^+$  secretion and the urine became acid. No significant change in  $HPO_4^{2-}/H_2PO_4^-$  reabsorption was observed. Following diuresis demonstrable tubular  $K^+$  secretion may persist, despite lack of edema and normal GFR.

These results suggest that multiple renal tubular transport systems for  $Na^+$ ,  $K^+$ , and  $H^+$  are normally present; e.g.  $K^+$  may be reabsorbed as a  $PO_4$ -complexing system which is inhibited by PAH excretion. CAI inhibition of  $H^+$  secretion is maintained despite PAH transport as long as  $K^+$  secretion does not occur.  $HCO_3^-$  can be reabsorbed without increased  $H^+$  secretion possibly because of rate-limited fixed cation transport during active PAH secre-

tion. In the nephrotic a temporary dissociation in  $Na^+$  and  $H^+$  transport systems largely referable to  $K^+$  may occur.

*Laboratory and Clinical Studies of a Hemolytic Test For Tuberculosis.* G. MIDDLEBROOK,\* H. OZERENGIN and R. OESTREICHER, Denver, Colorado.

A hemolytic modification of the hemagglutination test for antibodies against antigens of tubercle bacilli has previously been reported. The results of clinical applications of this hemolytic test have given rise to widely divergent views concerning its usefulness as a serologic aid in the diagnosis of mycobacterial diseases: the incidence of false positive reactions has varied in the experiences of different investigators from less than 2% to higher than 25%. Indeed, the frequency has varied markedly from time to time in the same laboratory.

The purposes of this paper are to report our own observations concerning some of the causes of these false positive reactions and to describe newly refined methods and materials for performing the hemolytic test.

Evidence will be presented to show that certain preparations of tuberculin contain material which sensitizes sheep red cells to give a positive reaction with the sera of rabbits injected with heat-killed Staphylococci. At least two lots of tuberculin have been found to contain no detectable amounts of this material. One of these lots, however, has been found to contain material which sensitizes sheep red cells to lysis by the sera of "normal" rabbits. Certain human sera from non-tuberculous individuals give positive reactions in this test before, but prove to be negative after, refrigeration at about  $4^\circ$  for 24 or 48 hours. Certain observations will be presented which confirm the previously described fact that serum specimens drawn from human beings within an as yet ill-defined period after skin tuberculin tests may give false positive reactions. Preliminary results of the application of this test as a useful aid for the diagnosis of tuberculosis will be presented.

*The Hepatic Inactivation of Antidiuretic Hormone.*

GEORGE E. MILLER and CHARLES E. TOWNSEND, Buffalo, N. Y. (Introduced by John D. Stewart).

In 1935 Heller first observed the *in vitro* inactivation of antidiuretic hormone by animal liver tissue, using the rat as an assay animal. This finding has subsequently been confirmed by others. It seemed appropriate to re-examine this problem using the water loaded dog for assay since determination of antidiuretic activity of biologic fluids by these two methods has sometimes given conflicting results. It was also desirable to extend the observations to include normal human liver.

Fresh liver tissue was iced, ground with sea sand and suspended in normal saline for ten minutes. The suspension was centrifuged and the supernatant fluid buffered at pH 6.7. Following incubation at  $37^\circ$  for thirty minutes with varying amounts of commercial pitressin (50 - 100 mU/0.1 gm. liver) a 1.0 ml. quantity of this fluid was administered intravenously to the trained, water loaded dog.



Minute urine volume was calculated from five minute collection periods. The experiments were controlled by the administration of standard amounts of pitressin, of similar amounts of pitressin incubated at 37°, and of liver homogenate alone. The antidiuretic activity remaining after incubation was quantitated by comparison of the change in urine flow with the reproducible alteration following known amounts of pitressin.

In twenty-five experiments rat liver inactivated 90—100 mU pitressin/0.1 gm. liver tissue. This result is comparable with previous findings by other methods. Likewise in five experiments normal human liver inactivated 90—100 mU pitressin/0.1 gm. liver tissue. Preliminary studies suggest that cirrhotic human liver may inactivate antidiuretic hormone at the same rate as the normal.

*The Effect of Carbohydrate Deprivation on the Metabolism of Fructose and Glucose in Normal Human Subjects.* MAX MILLER,\* JAMES W. CRAIG and HIRAM WOODWARD, JR., Cleveland, Ohio.

Starvation has long been known to produce an alteration in the ability of the organism to handle exogenous carbohydrate ("starvation diabetes"). The location of the metabolic defect responsible is not definitely known, although recently it has been suggested that pituitary respiratory-quotient depressing factor is formed which inhibits the transformation of hexosediphosphate to pyruvate. This would imply that the metabolism of both fructose and glucose would be impaired since fructose enters the metabolic scheme before the hexosediphosphate step.

Intravenous glucose and fructose tolerance tests (1 gm./K/1 hour) were performed in each of four human volunteers before and after two days of complete starvation or two days of a high protein, carbohydrate free diet. The glucose disappearance curves were abnormally *elevated* in every instance after carbohydrate deprivation. In contrast, the fructose disappearance curves were *unaltered*. After carbohydrate depletion, in three of the four cases the blood pyruvate rise was less after glucose administration, while in three of four cases fructose resulted in a greater pyruvate rise. In 6 of the 8 experiments carbohydrate deprivation resulted in a lowering of the fasting plasma inorganic phosphorus, but the fall in phosphorus during the glucose or fructose tolerance tests was not altered.

The finding that the handling of fructose is not impaired after starvation would militate against the hypothesis that there is a block after hexosediphosphate. Rather, it suggests that the block may be primarily at the glucokinase step, similar to that found in diabetes mellitus and in the loss of tolerance to glucose found in the "alarm reaction."

*Hereditary Nonspherocytic Hemolytic Disease.* ARNO G. MOTULSKY and OGDEN BRUTON, Washington, D. C. (Introduced by William M. M. Kirby).

Specific shape anomalies of the red cell characterize the conventional hereditary hemolytic syndromes, such as

sickle cell anemia, Cooley's anemia, hereditary spherocytosis and hereditary hemolytic elliptocytosis. Three cases from two families with an intra-erythrocytic hereditary hemolytic syndrome unassociated with specific red cell shape anomaly were studied. Mode of inheritance was dominant. Splenomegaly was present. One case (as well as several patients of another family from the literature) presented symptoms of severe hemolytic disease of the newborn.

Evidence of hemolysis (elevated indirect bilirubin, reticulocytosis, erythroid hyperplasia of bone marrow, elevated fecal urobilinogen) was found in all cases. Mean life span of the affected cells in a normal recipient was 13 days. Depending upon degree of bone marrow compensation, anemia varied from mild to severe. In contrast to hereditary spherocytosis, osmotic, mechanical and oxalate-fragility before and after incubation for 24 hours at 37 degrees was normal. Plasma hemoglobin was normal. Hemoglobin solution prepared from red cells of patients with this disease exhibited normal mobility on paper electrophoresis. In one instance fetal hemoglobin was found in slightly elevated amounts. Coombs test was negative and no abnormal antibodies could be detected. Fe<sup>59</sup> plasma clearance was determined in one case and found to be markedly accelerated. This patient exhibited significant hepatic hemosiderosis in the absence of previous transfusion or iron medication.

Splenectomy was performed in two cases. Splenic morphology differed from hereditary spherocytosis. The operation failed to produce significant improvement of hemolysis in both instances. Differential diagnosis from other hereditary and acquired anemias is discussed.

*Potassium Metabolism of Liver Mitochondria.* G. H. MUDGE,\* S. W. STANBURY and H. W. NEUBERG, New York, N. Y.

The electrolyte metabolism of an intracellular component has been examined in an attempt to obtain direct evidence on mechanisms of cellular K metabolism. Mitochondria were studied because of their high rate of aerobic metabolism, a function essential for K accumulation in many tissues.

Mitochondria, prepared by differential centrifugation of homogenates of rabbit liver, have a K/N ratio (mEq/gm) of 0.8 which is unaltered by repeated washes in chilled 0.150 N NaCl. During incubation in Warburg vessels exchange between mitochondrial and ambient K is facilitated by aerobic metabolism; the K/N ratios and apparent rate of exchange are partially depressed by incubation in nitrogen or without added substrate. After mitochondrial K is labelled with K<sup>42</sup> during incubation, the same radioactivity is recovered from the mitochondria whether subsequently washed with chilled 0.15 N NaCl or KCl, indicating minimal exchange in the absence of metabolic reactions despite a tremendous dilution by ambient cation.

The K/N ratio and respiratory rate are depressed by extremes of pH and of osmotic pressure; low concentrations of calcium and mercury are also inhibitory. Surprisingly, the mitochondrial K level was markedly de-

pressed by low concentrations of orthophosphate despite simultaneous respiratory stimulation. 2,4-dinitrophenol (DNP) had a biphasic action—K exchange was decreased at  $10^{-4}$ , and apparently increased at  $10^{-3}$  M DNP. This effect was specific for K and could not be demonstrated for Na. The increased K exchange produced by DNP is associated with an increase in the amount of mitochondrial ester phosphate, identified as phospho-enolpyruvate. The data suggest the possibility of a complex of K and organic phosphate, but definitive chemical evidence has not yet been obtained.

The precise relationship between the K metabolism of the mitochondria and that of the whole cell is at present quite obscure.

*The Effects of the Carbonic Anhydrase Inhibitor "6063" on Electrolyte and Acid-Base Balance in Two Normal Subjects and Two Patients with Respiratory Acidosis.* J. NADELL, New York, New York. (Introduced by David Seegal).

The effects of prolonged oral administration of the carbonic anhydrase inhibitor "6063" (2 acetylamino-1, 3,4 thiadiazole-5-sulfonamide) on electrolyte and acid-base metabolism have been studied in two normal subjects and two patients with pulmonary emphysema and respiratory acidosis. The effects in both groups were essentially the same.

Administration of "6063" caused a transient increase in urinary pH, increased urinary  $\text{CO}_2$ , sodium, potassium and phosphate and a fall in urinary ammonia. Associated with this there was a fall in arterial pH and  $\text{CO}_2$  content. The  $\text{CO}_2$  content remained depressed (5–10 mEq/L.) during the entire 1–2 month period of drug administration in 7 of 8 cases studied. The arterial pH rose to the control levels only after several weeks of drug administration. Upon cessation of "6063" there was a decreased urinary excretion of sodium, increased urinary excretion of ammonia and a rise of blood pH to above the control values for 3–7 days.

Most of the immediate effects of the administration of an inhibitor of carbonic anhydrase (such as sulfanilamide or "6063") on acid-base equilibrium can be explained by an interference with the renal mechanisms in which carbonic anhydrase is presumably involved. On prolonged administration of "6063", the persistent depression of the arterial plasma  $\text{CO}_2$  content despite the return of urinary electrolyte values to control levels suggests that some as yet undefined extrarenal factors are operative.

*The Escape of "Essential" and "Non Essential" Amino Acids in the Urine of Severely Burned Patients.* GEORGE L. NARDI, Boston, Massachusetts. (Introduced by Oliver Cope).

The severely burned patient excretes large amounts of amino acids in the urine. This aminoaciduria consists of both the "essential" amino acids not normally present as well as an increase in the "non essential" acids.

Seven hospitalized patients who had suffered extensive burns were placed on nitrogen balances and their urinary amino acid excretion studied by means of paper chromatography.

The qualitative and quantitative aspects of this aminoaciduria appears to correspond to the severity of the burn. Though the greatest aminoaciduria coincides with the period of greatest nitrogen loss, it is out of all proportion to the latter and suggests that in the negative nitrogen phase of burns the nitrogen containing components are not excreted proportionally.

The aminoaciduria has been studied in three patients with active Cushing's disease in order to obtain evidence regarding the role of the adrenal hormones in producing the increased excretion in the burned patient.

*The "Adhesion-Disappearance" Phenomenon: A Specific in vitro Immunologic Reaction Between Treponema pallidum, Antibody, and Normal Blood Cells.* ROBERT A. NELSON, JR., Bethesda, Md. (Introduced by Thomas Turner).

A phenomenon, tentatively termed the "adhesion-disappearance" reaction, occurs with virulent *T. pallidum* (either living or heat-killed) exposed *in vitro* to the action of syphilis serum (antibody) and to normal human whole blood (blood cells and complement).

Experimental assays indicate that a complex series of reactions take place wherein the treponemes react with antibody and complement and become adherent to normal erythrocytes. Upon addition of normal leucocytes to the treponeme-erythrocyte mixtures, the treponemes totally disappear. It is suggested that the latter reaction may be due to phagocytosis. Since no such disappearance occurs with leucocytes alone, it is concluded that erythrocytes may be a previously undetected prerequisite for phagocytic reactions with certain organisms.

Experiments are in progress to fractionate erythrocytes in order to definitively establish the role of the erythrocyte in this phenomenon.

Absorption experiments using cardiolipin antigen prove that the specific antibody essential for the reaction is separate and distinct from Wassermann antibody.

The adhesion-disappearance phenomenon has been applied to sera from 400 individuals as a method for detecting antibody. A high degree of correlation is present between the occurrence of antibody in the serum and the presence of syphilitic infection.

*Creatinuria in Patients with Leukemia and Lymphoma.* ROBERT E. OLSON and JAMES S. DINNING, Pittsburgh, Pa. (Introduced by I. Arthur Mirsky).

Since it has been demonstrated that susceptible mice with transferable acute lymphatic leukemia demonstrate excessive creatinuria at the time of maximum leucocytosis just prior to demise (Dinning, J. S. and Seagers, L. D., *Science*, 114, 502, 1951), it was thought profitable to

search for a similar metabolic abnormality in patients with various forms of leukemia and lymphoma.

23 adult male patients with lymphoma or leukemia, 4 children with acute leukemia, 2 adult males with aplastic anemia, 6 ambulatory adult male controls and 6 normal children were studied from the point of view of their 24-hour excretion of creatine, creatinine and glycoxyamine. 74% of the adult males with these diseases exhibited a significant creatinuria of the order of 140-650 mg per day whereas the adults with aplastic anemia and the normal controls exhibited none. All of the children with acute leukemia showed a significantly higher excretion of creatine than their corresponding controls. In some of the leukemic children, the excretion of creatine exceeded that of creatinine. No consistent pattern of glycoxyamine excretion was noted in leukemia.

The diseases represented in the adult group included acute and chronic lymphatic leukemia, acute and chronic myeloid leukemia, reticulum cell sarcoma, lymphosarcoma, and Hodgkin's disease. Patients with lymphosarcoma appeared to show consistently higher excretions of creatine than did those with reticulum cell sarcoma or Hodgkin's disease. Among the leukemics, those with lymphatic leukemia appeared to show more consistently higher excretions of creatine than those with myeloid leukemia, and in the chronic forms of either disease, the degree of creatinuria appeared to follow the "activity" of the disease more than the peripheral white cell count. All of the accepted methods of treatment appeared to decrease the initial creatinuria.

These preliminary data indicate that creatinuria is associated with malignancy of lymphoid tissue in man, and suggest that creatine formation is an important metabolic process of lymphocytes and lymphoid tissue.

*The Mode of Action of Streptomycin in Inhibiting the Respiration of Escherichia Coli.* THOMAS FITE PAINE, Jr., Boston, Massachusetts. (Introduced by Paul C. Zamecnik).

The antibacterial action of streptomycin has been postulated as being due to an interference with "terminal respiration at a pyruvate-oxaloacetate condensation stage." This hypothesis was based on an interpretation of *in vitro* experiments which showed inhibition of respiration of "resting" suspensions of coliform cells in the presence of certain substrates.

The experiments to be reported from this laboratory show that there is no correlation between the respiratory inhibition by streptomycin and its effect on cell viability. Interference with respiration of coliform organisms by streptomycin would appear to be due to interference with adaptive enzyme formation and not to an interference with "terminal" respiration at a pyruvate-oxaloacetate condensation stage.

*The Action of Insulin on Glucose Uptake by Muscle.* C. R. PARK, Nashville, Tenn. (Introduced by Hugh J. Morgan).

Levine *et al* have reported that the distribution of certain non-metabolizable sugars in the body water of

eviscerated dogs is greatly increased by insulin. The theory was advanced that insulin facilitated the transfer of these sugars (and glucose) across the cell wall. This theory, however, could not be tested in the case of glucose itself since this hexose does not ordinarily accumulate intracellularly but is rapidly removed by phosphorylation by the hexokinase system. Under the conditions used in the present experiments, we have obtained evidence that insulin does promote the transfer of glucose into the cell. The isolated rat diaphragm is incubated in a glucose containing medium with and without insulin and the disappearance of glucose from the medium and the accumulation of free glucose in the tissue are determined. It is found that when the concentration of glucose in the medium is raised to about 600 mg. per cent in the presence of insulin, free glucose begins to accumulate in the tissue in amounts that indicate that some has penetrated into the intracellular space. This implies that glucose is reaching the hexokinase system faster than it can be phosphorylated. In the absence of insulin, free glucose does not accumulate until glucose concentrations in the medium reach about 2000 mg. per cent. In another series of experiments the diaphragm was incubated at 10° C. in order to reduce hexokinase activity. It was noted now that insulin led to an increase in the amount of free glucose in the tissue, even with normal concentrations of glucose in the external medium. Using C<sup>14</sup> labelled glucose it was shown that the glucose in the tissue had penetrated from the external medium and was not of endogenous origin.

*Treatment of Typhoid Carriers with Penicillin and Benemid.* R. T. PARKER, J. C. FITZGERALD, H. D. PERRY and T. E. WOODWARD,\* Baltimore, Maryland.

*In vitro* growth of *S. typhosae* is inhibited by concentrations of penicillin of approximately 5 to 10 units/cc. whereas bacteriocidal effect occurs in concentrations of 20 units/cc. Penicillin in ordinary dosage regimens failed to favorably influence the clinical course of typhoid fever but several investigators have reported beneficial therapeutic effects with larger doses of penicillin and benemid achieving blood concentrations of the antibiotic to 60 units/cc.

Six typhoid carriers of long standing were treated by a regimen of 3,000,000 units of penicillin every six hours and 1.5 grams of benemid twice daily for 10 days. Determinations of antibiotic sensitivity of the typhoid bacillus, serial bio-assays of penicillin in the patients' blood serum, repeated stool cultures, cholecystograms, biliary drainage, liver biopsy and immunological tests of the patients' serum were performed in most instances.

One of the six carriers shed typhoid organisms 28 days after treatment stopped as determined by culture of the stool. Another carrier was unaffected throughout the course of treatment and observation. Of the remaining four, two have been under observation for 4 and 5 months respectively and have failed to shed the pathogens. Two additional persons have been under observation for only a short period but have remained free of *S. typhosae*.

The variability of the typhoid carrier state and the preliminary nature of this study make one cautious in drawing conclusions. However, the unsatisfactory nature of the ordinary procedures employed for typhoid carriers warrants further evaluation of the present approach.

*The Physiologic Effect of Rickettsia mooseri Toxin in Rabbits and Rats.* PHILIP Y. PATERSON and CHARLES L. WISSEMAN, JR., Washington, D. C. (Introduced by Joseph E. Smadel).

Rickettsial toxins produce death in experimental animals within a few hours but the physiologic changes culminating in exitus are poorly understood; indeed, the only changes recorded are limited to terminal hemoconcentration in mice. Furthermore, the hemolytic activity of rickettsiae, demonstrable *in vitro*, remains to be evaluated *in vivo*.

Rabbits, a new host, are less susceptible than mice to *R. mooseri* toxin but more suitable for physiologic studies. Inoculation of 4 rabbit LD<sub>50</sub> of toxin regularly produces convulsions and death within one hour. Less toxin, i.e., 1-2 LD<sub>50</sub>, elicits either similar rapid death or increasing prostration with relatively quiet exitus several hours later. Intravascular hemolysis, progressive and extensive, occurs in rabbits dying rapidly. Thus, plasma hemoglobin and plasma potassium concentrations rise sharply reaching levels of 500 mg% and 8 mEq/L, respectively, within 15 minutes and maximal levels of 3,000 and 15, respectively, terminally. In contrast, rabbits which survive several hours after smaller doses of toxin show less *in vivo* hemolysis. Within an hour these animals attain maximal plasma hemoglobin levels which rarely exceed 500 mg%. Moreover, their plasma potassium concentrations increase above normal limits only in the terminal hour with maxima of 10-12 mEq/L. Hyperkalemia, appearing early or late, is usually accompanied by prominent electrocardiographic abnormalities. Most rabbits show marked reduction in mean arterial blood pressure and slight decrease in whole blood specific gravity.

Rats never develop hemoglobinemia or hyperkalemia regardless of the amount of toxin inoculated. However, they show marked hemoconcentration with 20-30% increases in blood specific gravity and hematocrit values.

The abnormalities associated with fatal reactions from *R. mooseri* toxin vary considerably depending upon the amount of toxin and host employed. Under some circumstances *in vivo* hemolysis contributes materially to the fatal outcome while under other circumstances it is unimportant.

*Some Postural Adjustments of Salt and Water Excretion.* M. L. PEARCE and E. V. NEWMAN,\* Nashville, Tenn.

In the normal human subject undergoing moderate water diuresis quiet standing results in a slight depression of creatinine clearance and a marked depression of sodium, chloride, and water excretion as compared with

control measurements in the supine position. If the legs are wrapped with elastic bandages before the orthostatic position is assumed the depression of sodium and chloride excretion is inhibited without appreciable effect on the antidiuresis. On the other hand, administration of alcohol abolishes the orthostatic antidiuresis without significant effect on the orthostatic inhibition of salt excretion. In all experiments there was a slight fall in creatinine clearance during standing whether or not salt and water excretion were affected. Potassium excretion tended to follow sodium and chloride excretion, but changes were usually not as marked.

It is thought that there are separate postural adjustments of water and salt excretion, the former possibly under diencephalic posterior pituitary control and the latter under the control of some mechanism sensitive to the distribution of the blood volume and possibly residing in the legs themselves.

*The Role of Ovarian Function in the Growth of Mammary Carcinoma in Man.* OLOF H. PEARSON,\* CHARLES D. WEST and NORMAN E. TREVES, New York, N. Y.

Osteolytic tumors, by definition, grow by lysing bone. Bone destruction can be quantitated by measurement of calcium balance. The lysis of one gram of bone per day can readily be determined in this manner. Calcium loss is considered to be a reflection of the rate of growth of osteolytic tumors.

Calcium balance was measured in six women with osteolytic metastases from breast carcinoma during a menstrual cycle, following oophorectomy, and during ovarian hormone administration after castration. Initially, calcium balance was markedly negative in all patients. In 3 patients, calcium loss increased steadily with the progression of the menstrual cycle, reaching a peak just prior to menstruation. At menstruation, there was an abrupt decrease in the rate of calcium loss. Oophorectomy induced immediate cessation of osteolysis as indicated by lowering of calcium excretion to normal levels. 0.15 milligrams of ethinyl estradiole daily by mouth induced a prompt return of osteolysis as evidenced by hypercalcuria and hypercalcemia. Calcium loss subsided promptly on withdrawal of the hormone. Progesterone administration failed to alter calcium excretion.

In the 3 other patients calcium excretion did not fluctuate with the menstrual cycle, oophorectomy failed to inhibit the rate of osteolysis, and administration of estrogenic or progestational hormones did not increase the rate of osteolysis.

These observations indicate the existence of 2 types of mammary carcinoma in women which cannot be differentiated by the histological appearance of the tumors or by the clinical data. One type of tumor is dependent upon estrogen for growth maintenance, whereas, in the other type, the growth rate of the tumor is independent of estrogen. The clinical significance of these observations will be discussed.

*Chemical Observations on l-Hydrazinophthalazine.* H. MITCHELL PERRY, JR., St. Louis, Missouri. (Introduced by Henry A. Schroeder).

The renal excretion of l-hydrazinophthalazine has been measured in the urine of 40 hypertensive patients whose blood pressures were controlled by this agent and hexamethonium chloride. The recovery of hydrazinophthalazine approximates 3% whether given orally or parenterally. The reaction of the hydrazine radical with metal ions and soluble carbonyl and sulfhydryl compounds may explain the low values. With carbonyl groups a hydrazone is formed; the inactivation of pherentasin may occur thusly. Blood pyruvate levels were not depressed, perhaps because the amount of carbonyl compound in the body greatly exceeds that of drug. Effective therapeutic blood levels range from 0.05 to 0.2 micromolar.

A reaction occurs between substituted hydrazine and soluble mercaptan which is partially reversed by heavy metals. The urinary ratio of reversibly inactivated drug to free drug is high at the start of therapy and tends to diminish as though the sulfhydryl stores of the body were depleted. When BAL was given to one patient, no free urinary hydrazinophthalazine appeared.

In this connection the sulfhydryl content of urine as measured by amperometric titration with ammoniacal silver ion has been studied repeatedly in normal and hypertensive persons. In untreated hypertension the urine usually contains little or no mercaptan. The hypertensive titration curve can be returned to normalcy by the addition of cysteine to the urine.

Ferric iron oxidizes the terminal nitrogen atom of substituted hydrazine and leaves an heterocyclic amine; pervanadyl ion oxidizes the second nitrogen atom of the side chain. Other metals like copper, manganese, cobalt and selenium probably form complexes.

These observations are consistent with the hypothesis that certain hydrazines interact with sulfhydryl systems concerned in hypertension, perhaps with metals as intermediates.

*In Vitro Binding Capacity of Human Serum for Vitamin B<sub>12</sub>.* ROBERT PITNEY, MARION BEARD and EVERETT SANNEMAN, Louisville, Kentucky. (Introduced by Charles A. Doan).

Previous studies on urinary excretion of Vitamin B<sub>12</sub> following parenteral therapy, have indicated that the capacity of the body to retain the vitamin is limited. Vitamin B<sub>12</sub> occurs in the serum in both a free form and a form bound to serum protein. The free form is rapidly excreted in the urine.

The concentration of Vitamin B<sub>12</sub> in serum has been assayed microbiologically, using *Euglena gracilis* as test organism. This method of assay will distinguish free and bound forms of the vitamin. Normal and pathological serum have been incubated with varying amounts of crystalline Vitamin B<sub>12</sub>, and the mixtures assayed for bound and free concentrations. Results of these studies show relatively limited capacity of serum to bind Vitamin

B<sub>12</sub>. The significance of this finding in the treatment of Pernicious Anemia is discussed.

The capacity of the various fractions of serum to bind Vitamin B<sub>12</sub> and the location of naturally occurring bound B<sub>12</sub> has been investigated by paper electrophoresis.

*The Relationship Between Pathologic and Electrocardiographic Changes in Myocardial Disease.* MYRON PRINZMETAL,\* S. REXFORD KENAMER, CLINTON SHAW, MORTON MAXWELL and JACK BERNSTEIN, Los Angeles, California.

As observed by most clinicians, patients with essentially normal electrocardiograms occasionally die of heart disease while others with greatly distorted tracings exhibit relatively normal myocardial function. Such puzzling inconsistencies between the physiologic and electrocardiographic findings may be explained by the following experimental observations.

In 62 animals the anterior descending coronary artery was tied and electrocardiographic studies made several days or weeks after ligation. Histologic examination revealed that 8 of the infarcts were subendocardial, involving no more than the innermost two-thirds of the wall, while the remaining 54 were transmural. Leads from the precordium and intact surface overlying the subendocardial infarcts consistently presented essentially normal depolarization waves. In 7 other animals, subendocardial burns limited to the innermost two-thirds of the left ventricle were inflicted with an electric cautery. Again, epicardial leads recorded over the subendocardial lesions contained normal R or RS waves. On the other hand, subepicardial damage produced by mechanical, chemical or thermal trauma to the outermost third of the ventricle in 41 animals consistently caused markedly abnormal depolarization complexes in surface leads. In 10 such instances coronary QS waves were obtained.

These findings establish that, contrary to current belief, QR waves do not necessarily occur over subendocardial infarcts, and coronary QS waves do not always indicate a "hole" or transmural lesion in the underlying wall. Rather, depolarization of the large mass of ventricular muscle appears to be electrocardiographically "silent." Physiologically serious lesions involving the innermost two-thirds of the ventricle apparently fail to produce QRS changes if the epicardial region remains intact while, conversely, less important lesions affecting mainly the epicardial region produce ominous electrocardiographic signs. Thus the depolarization electrocardiogram may be affected primarily by location rather than magnitude of the lesion, thereby offering an explanation for certain discrepancies between the clinical, electrocardiographic and post-mortem findings in patients with myocardial disease.

*The Clinical and Physiological Contrasts Between Mitral Stenosis and Mitral Insufficiency.* MURRAY RABINOWITZ and LAWSON McDONALD, Boston, Mass. (Introduced by William P. Murphy).

Eighty-seven patients with mitral valvular disease were examined to determine whether stenosis or insufficiency

was predominant. Methods of investigation: clinical, electrocardiographic, radiological examinations, also cardiac catheterization, determination of cardiac output and measurement of pulmonary pressures. Respiratory symptoms occurred in severe mitral stenosis, in mixed valvular disease, and in mitral insufficiency in the presence of left ventricular failure. Severe stenosis caused a right ventricular type of apex beat and incompetence left ventricular. A loud first heart sound and opening snap was a very reliable indication of mitral stenosis, almost always excluding significant insufficiency. Apical systolic and rumbling diastolic murmurs were the rule in insufficiency and stenosis respectively: their absence did not exclude these conditions nor did their presence indicate either to exist in a hemodynamically significant degree. Right ventricular hypertrophy on the electrocardiogram indicated predominant mitral stenosis and left frequently pure mitral insufficiency, but left, combined, or no ventricular hypertrophy were less indicative than right in determining the predominant lesion. On x-ray, right ventricular enlargement and a prominent pulmonary artery and branches in mitral stenosis, contrasted with the left ventricular enlargement, inconspicuous pulmonary artery and clearer lung fields of insufficiency. Increased pulmonary arteriolar resistance and low cardiac output were common in advanced mitral stenosis; comparable pulmonary vascular disease was absent in severe insufficiency. It is concluded that the syndromes of pure mitral stenosis and insufficiency can easily be differentiated and that in mixed mitral valvular disease the predominant lesion can be determined in the great majority of patients.

*The Metabolism of Labeled l-triiodothyronine, l-thyroxine and d-thyroxine.* J. E. RALL, JACOB ROBBINS, DAVID BECKER and RULON W. RAWSON,\* New York, N. Y.

The metabolism of *l*-thyroxine, *d*-thyroxine and *l*-triiodothyronine labeled with radioiodine was studied in subjects with and without thyroids and in one individual with a complete biliary fistula.

Radioactivity was measured at various points over the body, in blood, urine and stool at intervals for three weeks after administering the substance intravenously.

Both isomers of thyroxine disappeared from the blood at a rate which could be decomposed into five exponential decay rates. Absence of the thyroid or drainage of bile to the outside reduced these to four. The first three rates were approximately the same with both isomers. The half life of the final rate for *l*-thyroxine was between seven and eleven days and between three and four days for *d*-thyroxine. The initial spaces into which both *d* and *l*-thyroxine were distributed were similar (35 to 60 cc/kg) but the final volumes of distribution (V.D.) varied markedly (*l*-thyroxine, 170 to 270 cc/kg; *d*-thyroxine, 570 to 830 cc/kg).

In a thyroidless individual, *l*-triiodothyronine disappeared from blood at a rate which could be decomposed into four exponential rates. The final rate was markedly faster than *l*-thyroxine (half time approximately two days). The initial VD (44 cc/kg) was similar to that

for both isomers of thyroxine but the final VD reached a value exceeding the body weight (1170 cc/kg).

The nature of the radioiodine in blood and urine was studied by chromatography. These observations will also be presented.

These data suggest that:

1. The optical isomers of thyroxine are metabolized at markedly different rates although they are distributed in a similar manner in the body pools.
2. Triiodothyronine is metabolized at a much faster rate than *l*-thyroxine and although initially is distributed in a space similar to that of thyroxine the final VD exceeds the body weight.

*Serum Hemolysin for Erythrocytes Coated with Non-species Specific Bacterial Products in Health and in Various Diseases.* LOWELL A. RANTZ,\* San Francisco, California.

A nonspecies specific heat stable substance is present in culture filtrates of most gram positive bacteria which prepares erythrocytes for hemolysis in the presence of complement and certain human sera. The serum hemolysin responsible for this reaction has been extensively studied. It possesses the characteristics of a true antibody. It is adsorbed on the coated red cells and the reaction requires complement. Titers are higher and comparable to those found in the mother in certain children at birth and wane rapidly during the first half year. A progressive increase occurs in healthy persons during life.

The actual antigen responsible for this antibody response in human beings is unknown. Mean concentrations are high in acute rheumatic fever, periarteritis nodosa, and disseminated lupus erythematosus, scleroderma, and acquired hemolytic anemia. They are normal or low in rheumatoid arthritis, acute nephritis, and nephrosis. It is probable that this antigen-antibody system is not intimately related to the pathogenesis of these disorders. The presence of these abnormal antibody patterns may well reflect an immunological hyperreactivity of persons who develop them. It is believed that these observations add further weight to the hypothesis that inappropriate immunological reactions are involved in the causation of certain of these disorders.

*Delayed Blood Coagulation in Multiple Myeloma.* OSCAR D. RATNOFF,\* Cleveland, Ohio.

Unexplained hemorrhagic phenomena have been described in occasional patients with multiple myeloma. Eleven patients with this disease were studied. Although minor bleeding was observed in six patients, none had a major hemorrhagic diathesis.

Defective coagulation was observed in 8 of 11 patients with myeloma, revealed by delayed coagulation in glass tubes in 4, and in silicone-coated tubes in 7. No single mechanism appeared to explain the prolonged clotting time. There appeared to be no abnormality in the conversion of prothrombin to thrombin. The concentration of fibrinogen was either normal or elevated. In 3 of 8 patients with delayed clotting, the one stage prothrombin time was slightly prolonged. In 5 of 7 patients with

delayed clotting in silicone, and all with delayed clotting in glass, the clotting time of a mixture of oxalated plasma and thrombin (thrombin time) was prolonged. This defect was described in a patient with myeloma by Luscher and Labhart. The abnormality which prolonged the thrombin time was present in the patients' defibrinated plasma. In all of the patients with defective coagulation, the concentration of serum globulin was elevated. However, the concentration of globulin in serum did not correlate with the clotting time. Globulin fractions of normal human plasma (Cohn) did not prolong the thrombin time when added to human fraction I or to normal plasma. The nature of the defect remained unexplained. Experiments in this and other laboratories have demonstrated that minor alterations in the milieu, such as small changes in the concentration of bivalent ions, alter the thrombin time. It is possible that such minor changes are responsible for the alteration in thrombin time, and, in turn, for the defect in clotting observed in these patients

*Effect of Rubidium on Acid-Base Balance in Potassium Deficient and Normal Rats.* ARNOLD S. RELMAN, ARLENE M. ROY and WILLIAM B. SCHWARTZ, Boston, Mass. (Introduced by Chester S. Keefer).

The close physico-chemical similarity of rubidium and potassium suggests the possibility of similar physiological roles for these elements. Others have shown that rubidium will prevent some of the pathological changes in experimental potassium depletion and that the distribution of administered rubidium is similar to that of potassium. The present study was designed to compare the effects of rubidium and potassium on acid-base balance.

Severe metabolic alkalosis and hypokalemia were produced in weanling rats by administration of potassium-free, alkaline ash diets. Intraperitoneal injection of the neutral chloride salt of rubidium, 5 mEq./kgm. daily for six days, resulted in a depression of plasma bicarbonate to levels below normal controls ( $p < .01$ ) and below those found in animals treated with equal amounts of potassium chloride ( $p < .01$ ). Alkalotic potassium-deficient rats given a total of 18 mEq./kgm. of rubidium or potassium chloride in a period of one day reduced their bicarbonate concentrations about equally, but slight alkalosis persisted in both groups.

Normal weanling rats given potassium chloride 5 mEq./kgm. daily for six days showed no change in blood bicarbonate, while those given equivalent amounts of rubidium developed a slight acidosis. Rubidium chloride appeared to be well-tolerated in all experiments.

Under the conditions of this study, rubidium appeared to have an acidifying effect equal to or greater than that of potassium. The mechanism of this action is under investigation.

*Histopathology and Bacteriology of Biopsied Parietal Pleura.* MARION L. RICE and FELIX HUGHES, Memphis, Tennessee. (Introduced by W. D. Sutliff).

Although biopsy of the pleura has been practiced from time to time by thoracic surgeons for the diagnosis of

pleural disease presenting unusual problems it was seldom used as an aid to medical diagnosis and no studies of technique or results were found in the literature. This report is a preliminary evaluation of the data with respect to the diagnosis and study of pleural disease. Technical procedures will be described elsewhere.

Cases were selected in which the results of other diagnostic studies were inconclusive and in which the response to therapy was incomplete. Definitive diagnoses, or confirmatory data, were obtained in 19 of 21 cases. In the age group 21 to 50, 14 cases were all found to be tuberculous. In the age group 51 to 65, 3 were found to have tuberculosis, 3 had carcinoma, and 1 had North American blastomycosis. Tubercle bacilli were found in 8 cases. In the remaining 9 cases of tuberculosis, the diagnosis was confirmed by recognition of chronic granulomatous inflammation, probably tuberculous, by histopathological examination. All the specimens were the sites of active granulomatous, or carcinomatous, reaction. The duration of the lesion varied in the cases of tuberculosis from 2 months to 11 months. The degree of fibrosis, caseation, and the type of cellular reaction did not vary with the age of the lesion. Tubercle bacilli were found microscopically only in the earlier cases, and extreme grades of pleural thickening and walled-off cavities were found only in the older lesions.

It was concluded: 1. Pleural biopsy was particularly valuable for differential diagnosis in the cancer age. 2. Specific evidence of tuberculosis, not otherwise obtainable, was found by bacteriological examination of biopsied pleural tissue.

*Acute Alterations in Blood Volume, Plasma Electrolytes and the Electrocardiogram Produced by Oral Administration of Hypertonic Solutions to Totally Gastrectomized Patients.* KATHLEEN E. ROBERTS, HENRY T. RANDALL and HOLLON W. FARR, New York, N. Y. (Introduced by Henry J. Tagnon).

Oral administration of hypertonic glucose, saline or protein hydrolysates to gastrectomized patients produces an immediate increase in jejunal motility as seen fluoroscopically or as measured with intraluminal balloons. Within ten minutes following the administration of such hypertonic solutions there occurs a progressive decrease in plasma volume and hemoconcentration which lasts approximately 20-30 minutes; this is followed by an increase in plasma volume and hemodilution which reaches a maximum in 70-80 minutes. Concomitant with the increase in plasma volume there is a fall in the plasma concentrations of sodium, potassium, chloride and bicarbonate. The clinical pattern of the "dumping syndrome" is produced simultaneously in most cases.

The electrocardiographic alterations which occur consist of tachycardia, elevation or depression of the S T segment, flattening or inversion of the T wave and prolongation of the Q T interval. The time sequence is such that the electrocardiographic changes begin within 5-10 minutes following hypertonic solutions and before alterations in

plasma electrolyte structure are measurable. Frequently the electrocardiogram returns to normal before the plasma alterations have returned to control values.

*Cerebral Metabolism and Hemodynamics in Pernicious Anemia.* EUGENE D. ROBIN and FRANK H. GARDNER, Boston, Massachusetts. (Introduced by Eugene C. Eppinger).

• Three patients with untreated pernicious anemia were studied by means of the Kety-Schmidt technique for determining cerebral blood flow and metabolism. Before treatment each patient showed a striking increase in cerebral blood flow. This increase in flow was in each case proportional to the decreased hematocrit. When these patients were transfused to relatively normal hematocrit levels, cerebral blood flow values rapidly fell to normal. Cerebral oxygen consumption was completely normal prior to treatment and remained normal following transfusions and after the administration of Vitamin B<sub>12</sub>. Even in the two patients with striking neurological changes normal cerebral oxygen consumption was present. It is therefore concluded that:

1. Cerebral blood flow is increased in the untreated patient with pernicious anemia.
2. The increased blood flow is proportional to the decrease in red cell mass.
3. Cerebral oxygen consumption is normal in this group of patients.

*The Utility of the Korotkoff Sounds in the Assessment of Cardiovascular Function.* SIMON ROBBARD, Chicago, Ill. (Introduced by Louis N. Katz).

The sounds heard at the brachial artery during sphygmomanometry are generally ignored except for the signalling of the levels of the systolic and diastolic pressures. Our studies on man and on circulation models have demonstrated that these sounds also contain information concerning the blood flow to the extremities and certain parameters of the state of the systemic circulation. Reduction of blood flow to the extremity beyond the point of auscultation results in a markedly reduced intensity and duration of the Korotkoff sounds. This effect was also produced in bloodless venesection and during surgery, when vascular collapse was impending, often 15 minutes or more before the descent of the blood pressure into shock levels. An increase in blood supply to the extremity causes an increased intensity and duration of the sounds. This was observed in reactive hyperemia and in states of vasodilatation, as for example in pregnancy. By recording the sounds simultaneously with the electrocardiogram, an accurate measure of the time required for the transmission of the pressure pulse is obtained. Plotting these values against the pressure in the sphygmomanometer provides a calibrated pressure-pulse contour. These studies indicate that the Korotkoff sounds provide considerable useful information, obtainable with little effort, concerning the state of the systemic and peripheral circulation.

*The Effect of Antibiotics on the Excretion of Urinary Phenols.* WALTER F. ROGERS, JR., MARY P. BURDICK and GEORGE R. BURNETT, Syracuse, N. Y. (Introduced by R. H. Lyons).

In the course of studying tyrosine metabolism it has been found that antibiotics given by mouth have a marked effect on the formation and excretion of volatile phenols (p-cresol and phenol). Fifteen subjects were given antibiotics (terramycin, aureomycin, chloramphenicol or penicillin). Urine in all and stools in three were analyzed for phenolic compounds before, during, and after treatment. After acid hydrolysis of urine and stools, phenols were separated into three fractions: Ether insoluble, ether soluble and extractable from the ether by 4% NaHCO<sub>3</sub>, and ether soluble requiring 2% NaOH for extraction. The last fraction contains the volatile phenols, chiefly phenol and p-cresol. The phenolic content of all fractions was estimated by a modified Millon's reaction. Normally the excretion of volatile phenols in the stools is only 1/4 of that in the urine. When an adequate amount of antibiotic is given orally (e.g. 2 gms. of terramycin daily) volatile phenol excretion in the urine falls from control values of 75 to 125 mg/day to levels of 0-8 mg/day, representing a 94 to 100% decrease in individual cases. The volatile phenol content of the stool also diminishes markedly while the ether insoluble fraction of the stool which contains tyrosine rises. Aureomycin, chloramphenicol, and penicillin (1.5 million units daily) by mouth have a similar effect whereas penicillin parenterally in the same dosage has little effect. These findings are consistent with the concept that volatile phenols are normally formed in the intestine by bacterial action on tyrosine, that they are rapidly absorbed, conjugated by the liver and excreted in the urine and that antibiotics can suppress their formation and thus their systemic metabolism.

*Hemodynamic Alterations Produced by a Plastic Valvular Prosthesis for Severe Aortic Insufficiency in Man.* JOHN C. ROSE, CHARLES A. HUFNAGEL, W. PROCTOR HARVEY and EDWARD A. PARTENOPE, Washington, D. C. (Introduced by Theodore J. Abernethy).

A plastic ball valve has been inserted into the thoracic aorta of patients with aortic insufficiency. Such patients still have regurgitation above the prosthesis but not below it. Since an estimated 60 per cent of the cardiac output traverses the valve, it was hoped that the reduced volume of regurgitation might permit recovery of cardiac efficiency. Thus far, studies have been completed pre and postoperatively in 4 patients in order to determine the overall hemodynamic effects of the operation.

Cardiac outputs (Fick), initially subnormal in all instances, increased significantly postoperatively. The mean increase was 46 per cent (range 14 to 77 per cent). Right ventricular pressure decreased in those patients who exhibited elevations preoperatively.

Prior to surgery pressure pulse contours in both brachial and femoral arteries revealed the typical wide pulse pressure and low diastolic pressure of aortic insufficiency. Following operation the femoral pressure pulses became



normal with the appearance of a sharp incisura. In the area above the valve the brachial pulse pressure widened postoperatively in all cases.

Preoperatively injection of T-1824 into the right ventricle produced dye concentration curves from the femoral artery which were abnormally flat and prolonged. Following surgery these curves became steeper and the average dye circulation time (Hamilton) decreased by 3.5, 7, 9 and 15 seconds respectively. Similar improvement in circulation time and configuration occurred in a simultaneous dye concentration curve from the brachial artery despite persistence of regurgitation in this area. This indicates that the broad, delayed, preoperative curves were not due to the regurgitation, *per se*, but more probably to pooling in the dilated left ventricle.

The increased cardiac output, widened pulse pressure in the arteries above the valve, and steeper dye concentration curves all suggest that more complete emptying of the left ventricle follows operation.

*A Comparison of Insulin Treatment with and without Added Carbohydrate in Human Diabetic Ketosis.*  
MARVIN ROSECAN and WILLIAM H. DAUGHADAY,\* St. Louis, Missouri.

A controversy exists whether the administration of carbohydrate to patients in diabetic acidosis actually speeds recovery from ketosis. To study this problem, the fall of blood ketones in diabetic ketosis during insulin therapy with and without the addition of carbohydrate has been measured.

A direct comparison of therapies was made possible by inducing ketosis 15 times in 5 patients by insulin withdrawal for 1-3 days. Blood total ketone levels at the start of therapy averaged 5.1 mM/L. Insulin was given to all patients in an average dose of 50 units and was constant for all studies on any one patient. Glucose or fructose was administered intravenously (0.8 mg/kg/hr) for four hours. Control patients received a comparable volume of normal saline solution. Blood levels and urinary excretion of glucose, fructose and total ketones were followed for 6 hours.

With insulin and saline therapy (5 patients) the blood ketones fell from 4.6 mM/L to 3.1 mM/L after 6 hours. When insulin and fructose were given (5 patients) the blood ketones fell from 5.9 to 2.7 mM/L. Insulin and glucose administered to 3 patients resulted in a fall in blood ketones from 5.2 to 1.8 mM/L. Analysis of the results indicates that the addition of carbohydrate to insulin in the treatment of diabetic ketosis results in a significantly faster fall of blood ketone levels. No superiority of fructose over glucose in respect to rate of decrease in ketonemia could be demonstrated in this small series.

The total reducing sugar of the blood was significantly higher when insulin and glucose were given as compared to insulin and fructose. Seventy-eight per cent of the administered carbohydrate was retained when fructose was given as compared to 32 per cent when glucose was given.

*Metabolic Effects of an Anterior Pituitary Extract.* I. N. ROSENBERG, Boston, Massachusetts. (Introduced by E. B. Astwood).

The material (preparation D or oxycel-purified corticotropin) which is extracted from hog anterior pituitary by glacial acetic acid, adsorbed by oxycellulose and eluted by acid, is not only rich in corticotropin and intermedin but also produces marked adipokinetic and respiratory metabolic effects. Administered intraperitoneally to adult female mice in doses of 3 to 15 micrograms, the extract causes the concentration of hepatic lipid to rise 40 to 50 per cent above control values within three to four hours. The adipokinetic potency of preparation D is approximately 100 times that of the starting pituitary powder. Since preparation D contains no detectable gonadotropic, thyrotropic, or growth activity, the adipokinetic effect is not attributable to these principles. Potent growth hormone preparations were only 1 to 5 per cent as active as preparation D in adipokinin. The adipokinetic effect is not mediated by the adrenal since preparation D produces fatty liver in adrenalectomized mice maintained with cortisone. Adipokinin is not intermedin since these activities are partially separable, and conditions of high pH and temperature which potentiate intermedin decrease adipokinin activity. Peptic or tryptic digestion decreases the adipokinetic activity of preparation D.

The administration of preparation D (10 to 25 micrograms) to mice results in an appreciable increase in the rate of oxygen consumption unassociated with increased activity or with a rise in body temperature. The survival time of injected animals placed in closed vessels is considerably shorter than that of controls, and the former die at a somewhat higher concentration of residual oxygen. Pretreatment of mice with preparation D prevents or reduces the rise in respiratory quotient otherwise induced by glucose.

It is not clear whether the factor which induces fat mobilization is the same as that which affects the respiratory quotient and metabolic rate.

*Studies on Hemorrhagic Diseases Caused by Deficiencies of Plasma Thromboplastin Factors.* ROBERT L. ROSENTHAL, O. HERMAN DRESKIN and NATHAN ROSENTHAL, New York, New York. (Introduced by Arthur M. Fishberg).

Studies were performed on 4 patients with hemorrhagic diseases and with blood coagulation findings resembling hemophilia: prolonged clotting times, impaired prothrombin consumption, and normal values for platelet count, prothrombin time, tourniquet test and bleeding time. No anticoagulants were present. However, matching experiments revealed that the bloods of these patients mutually corrected the clotting defect in true hemophilic or anti-hemophilic globulin (AHG) deficient blood. Three of these patients are members of one family (Family A), a 50 year old maternal uncle and 2 nieces, aged 25 and 29 years. Their hemorrhagic manifestations have been relatively mild and marked chiefly by profuse bleeding after tooth extractions. Their coagulation findings

are alike and mixtures of their bloods with one another do not correct the clotting defect. The fourth patient is a 4 year old male (D. R.) who has had intermittent episodes of severe bleeding dating back to the first week of life. There is no family history of bleeding. Further matching experiments revealed that mixtures of the blood of D. R. with bloods from members of Family A mutually corrected each other. D. R. has been positively identified as a case of plasma thromboplastin component (PTC) deficiency. A third plasma thromboplastin factor, deficient in members of Family A, has been designated as plasma thromboplastin antecedent (PTA).

Further identification of the 3 types of plasma thromboplastin clotting deficiencies can be made by the effects of BaSO<sub>4</sub>-treated normal plasma and normal serum. AHG deficiency is corrected by BaSO<sub>4</sub> plasma but not by normal serum. PTC deficiency is corrected by normal serum but not by BaSO<sub>4</sub> plasma. PTA deficiency is corrected by both. Fraction I does not correct PTC or PTA deficiency.

Identification of 3 plasma thromboplastin clotting factors should clarify the diagnosis of patients with hemorrhagic diseases resembling hemophilia.

*New Cytologic Techniques for In Vivo Study of the Pathogenesis of Gastrointestinal Neoplasia.* C. E. RUBIN, B. W. MASSEY, D. D. STONECYPHER and R. L. DOBSON, Chicago, Illinois. (Introduced by J. B. Kirsner).

Techniques for cell collection and tissue biopsy have been developed after studies in more than 250 patients with gastrointestinal disease. Any chosen area of the esophagus or stomach is accessible to repeated harmless biopsies under fluoroscopic control using a flexible tube developed in this laboratory. A knuckle of mucosa including some muscularis is sucked into the tube and excised. The excellent histologic preservation differs markedly from the usual autopsy and surgical material.

Proper preparation of the patient for gastrointestinal cytologic examination is essential. It avoids contamination by food and detritus. Better cell collection techniques and rapid processing minimize cell digestion. In the esophagus, lavage provides excellent cytologic material. In the stomach, a net-covered, mercury-weighted balloon abrades both the proximal and distal portions, the latter being an area of predilection often inaccessible to other methods. Lavage with suitably buffered chymotrypsin digests the gastric mucous barrier, releasing well-preserved mucosal cells. In the duodenum, a mercury-weighted tube with an inner "bleeder" tube is passed under fluoroscopic control. Cell drainage from the liver, biliary tract and pancreas is stimulated by the administration of amyl nitrite, magnesium sulfate, intravenous secretin and decholin. The duodenal tube is adaptable to small bowel cytologic study. In the left colon, high enemas accompanied by abdominal massage secure useful cytologic material. Since the latter technique is not consistently successful in right colon study, an enema tube is passed to

the splenic flexure by using a flexible obturator, an iron tip and external magnetic control.

Repeated study of cell changes in the gastrointestinal mucosa of cancerprone patients (those with pernicious anemia, familial polyposis, etc.) should contribute to a more dynamic understanding of tumor development.

*Anomalous Serum Proteins in Malignant Lymphomas and Leukemia.* R. W. RUNDLES,\* TULLIO ARENDS and EVELYN V. COONRAD, Durham, North Carolina.

Abnormal serum protein components, comparable to those occurring in multiple myeloma, were sought for in the sera of patients with other hemopoietic malignancies. Ninety-two sera from 61 patients with Hodgkin's disease, malignant lymphomas, and leukemia were studied by electrophoresis. The findings were analyzed in relation to the clinical status and changes in disease activity induced by therapy.

Forty-one patients had abnormal electrophoretic serum patterns. Non-specific abnormalities, reversible by effective therapy, were characteristically present in seriously ill patients. These included albumin concentrations of less than 3 gm.% in 19 instances, increased alpha or beta globulins in 23, and gamma globulin of over 1.5 gm.% in 20. The gamma increments were usually of less than average mobility. Increased gamma globulin was present in 11 of the 25 patients with Hodgkin's disease or malignant lymphoma. One patient had agammaglobulinemia.

Abnormal components of gamma or "M" mobility in quantities of 0.5-7.8 gm.% were found in the sera of 6 patients. One was associated with cryoglobulin. Four patients had sub-leukemic lymphocytic leukemia, one atypical lymphocytic leukemia, and one erythroleukemia with some of the primitive elements resembling plasma cells. Three patients improved with TEM or urethane chemotherapy, and the quantity of abnormal protein in their sera decreased.

Anomalous serum proteins have been found only in those hemopoietic malignancies in which there is proliferation of primitive lymphoid or plasma cells. Their occurrence has no correlation with the anatomic extent of the disease, clinical course, amenability to therapy, etc. The abnormal proteins may signify, accordingly, a perversion in normal protein synthesis rather than represent an abnormality intrinsic to the malignant process.

*The Determination of Albumin in Human Serum. A Method Based on the Interaction of Albumin with an Anionic Dye—2-(4'-hydroxybenzeneazo) Benzoic Acid.* DAVID D. RUTSTEIN,\* ESTELLE FASOLINO INGENITO and JEANNE M. BOURNEUF, Boston, Massachusetts.

A new and simple method has been devised for the quantitative measurement of human serum albumin, based on the colorimetric reaction of serum albumin with an anionic dye, 2-(4'-hydroxybenzeneazo) benzoic acid, hereinafter termed "HBABA."

The validity of this method has been established through

the following series of experiments on the spectrophotometer, which demonstrate:

I. The spectrophotometric absorption curve of the free dye, HBABA.

II. That the spectrophotometric absorption curve of the dye-albumin complex differs sufficiently from that of the free dye, HBABA, to permit measurement of albumin in an excess of the free dye.

III. The linear relationship between concentrations of albumin and optical density of the dye-albumin solution, i.e., establishment of a working curve which follows Beer's Law, in a range adequate for measurement of human serum albumin.

IV. The non-interference of human serum with the dye-albumin reaction.

V. The non-reaction of other plasma fractions with HBABA and absence of their interference with the dye-albumin reaction.

VI. The method of albumin measurement in normal serum, and correlation of results with those obtained by electrophoresis.

VII. The reproducibility of the method.

VIII. The comparison of results in abnormal bloods with the usual clinical laboratory method.

Finally, this spectrophotometric method has been modified for use in the Evelyn photoelectric colorimeter.

This simple colorimetric method, adapted for use in the usual clinical laboratory, depending upon the reaction of an anionic dye, 2-(4'-hydroxybenzeneazo) benzoic acid, and serum albumin, does not require salting out or Kjeldahl determination, correlates better with electrophoresis than the usual clinical laboratory methods, and should provide a useful tool for the clinical investigator.

*The Incidence of Hepatitis in Korean Casualties Following the Use of Pooled Plasma.* VICTOR M. SBOROV, BURTON GIGES and JOSEPH MANN, Washington, D. C. (Introduced by F. W. Hoffbauer).

The outbreak of hostilities in Korea with the resulting casualties afforded an opportunity to determine the incidence of viral hepatitis following the use of pooled plasma and whole blood. A study covering the period from November 1950 to June 1951 was made in three Army Hospitals in the United States. Patients were included in this study if they were wounded and were known to have received whole blood or plasma transfusions. It was possible to follow the majority of these patients from thirty to one hundred eighty days from the time of wounding. Careful clinical examinations were made at weekly intervals for evidences of liver disease. Urines were tested twice weekly for urobilinogen and bilirubin; in doubtful cases other liver function tests and liver biopsies were taken to clarify the diagnosis.

It was possible to follow a total of 587 casualties in this manner. Of these 255 had received plasma and blood, and 332 had received blood alone. Fifty-six, or 21.9 percent, of those who received plasma and blood, developed hepatitis with jaundice (serum bilirubin greater than 2

mg. percent) while only 12, or 3.6 percent, of those who received blood alone developed hepatitis with jaundice. Other than the type of transfusion received, there was no difference in the composition of the two groups. The mean "probable incubation period" for this hepatitis was  $90.3 \pm 11.7$  days.

In the first months of this survey only unirradiated pooled plasma was used but in the later months this was withdrawn and replaced with plasma that had been reconstituted, pooled, irradiated with ultraviolet light and lyophilized. No difference in the incidence of hepatitis could be related to the use of plasma which had been irradiated.

This study is of significance because it emphasizes the impressive risk of hepatitis following the use of pooled plasma and demonstrates that there is apparently a lack of protection by ultraviolet irradiation against the virus of hepatitis in pooled plasma.

*Endothelial Permeability in Man Studied with Albumin-<sup>125</sup>I Injected Intravenously or Intraperitoneally.* JAMES A. SCHOENBERGER, ROBERT M. KARK,\* GEORGE KROLL and EDWARD ECKERT, Chicago, Illinois.

Albumin-<sup>125</sup>I was used to study endothelial permeability in 9 non-edematous controls and in 10 patients ill with cirrhosis and ascites. Tracer doses were injected intravenously or intraperitoneally and radioactivity in blood and ascitic fluid was measured at frequent intervals, up to 14 days post injection. To observe the effects of rehabilitation with high-protein diet therapy these experiments were repeated at intervals in four cirrhotic patients, treated in a metabolic ward.

The studies disclosed a dynamic equilibrium between albumin in intravascular and extravascular sites. Mathematical analyses (Gastroenterology, 22:607, 1952) yielded quantitative rates of exchange of albumin across peritoneal membranes and the vascular endothelium.

Following intravenous injection in controls, 3.2 gm of albumin were exchanged per hour (range 2.2 to 4.6 gm/hr). In cirrhotics the average was 3.4 gm/hr (range 1.05 to 7.1 gm/hr). During dietary rehabilitation of four cirrhotics, alterations in permeability were observed. For example, in patient F.H., studied in March, May and November, 0.61, 0.51 and 0.21 gm albumin/hr, respectively, were exchanged between plasma and ascitic fluid.

These changes in permeability in cirrhosis were confirmed by parallel studies of electrophoretic patterns and osmotic pressure measurements of plasma and ascitic fluid, made serially during dietary rehabilitation. In F. H., who was "highly" permeable by radioactive albumin studies, ratios of serum albumin and gamma globulin to ascitic albumin and gamma globulin were close to unity, showing that the peritoneal membrane was equally permeable to both large and small protein molecules. On the other hand, in patient R. E., who was "poorly" permeable, the ratio for albumins was 0.6 and for gamma globulins was 2.2, indicating less permeability to globulin than albumin.

*Renal Retention and Diuresis Occurring in Association with Certain Life Situations.* WILLIAM W. SCHOTTSTAEDT and LAWRENCE E. HINKLE, JR., New York, N. Y. (Introduced by Harold G. Wolff).

The rates of renal excretion of water, sodium, and potassium were followed in five healthy subjects carrying on their daily routine activities over periods ranging from 10 to 46 days and in more than 30 patients under carefully controlled conditions for short periods of 2 to 4 hours. Rates of excretion observed under neutral or tranquil conditions were used as a baseline for comparison. Situations evoking excitement or apprehension were accompanied by increased rates of excretion of water and sodium whereas situations evoking resentment were associated only with an increase in sodium excretion. Working under circumstances evoking feelings of being "pressed" also was associated with a sodium diuresis. Situations accompanied by feelings of tension were associated with decreased rates of excretion of water and sodium while those accompanied by feelings of depression were associated with decreased rates of excretion of water, sodium, and potassium. Active relaxation, i.e., the release of tension, was accompanied by a water diuresis with little change in electrolyte excretion. These variations in fluid and electrolyte excretion were sufficiently constant to permit fairly reliable prediction of feeling state from excretory rates alone.

*The Clearance of Urea by the Sweat Glands.* IRVING L. SCHWARTZ, JORN HESS THAYSEN and VINCENT P. DOLE,\* New York, N. Y.

The clearance of urea through the skin has been used to trace the movement of water within sweat glands. Methods have been developed for quantitative measurement of excretory rates of urea and water from a region of skin 1 inch in diameter. Collection times of 10 to 30 minutes provide enough sweat for accurate analysis, and thus allow an experiment to be divided into a series of timed periods.

It was found that the concentration of urea in sweat, S, was about twice that of the concentration of urea in plasma, P. This proportional relationship was maintained when P varied from 12 to 300 mg per cent. Data from 200 collection periods, representing 42 experiments on 13 subjects, yielded the regression  $S = (1.82 \pm 0.019)P - (6.6 \pm 2.6)$ . An analysis of variance showed the ratio S/P to be independent of the absolute fluxes of urea and water, and therefore to be independent of the expenditure of energy.

The delay in transfer of urea from plasma to sweat was shown to be only 5 minutes or less. In two experiments S followed a rising P with a time lag of about this magnitude.

Lack of saturation of the transfer mechanism despite 180 fold increase in the rate of excretion suggests that urea is concentrated in the sweat by reabsorption of water from a precursor solution, and not by specific secretion. If this is correct, the precursor solution must be concentrated by the reabsorption of a constant fraction of

water, since S is independent of the sweating rate. Unlike the kidney, in which change in urine flow is due to variable reabsorption of water from a constant filtrate, the sweat gland appears to change flow by variation in the rate of formation of precursor solution.

*Electrolytes, Ammonia and Glutamine in Hepatic Coma.* R. SCHWARTZ, J. E. SEEGMILLER, G. B. PHILLIPS, G. J. GABUZDA, JR. and C. S. DAVIDSON,\* Boston, Massachusetts.

Since abnormalities in blood acid-base equilibrium, electrolytes and ammonia may be of importance in the genesis of hepatic coma, studies were undertaken to determine the biochemical abnormalities associated with this state. Patients were observed with impending coma (mental confusion and a "flapping" tremor) later progressing to coma. The mean data of initial observations for fifteen patients with impending coma and for thirteen patients with coma fell within normal limits with respect to serum pH, CO<sub>2</sub> content, and potassium concentration, although the ranges were greater than normal. As in non-comatose cirrhotics with ascites, serum sodium concentrations were decreased in both groups with mean values of 133.8 and 128.8 mM/l. respectively. Parenteral administration of hypertonic salt was without effect upon the clinical status.

Blood "ammonia" (modified Conway micro-diffusion technique) was elevated in these patients. The mean for normals was: 2 gamma-N/ml. (Range 1.0-3.0), for impending coma: 4.3 (R. 2.0-10.0), and for coma: 6.0 (R. 2.0-15.4). No consistent correlation in individual serial measurements was found between clinical status and blood "ammonia."

Since ammonia and glutamine are metabolically inter-related, plasma glutamine was determined using the specific glutaminase of Cl. Welchii to elucidate further the relationship of ammonia metabolism to hepatic coma. The mean plasma glutamine concentration for eleven normals, ten uncomplicated cirrhotics, twelve with impending coma, and nine with coma were: 8.0, 7.0, 7.6 and 11.0 gamma amide-N./ml. respectively, while the plasma "ammonia" blank was 1.3, 2.2, 2.6, and 3.5 gamma-M/ml. respectively. The plasma glutamine was not invariably elevated in deep coma.

Conclusions: 1) The abnormalities in acid-base equilibrium and serum electrolyte concentration noted do not appear to be of primary importance to the genesis of hepatic coma.

2) Plasma glutamine was elevated in patients with deep coma only.

3) Blood or plasma "ammonia" correlated best with the clinical condition.

*Renal and Extrarenal Adjustments to Acidifying Salts in Sodium-Depleted Subjects.* WILLIAM B. SCHWARTZ, ROBERT L. JENSON and ARNOLD S. RELMAN, Boston, Mass. (Introduced by Maurice B. Strauss).

Normal subjects were depleted of sodium with mercurial diuretics and sodium-free diets and then given

large oral loads of ammonium chloride or ammonium sulfate.

In both the chloride and the sulfate experiments renal conservation of fixed cation by ammonium substitution was much more efficient than that previously observed in subjects ingesting salt. Ammonium covered half or more of the urinary anion increment on the first day of anion diuresis and was related closely to the magnitude of the anion excretion rather than to extracellular acidosis. In the ammonium sulfate subjects, who had little or no acidosis, the initial ammonium response was at least as large as in the more acidotic ammonium chloride subjects.

During the first two days of anion diuresis there was no urinary loss of sodium with anion loads of less than 150 mEq. per day; sodium excretion with larger loads was roughly proportional to the load but constituted only about 10-15 per cent of the total cation increment. Most of the fixed cation increment was intracellular potassium which, by calculation, had been replaced by hydrogen ions. Immediately after withdrawal of the acidifying salt, without consistent decrease in serum potassium, there was a striking retention of potassium. At this time, ammonium replaced potassium in the urine, with consequent progressive correction of the intracellular acidosis. Calculations of internal sodium shifts suggested that some of the administered hydrogen may have exchanged with sodium.

*Microchemical Study of Constituents of Liver in Patients with Hepatic Disease.* ROBERT E. SHANK,\* ALBERT I. MENDELLOFF and MICHAEL M. KARL, St. Louis, Missouri.

Changes in chemical composition of specimens of liver obtained by punch biopsy from sixteen patients with hepatic disease have been investigated using microchemical techniques. A single biopsy specimen providing approximately 5 to 15 mg. of liver tissue is homogenized and aliquots taken for analyses for deoxyribose nucleic acid (DNA), ribose nucleic acid (RNA), glycogen, protein, diphosphopyridine nucleotide (DPN), flavin adenine dinucleotide (FAD), free riboflavin, and hemoglobin. Values are corrected for the blood present in the specimen on the basis of hemoglobin analyses, and are expressed in terms of micrograms per microgram DNA or per microgram protein. Similar analyses done on specimens of liver from eight individuals without hepatic disease obtained either by surgical biopsy or by punch biopsy provide control observations. Patients with liver disease included nine patients with portal cirrhosis, three with acute fatty liver, and five with primary hepatic carcinoma.

Liver specimens from patients with cirrhosis were found to have significantly lower concentrations of RNA, glycogen, protein, DPN and FAD than those from normal subjects when values were related to DNA as a standard of reference. Concentrations of DPN and FAD, coenzymes important in systems of carbohydrate and protein metabolism, decreased to very low levels late in the course of this disease. Earlier in the course there was an

apparent increase in DNA per milligram protein. The changes observed in five patients with primary carcinoma of the liver were similar to those found in cirrhotics, while little change from normal values was observed in patients with fatty liver. The results presented will be discussed in terms of their possible significance in alterations of hepatic structure and function.

*Hemoglobin Tolerance in Various Types of Anemia.* SHU CHU SHEN, Boston, Mass. (Introduced by Henry Jackson, Jr.).

Twenty-six patients with anemia of various types and 5 without anemia were given by intravenous infusion a hemoglobin solution in physiological saline prepared from human red cells of homologous group. Constant rates of infusion were maintained by an adjustable mechanical pump during successive 1 hour periods. The amount of hemoglobin infused during each hour was increased by 0.325 gm. at the beginning of each subsequent hour for 5 hours. Observations were made of the plasma hemoglobin and bilirubin levels at the end of each hour for 5 hours, and at the end of 2, 4, and 19 hours after the termination of infusion.

At the end of infusion 12 of the patients with non-hemolytic anemia showed increases of the plasma hemoglobin level of between 35.9 and 57 mg.% above the initial level; and in 5 patients without anemia there were increases of between 61 and 100.9 mg.%. In 11 of the 13 with hemolytic anemia and in 1 patient with massive splenomegaly the increases were below 35 mg.%. In the other 2 patients, both with paroxysmal nocturnal hemoglobinuria, the high initial plasma hemoglobin levels increased by over 50 mg.%. However, in most of the patients with hemolytic anemia the initial rate of hemoglobin infusion could be increased 2 or 3 times without producing a detectable increase in hemoglobinemia.

Four patients with non-hemolytic anemia showed hemoglobinuria either during or shortly after infusion. None of the patients with hemolytic anemia who exhibited increases of the plasma hemoglobin level of less than 35 mg.% showed hemoglobinuria. The two patients with paroxysmal nocturnal hemoglobinuria who had perpetual hemoglobinemia displayed marked hemoglobinuria shortly after the termination of the first hour of infusion.

*The Nature of the Action of Thrombin. Enzymatic Attack on a Synthetic Substrate.* SOL SHERRY\* and WALTER TROLL, Cincinnati, Ohio.

Several investigators have demonstrated that the major enzymatic action of thrombin is proteolytic. The data suggest that one or more peptides are split from fibrinogen, resulting in an altered fibrinogen which is spontaneously capable of clotting. The studies summarized below describe, for the first time, a synthetic substrate which thrombin hydrolyzes, and firmly establish the proteolytic action of this enzyme. The hydrolysis of the synthetic substrate can be measured by simple titration or colorimetry, and makes available a new tool for the

study of thrombin. The methods are readily adapted to plasma for the quantitative measurement of prothrombin in clinical states.

While characterizing the streptokinase activated plasma proteolytic enzyme (plasmin) by its ability to split specific amino acid esters, it was found that one of the plasmin substrates, tosyl arginine methyl ester (TAME), was split enzymatically by thrombin preparations of human, bovine, and rabbit origin. The enzyme in the thrombin preparations, capable of hydrolyzing this new type of synthetic substrate for proteolytic enzymes, was not plasmin. When plasmin and thrombin preparations of equal TAME splitting activity were compared, only the plasmin preparations were actively fibrinolytic, proteolytic for casein, and capable of splitting lysine ethyl ester.

Four lines of evidence indicate that the TAME splitting action of thrombin preparations is an integral part of the clotting activity of thrombin.

1. Extensive purification of thrombin results in a purification of TAME activity.

2. The activation of human plasma prothrombin by calcium and rabbit thromboplastin, or of highly purified prothrombin by 30% citrate, is associated with the appearance of a markedly increased enzymatic action on TAME.

3. In the presence of TAME, thrombin does not clot fibrinogen. After the TAME is hydrolyzed, clotting occurs.

4. Adsorbents which remove the clotting activity of thrombin preparations remove equivalent amounts of TAME splitting activity.

*The Relationship Between Fibrinolysis and Coagulation Defects.* N. RAPHAEL SHULMAN, Bethesda, Maryland. (Introduced by Thomas McP. Brown).

Fibrinolysis is the term used to describe the presence of an active proteolytic enzyme intravascularly. This enzyme, which digests fibrin clots, may occur in patients with a wide variety of diseases. It is frequently associated with low fibrinogen and prothrombin and occasionally with afibrinogenemia and fatal hemorrhage. Since a proteolytic enzyme which is capable of digesting fibrinogen and prothrombin can be derived from normal serum, it has been assumed that the enzyme responsible for fibrinolysis is also responsible for any concurrent decrease in fibrinogen and prothrombin.

In the present study, intravenous typhoid vaccine was used as a stimulus in human beings to provoke fibrinolysis and decreases in fibrinogen and prothrombin. It was found that there was no temporal or quantitative correlation between fibrinolysis and changes in these coagulation factors. In some instances it was possible to produce either effect without producing the other. It was demonstrated by *in vitro* methods that the enzyme responsible for fibrinolysis does not digest fibrinogen or prothrombin. (Additional differences between this enzyme and other serum proteolytic enzymes will be reported elsewhere.) Therefore, decreases in the coagulation factors, although frequently found in association with

fibrinolysis, must have some explanation other than the action of the fibrinolytic enzyme.

It was found that decreases in fibrinogen and prothrombin were always associated with a hypercoagulable state of the blood in which the clotting time decreased and platelets fell. It appears that the loss of coagulation factors can be attributed to their consumption in intravascular coagulation.

Thus it was shown that the enzyme responsible for fibrinolysis is incapable of causing hypoprothrombinemia and hypofibrinogenemia. Since fibrinolysis and decreases in these coagulation factors are so frequently associated, it appears that intravascular coagulation may be involved in the production of fibrinolysis. These observations offer an approach to the understanding of the mechanism and physiological significance of fibrinolysis.

*Transfers of Cellular Cations During Acute Respiratory Alkalosis and Acidosis Experimentally Produced in Man.*

R. B. SINGER, J. R. ELKINTON,\* E. S. BARKER and J. K. CLARK,\* Philadelphia, Pa.

In six normal subjects acute respiratory alkalosis was induced by hyperventilation and in another six subjects acute respiratory acidosis by inhalation of 7.5% CO<sub>2</sub>. The changes produced in urinary excretion of electrolytes, which have already been reported (*J. Clin. Invest.*, 1952, 31, 663 and *Fed. Proc.*, 1953, *in press*), consisted primarily of an increase (hyperventilation) or decrease (CO<sub>2</sub> inhalation) in the excretion rate of bicarbonate in association with potassium, as well as, or instead of, with sodium. The average change in excretion of potassium per period was +1.2 and -2.2 mEq., respectively. The changes in excretion of ammonia and titratable acid (hydrogen ion) were in a reciprocal direction to those of potassium.

In the same subjects, the concomitant transfers of cellular cations were calculated in relation to changes in the chloride space. Of these, hydrogen ion transfers were estimated from the difference between extrarenal changes in total "extracellular" bicarbonate and the release or uptake of hydrogen ion by buffer protein in blood. The results are indicated by the following average values in mEq. for changes in *respiratory alkalosis* and *respiratory acidosis* respectively:  $\Delta$  total "extracellular" bicarbonate - 124 and + 32,  $\Delta$  hydrogen of buffer protein - 37 and + 12,  $\Delta$  "cellular" hydrogen (or minus "cellular" bicarbonate) - 82 and + 23,  $\Delta$  "cellular" sodium + 67 and - 24, and  $\Delta$  "cellular" potassium - 2 and - 3. While ions outside of the chloride space are labelled "cellular," it is recognized that other depots such as bone may be involved.

Thus, *acute* respiratory acid:base disturbances result in both renal and body cellular cation responses, the former involving mainly potassium and hydrogen, the latter sodium and hydrogen (or bicarbonate). In relative magnitude, however, the cellular sodium:hydrogen exchange is by far the greatest, constituting 90 per cent or more of the total cation exchange involved.

*Effects of Phenylbutazone (3,5-dioxo-1, 2-diphenyl-4-n-butapyrazolidine) on Urate Clearance and other Discrete Renal Functions in Gout.* J. H. SIROTA and T. F. YU, New York, N. Y. (Introduced by A. B. Gutman).

The effects of phenylbutazone on the renal clearances of urate, inulin, endogenous creatinine, p-aminohippurate, sodium, potassium, chloride and phosphate, and upon Tm PAH, were studied in 13 gouty subjects. Intravenous phenylbutazone in 1-2 gm doses produced a rise in the clearance ratio Curate/C<sub>in</sub> from a mean control of 0.063 to peak values averaging 0.153 in 7 subjects, without significant alteration in C<sub>in</sub>. Curate/C<sub>in</sub> and/or Curate/C<sub>Cr</sub> were consistently elevated for 1-10 hours following the drug. Inconstant elevations of these ratios persisted for 18-20 hours. Tm PAH measured in 2 subjects revealed depressions following phenylbutazone from control values of 61.0 and 79.0 mg/min. to 14.0 and 12.9 mg/min. respectively. C<sub>PAH</sub> fell from a mean of 437 to 349 cc/min.

In 3 subjects maintained on a constant diet, intravenous phenylbutazone, 800 mg per day in divided doses, resulted in elevations of the urate clearances after one day in 2 subjects, and after 3 days in another. Depressions of sodium and chloride clearances occurred after the first day, producing a fall in the mean output of sodium from 72.8 to 19.5 meq/day, and chloride from 72.8 to 32.8 meq/day. Elevation of Curate/C<sub>Cr</sub> and depression of C<sub>Na</sub>/C<sub>Cr</sub> and C<sub>Cl</sub>/C<sub>Cr</sub> were still demonstrable 72 hours after discontinuance of the drug. The clearances of potassium and phosphate remained unaffected. In 4 subjects the administration of intravenous phenylbutazone either prior or subsequent to Benemid did not alter Benemid uricosuria.

It is concluded that phenylbutazone given intravenously is uricosuric, due to inhibition of renal tubular reabsorption of urate. In addition, phenylbutazone inhibits tubular transport of PAH, and produces sodium and chloride retention without affecting glomerular filtration rate.

*The Effect of l-Norepinephrine on Estimated Hepatic Blood Flow in the Normal Anesthetized Dog.* C. M. SMYTHE, J. P. GILMORE and S. W. HANDFORD, Camp Lejeune, N. C. (Introduced by George A. Perera).

Investigation of the circulatory effects of l-norepinephrine has revealed that it is a powerful vasoconstrictor of all vascular beds except the coronary circulation. However there has been little study of the effects of the pressor amines on the splanchnic vasculature. Estimated hepatic blood flow (EHBF) has been determined by the bromsulphalein (BSP) removal method in 27 mongrel dogs anesthetized with intravenous pentobarbital or a morphine-chloralose-urethane mixture. After three control determinations had been made, an intravenous infusion of l-norepinephrine was started (mean dose 0.91 μ/Kg./min.) which caused an increase of mean arterial pressure from 109 to 144 mm. Hg. After arterial pressure had reached a plateau (5 minutes), three more determinations were made over the next 20 minutes. Although in some experiments EHBF increased or decreased as much as 30% of the control figures, in most experiments

there was little change of EHBF. The mean figure for all experiments (32.4 ml./Kg./min.) showed no change from control. This was also true of extraction per cent of BSP. There was a significant increase of hepatic arterio-venous oxygen difference from 4.8 to 6.5 volumes per cent ( $t = 2.98$ ,  $P < .0015$ ). In turn, there was also a significant increase of splanchnic oxygen consumption (EHBF × Hepatic A-VO<sub>2</sub> Diff.) from 23 to 31 ml./min. ( $t = 2.60$ ,  $P < .0046$ ). Increases of hepatic venous oxygen unsaturation and hematocrit were not statistically significant. Splanchnic vascular resistance increased from 21,400 to 29,100 dynes sec. cm.<sup>-2</sup>, and this change is significant ( $t = 2.43$ ,  $P < .0075$ ).

In summary, l-norepinephrine was found to effect hepatic vasoconstriction and an increase of splanchnic oxygen consumption but no change of hepatic blood flow.

*Metabolic Balance Studies on a Patient Sustaining Large Renal Losses of Potassium.* RUSSELL D. SQUIRES, EDWARD J. HUTH and J. RUSSELL ELKINTON,\* Philadelphia, Pa.

Studies are reported on a patient sustaining large renal losses of potassium with serum concentrations of 1.8 to 3.0 mEq./l., a history of episodes of muscular weakness, dry mouth and tachypnea. Potassium intake of 214 mEq./day prevented recurrence of symptoms. Intake of 40 mEq./day reduced serum concentration to 1.2 mEq./l. and resulted in depletion of 4.0-5.7 mEq./kilogram body weight within four days with symptoms as described above. Resumption of augmented intake restored serum concentration to previous level, alleviated symptoms but failed to effect a positive balance. Blood pH revealed mild respiratory alkalosis during depletion.

Renal studies revealed no proteinuria or abnormal formed elements, daily specific gravity from 1.004-1.007 with attenuated response to water deprivation and pitressin and markedly delayed dye excretion. Acute studies demonstrated C<sub>PAH</sub> 167, PAH extraction 67%, Tm<sub>PAH</sub> 15 and, during a partially successful period of repletion, simultaneous C<sub>in</sub> 36, C<sub>Cr</sub> 44, C<sub>K</sub> 93, C<sub>K</sub>/C<sub>in</sub> = 2.60 and C<sub>K</sub>/C<sub>Cr</sub> = 2.20; 24-hour C<sub>K</sub>/C<sub>Cr</sub> ratios of 1.66 have been obtained. During ten 24-hour balance periods on augmented potassium intake, C<sub>K</sub>/C<sub>Cr</sub> ratios > 1.16 were obtained. In five of these periods the UV<sub>NE<sub>4</sub></sub> plus titratable acid was definitely reduced.

Negative potassium balances were associated with reduced values for UV<sub>NE<sub>4</sub></sub> plus titratable acid, an increasing urine pH and no change in undetermined anion, (UV<sub>Na+K+NE<sub>4</sub></sub>) - (UV<sub>Cl+PO<sub>4</sub></sub>). Negative sodium balances occurred independently of changes in potassium balance and were associated with marked increase in undetermined anion without commensurate increase in urine pH. Methyltestosterone failed to produce a positive potassium balance or to reduce the magnitude of loss during a depletion period, but caused large transfers of sodium out of the chloride space.

This patient's response will be compared to potassium depletion in normal subjects carried out in conjunction with this study.

*An Indicator Method for Study of Intestinal Absorption in Man.* MALCOLM M. STANLEY,\* Boston, Mass.

Absorption of individual food constituents can be measured by mixing a non-absorbable indicator with the diet and determining in the stools the ratio of indicator to various other appropriate fecal components. Quantitative collections of stools are unnecessary if a representative sample of indicator-labeled stool is obtained.

Three identical meals, each mixed with chromium sesquioxide, Cr<sub>2</sub>O<sub>3</sub>, a completely inert, non-absorbable indicator, are fed during a one-day period. The indicator is uniformly blended with the food, a crucial point especially in patients with malabsorption. Carmine is given before the second meal to identify further the mid-portion of the labeled stools. The reddest stools are analyzed. Different parts of such stools contain identical ratios of unabsorbed food constituent to indicator, thus confirming their representative character. Under usual conditions a satisfactory study of absorption can be carried out during a two-day period.

The ingested food contains known quantities of constituents (fat, protein, vitamins, etc.) and is mixed with known amounts of indicator. From (I), quantity of indicator ingested, and concentrations in the stool of a food constituent, (f), and indicator, (i), the absolute quantity of this food substance unabsorbed is calculated as  $\frac{I(f)}{(i)}$ . If (F) = quantity of this food component ingested, the percentage of this constituent unabsorbed equals  $\frac{I(f)}{(i)} \times 100$ . Absorption of several constituents can be studied simultaneously.

*Example:* Diet contained 100 grams fat, 6 grams indicator. Dried stools contained 140 mg./gm. Cr<sub>2</sub>O<sub>3</sub>, 120 mg./gm. fat. Percentage fat unabsorbed equals  $\frac{6 \times 120}{140} \times \frac{100}{100}$ , or 5.14.

Data concerning various indicators, minimum time necessary to feed the labeled diet, adequacy of dispersion of indicator in the food and stools, and comparison of indicator technique with the usual "balance" method will be presented.

*Antigenicity of Platelets and Evidence for Platelet Groups in Humans.* MARIO STEFANINI and GERALD PLITMAN, Boston, Mass. (Introduced by William Dameshek).

The antigenicity of platelets is demonstrated by production of specific antisera following their administration into heterologous species. We have also observed that patients with "amegakaryocytic" thrombocytopenia exhibit progressively less response to successive platelet transfusions and eventually become resistant to transfused platelets from any donor. Plasma from such patients may then induce significant thrombocytopenia in normal recipients. Transient thrombocytopenia may develop in non-thrombocytopenic patients after multiple transfusions. Platelet pan-agglutinins are demonstrable in all these individuals. Finally, agglutinins found in 48% of 39 cases

of "chronic" idiopathic thrombocytopenic purpura may imply platelet auto-sensitization.

Normal plasma causes transient thrombocytopenia in 80% of normal recipients, suggesting platelet incompatibility and specific platelet groups. Platelet-free plasma and platelet-rich plasma obtained from 215 different, normal, never previously transfused humans, using 1/10 volume Sequestrene-Na<sub>2</sub> 0.7% and Siliconized surfaces, were incubated with each other (for 90 minutes, at room temperature). This technic demonstrated agglutinins in 6% of these plasmas; these were consistently specific for only certain platelet suspensions. Thus they were different from platelet pan-agglutinins mentioned above. No stimulus for the formation of these agglutinins could be established and thus they were probably naturally occurring. In all instances, platelets of the agglutinators were *not* reciprocally clumped by the plasmas of the agglutinated individuals. Statistical analysis demonstrated apparent existence of two platelet agglutinogens (I and II). On this basis, four platelet groups are tentatively proposed (unrelated to red cell groups): I—9%, II—3%, III(I + II)—4%, IV(O)—84% of white population.

Repeated administration of compatible platelets to normal humans failed to induce iso-immunization. Otherwise normal plasma, infused into a platelet incompatible recipient, produced striking temporary thrombocytopenia.

*The Relationship of Streptococcal Infections to Acute Glomerulonephritis.* CHANDLER A. STETSON, CHARLES H. RAMMELKAMP, JR.,\* WILLIAM D. PERRY, RICHARD M. KRAUSE and ROLAND J. KOHEN, Cleveland, Ohio.

An epidemic of acute glomerulonephritis occurring in a military population was studied to determine the relationship between this disease and streptococcal infections. A total of 401 patients with exudative pharyngitis was observed during the acute illness and at 14 and 21 days after the onset of the respiratory infection. Throat cultures and serum samples were obtained at weekly intervals, and modified Addis counts were performed on urine obtained each day. It was found that types 3, 6, 12 and 19 group A streptococci were responsible for the majority of the cases of streptococcal pharyngitis. No cases of acute nephritis were observed in 118 patients infected with types 3, 6 or 19 streptococci, but 15 of 191 patients with type 12 streptococcal infections developed nephritis with albuminuria, red blood cell casts and persistent hematuria. A latent period, averaging 10.5 days, intervened between the onset of the acute streptococcal pharyngitis and the onset of hematuria in these cases.

The administration of penicillin to 50 of the 191 patients with type 12 streptococcal infections appeared to prevent the subsequent development of acute nephritis, while gamma globulin administration in 30 patients was followed by the development of acute nephritis in six cases.

These findings indicate that the capacity to produce acute glomerulo-nephritis is related to the type of infecting streptococcus, and thus suggest that there are basic differences in the nature of the relationship between strepto-



coccal infections and the "non-suppurative complications, nephritis and rheumatic fever."

*The Use of Hemolysis Inhibition in the Study of Experimental and Clinical Hyperlipemia.* GENE H. STOLLERMAN, New York, N. Y. (Introduced by Colin M. MacLeod).

The nature of the inhibition of certain hemolytic systems by hyperlipemic sera of rabbits and of human beings may reflect changes in the composition and physicochemical state of serum lipids which are not readily detectable by conventional analytical methods.

The sera of cholesterol-fed rabbits become intensely inhibitory against streptolysin O when serum cholesterol concentration reaches 200 to 300 mg. per cent and exceeds the concentration of serum phospholipids. Injection of heparin intravenously enhances this inhibition in the absence of changes in the absolute or relative concentrations of cholesterol and phospholipids.

In contrast, the sera of alloxan diabetic rabbits and of rabbits injected intravenously with Triton WR 1339 (a non-ionic detergent) do not inhibit streptolysin O despite comparable elevation in serum cholesterol. In these animals the concentration of serum phospholipids usually exceeds that of cholesterol and they are less prone to develop atherosclerosis. The sera of Triton treated rabbits produce a "prozone" effect whereby streptolysin O inhibition is apparent only when serum is assayed in high dilution.

Sera obtained from patients with the nephrotic syndrome and with other hyperlipemic states do not inhibit streptolysin O despite extreme degrees of hypercholesterolemia except in rare instances where serum cholesterol concentration significantly exceeds the concentration of phospholipids. The reported marked non-specific inhibition of streptolysin O by the sera of some patients with infectious hepatitis may be related to the above observations.

Inhibition of the hemolytic activity of saponin by human and by rabbit sera, in contrast to streptolysin O inhibition, reflects in general the absolute concentrations of cholesterol and phospholipid regardless of the method by which hyperlipemia is induced and is unaffected by the administration of heparin.

*Studies of the Mechanisms of Destruction of Transfused Incompatible Erythrocytes by Isoantibodies.* SCOTT N. SWISHER, JR., WILLIAM A. O'BRIEN, JR. and LAWRENCE E. YOUNG,\* Rochester, New York.

Transfusions of incompatible dog red cells were given in 20 experiments to immunized recipient dogs in an effort to elucidate the mechanisms of *in vivo* destruction of erythrocytes by isoantibodies. The isoantibodies studied were of 6 different specificities and differed considerably in *in vitro* behavior. Disappearance of transfused incompatible red cells was measured by a method of quantitative differential agglutination. Observations on erythrocyte fragility, plasma heme pigment concentrations, serum antibody and complement content, and erythrophagocytosis

by peripheral blood leukocytes were made on blood samples from the recipient during many of the experiments. In some experiments, observations designed to detect splenic erythrophagocytosis were made.

Some dog isoantibodies (canine anti-A and alpha.) were found capable of destroying large volumes of transfused incompatible red cells within a few minutes. Other antibodies destroyed incompatible cells much more slowly, the cells disappearing over periods ranging from a few days to several weeks. One isoantibody (canine anti-C) had no effect upon the survival of incompatible erythrocytes, in spite of the fact that an excess of the antibody was present in the plasma of the recipient during the time that the "incompatible" cells were circulating. This antibody nevertheless produced well marked hemagglutination *in vitro* at body temperature.

These observations show that the canine isoantibodies differ widely with regard to their capacity to promote destruction of incompatible red cells *in vivo*. Red cells that have reacted with an hemolysin, such as canine anti-A, are rapidly destroyed by the action of complement. Antibodies that fail to fix complement or to hemolyze red cells *in vitro* may nevertheless promote erythrocyte destruction *in vivo* and probably do so by a number of mechanisms. The mechanisms operating in such instances are of general biological interest and are of clinical importance since most human isoantibodies, like most dog isoantibodies, are agglutinins not hemolysins *in vitro*.

*The Response of the Kidney and Body Fluids to Hypertonic Saline in Normal Subjects and in Patients with Diabetes Insipidus.* ROBERT TARAIL and GEORGE R. LOGSDON, Pittsburgh, Pa. (Introduced by Jonas E. Salk).

Excretion and exchanges of electrolytes and water were compared in normal adults to subjects with untreated diabetes insipidus in nine studies involving rapid intravenous infusion of large quantities of 2.5% saline (4.0 to 6.3 millimoles of NaCl per kilogram). Experimental preconditions included mild hydropenia in normals only, and water loading with or without prolonged potassium chloride administration in both groups. Antecedent diets were uncontrolled.

Diabetes insipidus appeared neither to impair nor enhance slow renal disposition of loads of sodium or chloride under all circumstances. Nor did prior ingestion of potassium chloride appear to affect excretion of saline loads. Nevertheless, sodium chloride disposal was diminished in one hydropenic normal whose previous diet and control urines were rich in salt.

The apparently normal urinary excretion of injected saline in diabetes insipidus entailed, however, sacrifice of body water—especially intracellular water presumably. Thus, whether or not potassium chloride was taken, saline augmented urine flow sufficiently to accelerate negative water balance. Therefore hypertonicity of extracellular fluid, defined by unchanged or increased concentrations of extracellular sodium, persisted for hours after saline administration in subjects with diabetes insipidus.

This contrasted with: (1) Fall in extracellular sodium toward control concentrations in all other experiments except when excessive salt retention occurred; (2) Increased positive water balance produced by saline in all normal subjects.

Based upon chloride space and urinary excretion data, cellular potassium losses continued after saline administration in all experiments, potassium excretion was not profoundly altered, and changes in intracellular sodium appeared erratic and insignificant.

The stimulus of salt loads in diabetes insipidus, therefore, produced no discernible disturbance of excretion or metabolism of solutes measured. These findings are consistent with the hypothesis that reduction in effective anti-diuretic activity did not directly or strikingly alter excretion or metabolism of sodium, potassium, or chloride.

#### *Tissue Electrolytes in Renal and DCA Hypertension.*

LOUIS TOBIAN, JR. and JOHN BINION, Boston, Massachusetts. (Introduced by Marian Ropes).

Cells of the normal rat aorta, mainly muscle cells, exhibit an extremely atypical intracellular composition. The average intracellular concentration must be somewhere between 150-151 meq./l. for sodium, 81-93 meq./l. for chloride, and 102-150 meq./l. for potassium, assuming that there are no insoluble extracellular salts of both sodium and chloride. Such salts have never been described in aortas. Chloride values were corrected for collagen chloride. Analyses of human aorta surgical specimens and necropsied renal arteries also indicate intracellular sodium concentrations around the same order of magnitude.

In a group of rats with severe renal hypertension, the aorta stripped of adventitia contained 34% more water, 16% more sodium, 43% more potassium, and 13% less magnesium per gram of fat-free solids than aortas from normotensive rats. Even rats with the kidney operation that did not develop hypertension showed 7% more water and 20% more potassium than normal. In contrast to aorta, brain and skeletal muscle showed relatively insignificant chemical alterations, in relation to renal hypertension.

Aortas in DCA hypertensive rats showed similar chemical alterations: 19% more water, 22% more sodium, and 15% more potassium than normal. Brain potassium in these rats was 10% above normal: muscle potassium, 17% below normal. Thus in brain and aorta, DCA produces a rise instead of the expected decrease in potassium content.

The aorta and plasma chloride content was not significantly abnormal in any of these groups, making it unlikely that the increased sodium and water content of the hypertensive aortas is due to a plethora of extracellular fluid.

Most of the increased water, sodium, and potassium in hypertensive aorta is located intracellularly, inside arterial muscle cells. These alterations in composition may be fundamentally related to arteriolar narrowing in hypertension.

#### *Potassium Exchange in Sickle Cell Anemia Red Cells.*

D. C. TOSTESON, Upton, New York. (Introduced by Lee E. Farr).

Potassium exchange in red cells from 6 patients with sickle cell anemia (SCA) was studied in vitro using  $K^{42}$  as a tracer. Heparinized blood diluted with an isotonic bicarbonate buffer was incubated under controlled conditions of temperature, gas composition and pH. Measurements made on packed cells were corrected for trapped plasma as determined by  $I^{125}$  labelled human serum albumin. Rates of K entrance into ( $\rho_{in}$ ) and exit from ( $\rho_{out}$ ) red cells were calculated in terms of mEq. K/1 RBC. hr.

SCA red cells incubated in  $O_2$  remained unsickled and in the steady state with respect to K exchange ( $P_{in} = P_{out} = 2.0$  to  $2.5$  at  $37^\circ C$ ) as was the case with normal human cells in  $O_2$  or  $N_2$  ( $\rho = 1.5$  to  $2.0$  at  $37^\circ C$ ). SCA red cells incubated in  $N_2$  became sickled and lost K rapidly for the 1st 2 hours and slowly for the next 48 hours.  $\rho_{out}$  is initially very high (15 at  $37^\circ C$ ), dropping in the first 2 to 3 hours to a value only slightly above  $\rho_{in}$ .  $\rho_{in}$  is 4 to 6 throughout the first 6 hours of incubation at  $37^\circ C$ .  $\rho_{in}$  trebled with a tenfold increase in the [K] in the medium in deoxygenated but not in oxygenated SCA red cells.  $\rho_{out}$  was not clearly related to [K] in the SCA cells in either  $O_2$  or  $N_2$ . Apparent activation energy of inward and outward K transport processes was equal for SCA and normal red cells in  $O_2$  or  $N_2$ . The acceleration of K exchange caused by deoxygenation of SCA red cells in vitro persisted despite reduction of reticulocytosis effected by having patients breathe 50%  $O_2$  for 1 to 2 weeks. These data are discussed with relation to the mechanism of transport of K across the surface ultra-structure of the red blood cell.

#### *Adrenal Cortical Reserve and Its Relation to Stress.*

FRANK H. TYLER,\* AVERY A. SANDBERG and KRIS EIK-NES, Salt Lake City, Utah.

By measurements of 17-hydroxycorticosteroid levels in plasma we have demonstrated that epinephrine does not cause its adrenocortical-like effects in humans by raising the concentration of these substances. This fact raises the question of the relation of the adrenal cortex to other types of stress in the human. We have studied this problem in two ways. (1) Plasma levels of 17-hydroxycorticosteroids have been determined during and after clinical stresses such as infections and during and after artificial stress such as intravenous insulin tolerance tests. Most of these were not associated with significant elevations of steroid levels. On the other hand prolonged surgical operations, particularly when complicated by bleeding or shock, and certain other events which are best summarized as "life-threatening" were associated with marked elevation of circulating adrenal steroids. (2) In an attempt to determine the role of disposition of these compounds, in contrast to endogenous secretion of ACTH and cortical steroids, a technique has been devised for the estimation of acute adrenal cortical reserve. The continuous intravenous administration of twenty-five units

of ACTH over a six hour period results in a predictable rise in plasma steroids in humans with normal adrenal function when they are in the basal state. The amount of this rise is not increased by giving larger doses over the same period. Thus it appears that this dose produces maximal stimulation of the adrenal. However, we have observed that the rate and the magnitude of the rise of plasma steroids during severe stress may exceed that which is produced by intravenously administered ACTH. Nevertheless, in such cases, when the plasma steroid level is already greatly elevated an additional increment of the usual degree can be induced by the intravenous administration of ACTH. These findings suggest that factors other than adrenal secretion may play an important role in the production of elevated steroid levels.

*Direct Effects of the Anterior Pituitary on the Ventral Prostate in Rats.* W. P. VANDERLAAN, Boston, Massachusetts. (Introduced by Samuel Proger).

In rats castrated at 21 days the administration of oxycel-purified corticotropin (Fraction D) for 9 days resulted in 5-fold enlargement of the adrenals, but no significant hypertrophy of the ventral prostate. Whole anterior pituitary powder and crude corticotropin (Fraction A) caused slight enlargement of the ventral prostate in castrated rats; neither growth hormone nor prolactin (Schering) preparations were more active than the whole gland powder.

To obtain a more sensitive test object 21-day-old rats were hypophysectomized and castrated and were found to respond over a 10-day injection period to graded doses of testosterone propionate with less ventral prostate enlargement than rats castrated but not hypophysectomized.

The alkaline phosphatase activity of the ventral prostate was lowered by castration and still further lowered by hypophysectomy and castration.

In hypophysectomized castrated rats a crude extract of beef anterior pituitary nearly restored the responsiveness of the ventral prostate to testosterone propionate. Corticotropin, growth hormone and prolactin were not active in this regard.

No evidence for an androgen of adrenal origin in the rat was found in these experiments. The pituitary has a direct effect upon the ventral prostate, and crude pituitary extracts afforded partial replacement after hypophysectomy. It is not known whether the effect is due to a single or to multiple pituitary factors.

*Studies on the Mechanism of Hemolysis in Primaquine-Sensitive Negroes.* I. M. WEINSTEIN, CAPT. R. J. DERN and D. W. TALMAGE, Chicago, Ill. (Introduced by Alf S. Alving).

The peculiar sensitivity of certain Negroes to the antimalarial drug, primaquine, as evidenced by hemolysis of their red blood cells has been investigated with the aid of  $\text{Na}_2\text{Cr}^{54}\text{O}_4$  tracer studies. Preliminary work has shown that this isotope specifically tags the red blood cell and affords an accurate method of studying red blood cell survival *in vivo*.

Red blood cells from Negroes known to hemolyze when given primaquine were labelled with  $\text{Na}_2\text{Cr}^{54}\text{O}_4$  and injected into Negro and white subjects known not to hemolyze when given the drug. After a control period therapeutic doses of primaquine (30 mg. base daily) were given to one-half of these subjects. Hemolysis of the injected cells began in about 24 hours, as demonstrated by a marked fall in the concentration of the  $\text{Na}_2\text{Cr}^{54}\text{O}_4$  per cc. of the recipient's blood, whereas the decline of the concentration in the subjects not receiving the drug represented a normal survival curve.

When red blood cells from subjects not sensitive to primaquine were similarly labelled with the isotope and injected into other non-sensitive subjects, no evidence of hemolysis was obtained whether the recipients received primaquine or not. When these non-sensitive cells were injected into sensitive Negroes, who were not receiving primaquine, the red cell survival curve was also normal. However, if primaquine was then given, the concentration of the  $\text{Na}_2\text{Cr}^{54}\text{O}_4$  in these Negroes increased, indicating that only their own cells were hemolyzed.

Osmotic fragility was normal, and the sickle and Coombs tests were negative in all subjects throughout the investigations. These results indicate that a specific idiosyncrasy exists between the red cells from certain Negroes and the drug primaquine, unrelated either to sickling or to an aberrant breakdown of the drug.

*The Use of Calcium Pantothenate for Myasthenia Gravis.* DAVID R. WEIR, Cleveland, Ohio. (Introduced by Joseph T. Wearn).

If the basic metabolic defect in myasthenia gravis is accepted to be deficiency of acetylcholine, it is logical to attempt to increase the production of this substance, hoping that the defect may be corrected. In tissue homogenates acetylation of choline occurs in the presence of acetate, coenzyme A, and ATP. A basic component of coenzyme A is pantothenic acid. As this substance is easily available it was selected as a therapeutic agent which might possibly promote acetylation *in vivo*.

Eight myasthenia gravis patients have been treated for periods up to nine months in duration with calcium pantothenate in oral and parenteral doses up to 1.5 grams daily. Two patients have shown remarkable improvement; two others have shown moderate improvement; the other four have shown no improvement. For one of the patients complete remission occurred while the vitamin was being given, followed by relapse with placebos, then a second remission with vitamin, then a second relapse with placebos, then a third remission with vitamin. For a second patient complete remission occurred while the vitamin was being given. With placebos such a severe relapse occurred that she has refused to stop her neostigmine intake, although she has had complete remission while taking both neostigmine and calcium pantothenate. In these two patients remissions occurred within one to five weeks after starting the vitamin; relapses occurred within seven weeks after starting placebos.

If calcium pantothenate has had an effect on some of

the patients, the failures might be explained on the basis of the existence of more than one metabolic defect capable of producing the myasthenia syndrome. Also, if an effect has occurred, it might be assumed that a local rather than a general defect in acetylation exists or that there is a higher requirement for acetylcholine at the myoneural junction.

*Utilization of S<sup>35</sup> Labelled L-Cystine by Normal and Leukemic Leukocytes.* AUSTIN S. WEISBERGER and BENNETT LEVINE, Cleveland, Ohio. (Introduced by Joseph M. Hayman, Jr.).

Disorders of sulfhydryl metabolism have frequently been implicated in leukemia. The observation that *l*-cysteine modifies nitrogen mustard induced leukopenia and that this effect is dependent upon a specific structural configuration suggests that *l*-cysteine or related compounds may have a unique role in leukopoiesis. Accordingly, the utilization of radioactive *l*-cystine by normal and leukemic leukocytes was investigated.

S<sup>35</sup> labelled *l*-cystine was administered orally in doses of 3 microcuries per kg. and blood samples obtained serially. Leukocytes were separated following sedimentation of erythrocytes and the radioactivity per ml packed leukocytes was determined.

A rapid uptake of S<sup>35</sup> by leukocytes was observed in acute leukemia. High counts were obtained in 10 minutes and maximum values in one hour. By comparison, normal leukocytes incorporated *l*-cystine S<sup>35</sup> slowly. Low counts were observed initially and counts comparable to those observed within 30 minutes in acute leukemia were not obtained for 3 days. Maximal counts were obtained in 5 to 6 days.

Although the rate of incorporation of S<sup>35</sup> in leukemic leukocytes was more rapid, the total amount of S<sup>35</sup> incorporated was greater in normal leukocytes. This may be due to dilution of S<sup>35</sup> by the known increased sulfhydryl pool in leukemia or to a greater demand on available S<sup>35</sup> by increased numbers of leukocytes with an increased avidity for *l*-cystine.

The pattern of utilization of labelled *l*-cystine in chronic myeloid leukemia was similar to that of normal leukocytes. In blastic chronic myeloid leukemia, the utilization was similar to that of acute leukemia. Preliminary studies with S<sup>35</sup> methionine and C<sup>14</sup> formate reveal similar patterns of utilization.

The data indicate that *l*-cystine is readily utilized by normal and leukemic leukocytes and that *l*-cystine or related compounds may have an important role in leukopoiesis.

*Splanchnic Ketone Production Before and During the Intravenous Infusion of Sodium Octanoate.* EMILE E. WERK, JR., LADD W. HAMRICK, JR., J. D. MYERS \* and FRANK L. ENGEL,\* Durham, North Carolina.

The ability of the human liver to manufacture ketones under essentially normal physiological conditions has not, heretofore, been measured quantitatively. The availability of (1) hepatic venous blood sampling (by catheter)

and (2) a substrate for ketone production (sodium octanoate), which can be administered intravenously, has made it possible to estimate splanchnic ketone production in man, fasting and during sodium octanoate loading.

Seven mildly ill patients between ages 25 and 62, without metabolic abnormality, fever, hepatic or renal disease, have been studied. All but one were studied after an overnight fast; the exception, after a six hour fast. In each subject after control measurements, 500 ml. of 1.5 percent solution of sodium octanoate was administered intravenously over approximately one hour, during which time the various measurements were repeated.

The mean control ketone levels ( $\mu$ M% whole blood, as acetone)  $\pm$  S.E. were: hepatic vein  $15.3 \pm 2.66$ ; femoral artery  $12.0 \pm 1.88$ ; difference  $3.3 \pm 0.85$  ( $p < .01$ ). During octanoate infusion: hepatic vein  $58.8 \pm 4.53$ ; artery  $37.8 \pm 3.54$ ; difference  $21.0 \pm 2.13$  ( $p < .01$ ). The increase in A-V difference was  $17.7 \pm 2.03$  ( $p < .01$ ).

The mean splanchnic ketone production (A-V ketone difference multiplied by hepatic blood flow, estimated by the BSP extraction method), expressed as  $\mu$ M/min/m<sup>2</sup>  $\pm$  S.E., was control  $26 \pm 5.3$  and during octanoate infusion  $163 \pm 15.4$  ( $p < .01$ ).

Further mean results  $\pm$  S.E., control and during octanoate infusion, respectively, were: hepatic blood flow (ml/min/m<sup>2</sup>)  $811 \pm 68.3$  and  $778 \pm 83.4$  ( $p > .5$ ); splanchnic glucose production (mgm/min/m<sup>2</sup>)  $73 \pm 8.9$  and  $75 \pm 10.7$  ( $p > .5$ ) and splanchnic oxygen consumption (ml/min/m<sup>2</sup>)  $41 \pm 3.8$  and  $45 \pm 2.3$  ( $p > .05$ ).

Thus, a small but significant endogenous splanchnic ketone production was measured in seven mildly ill patients in the post-absorptive state and was increased an average of six-fold during the intravenous administration of sodium octanoate. The hepatic blood flow, splanchnic glucose production, and splanchnic oxygen consumption remained essentially unchanged.

These data should serve as a useful baseline in assessing the effects of various factors on hepatic ketone production in man.

*Experimentally Produced Phlebothrombosis in the Study of Thromboembolism.* STANFORD WESSLER, Boston, Massachusetts. (Introduced by Herrman L. Blumgart).

A method has been developed whereby intravascular thrombosis may be routinely produced in dogs without initial endothelial injury. The technic is based on the use of isolated venous segments and the systemic infusion of serum fractions containing clot accelerator substances. In veins examined one to 43 days after the induction of thrombosis, the sequential histopathology was that of a phlebothrombosis.

Studies have demonstrated that retarded venous flow combined with a temporary increase in serum accelerator activity can induce intravascular thrombosis in the absence of both endothelial damage and complete stasis. Since serum and stasis are common by-products of major surgery, they may together represent a trigger mechanism

whereby post-operative thrombosis is initiated. Both dicumarol and heparin block this mechanism.

Intravenously administered enzar, a purified trypsin preparation, has recently been reported to dissolve intravascular clots in animals and man. Rapid systemic infusion of 100,000 units of enzar routinely induced striking fresh clot propagation upon preformed thrombi in jugular veins. Moreover, this enzyme preparation successfully replaced serum fractions in the induction of experimental intravascular coagulation. Dicumarol, in toxic doses, failed to inhibit these trypsin-induced thrombi, whereas heparin, in therapeutic amounts, effectively blocked their production. This suggests that dicumarol which depresses prothrombin conversion may not provide as optimal an anticoagulant effect as heparin which blocks fibrin deposition through other mechanisms.

These studies have provided a new method for the investigation of the morphology, pathogenesis and therapy of thromboembolism.

*Natriuresis and Chloruresis Following Pitressin-Induced Water Retention in Non-Edematous Patients: Evidence of a Homeostatic Mechanism Regulating Body Fluid Volume.* RAYMOND E. WESTON, IRWIN B. HANENSON, JACOB GROSSMAN, GLORIA A. BERDASCO and MORRIS WOLFMAN, New York, N. Y. (Introduced by Louis Leiter).

To determine the response of non-edematous patients to expansion of total body fluid by primary water retention, the effects of giving 3-10 units of Pitressin Tannate in Oil (PTO), in divided doses for 5-20 days, on body weight, blood electrolytes, eosinophil counts and metabolic balances for sodium, chloride, potassium, phosphorus, nitrogen and water were studied. On intakes of 2000-3000 ml. of fluid and either 15 m.Eq. or 75-170 m.Eq. of sodium, the typical response to PTO was as follows: 1) Initially, oliguria without change in electrolyte excretion; 2) weight gain due to water retention; 3) despite the continuing 20 to 30 m.Eq./l. drops in serum electrolyte levels, 70 to 370 m.Eq./day increases in urinary sodium and chloride excretion, with resulting osmotic water diuresis; 4) within 24-48 hours after discontinuing PTO, profuse diuresis of water with little sodium until 4th-5th day when weight fell below control levels, because of the preceding natriuresis; 5) subsequently, persistent sodium and chloride retention even after serum electrolytes returned to normal.

In several patients, profuse electrolyte diuresis occurred after the body water expansion, despite eosinopenia and metabolic evidence of adrenocortical discharge. In one patient on high salt intake, 120-160 units of corticotropin daily did not prevent the natriuresis and chloruresis. However, another, who on DOCA (5-7½ mgm. b.i.d.) and PTO retained water and gained 5 kgm. in weight, did not develop the electrolyte diuresis she exhibited on PTO alone.

Apparently, PTO-induced expansion of total body fluid, in non-edematous subjects as contrasted to cardiac or congestive failure, may activate homeostatic mechanisms

which attempt to regulate fluid volume by producing natriuresis and chloruresis, without regard to the hypotonicity of the extracellular fluid and despite increased adrenal cortical discharge.

*The Prevalence and Significance of Hypercholesterolemia Among Children and Siblings of Patients with Hypercholesterolemic Xanthomatosis.* EDWIN O. WHEELER and HOWARD B. SPRAGUE, Boston, Massachusetts. (Introduced by Edward F. Bland).

Studies have been made to determine the prevalence of hypercholesterolemia in 166 relatives of 10 patients who have a disease characterized by hypercholesterolemia, clear serum, and tendon nodules. Eight of the 10 had xanthelasma; 2, xanthoma tuberosum. Hypercholesterolemia is defined as a serum cholesterol greater than 309 mg.% (mean plus two standard deviations of a control series). In 10 families in which one parent was affected (hypercholesterolemic xanthomatosis) and one parent was normal, 13 (46.4%) of 28 children were hypercholesterolemic. In 14 families in which one parent was affected or probably affected and one parent was normal or probably normal, 19 (51.4%) of 37 children were hypercholesterolemic. In 10 families in which both parents were normal or probably normal, one of 21 children was hypercholesterolemic.

The prevalence of hypercholesterolemia among the siblings of affected persons was compared with the expected prevalence computed by Hogben's method, assuming simple Mendelian dominance and one affected parent. Of 40 members of 9 sibships, 22 were hypercholesterolemic whereas 21.2 were expected to be so affected.

Hypercholesterolemia was not significantly related to sex or age. However, tendon nodules were related to age, occurring in 75.0% of hypercholesterolemics over 40 but in only 10.0% of hypercholesterolemics under 20 (index cases omitted).

Lipoproteins of the Sf<sub>12-20</sub> class measured in 108 of these subjects were not reliable indexes of this disorder, being elevated (greater than the mean plus two standard deviations of a control series) in only 3 of 22 persons with hypercholesterolemic xanthomatosis, in 3 of 24 persons with hypercholesterolemia alone, and in none of 62 normocholesterolemics.

It is concluded that hypercholesterolemia in the relatives of patients with hypercholesterolemic xanthomatosis is synonymous with this disorder, and that hypercholesterolemic xanthomatosis is a familial disorder which if inherited is probably transmitted by simple Mendelian dominance.

*Impaired Renal Response to the Antidiuretic Hormone in Chronic Renal Disease.* ABRAHAM G. WHITE, MARTIN KURTZ and GEORGE RUBIN, New York, N. Y. (Introduced by Milton Mendlowitz).

A deficiency of the neurohypophysial antidiuretic hormone (ADH) diminishes the "facultative" reabsorption of water in the renal distal tubule and causes diabetes

insipidus. "Nephrogenic" or "congenital" diabetes insipidus results from a congenital defect in the renal tubule making it unresponsive to ADH, thus producing polyuria, dehydration and polydipsia. We find that in patients with chronic renal disease the renal tubule also may be unable to respond to circulating ADH, resulting in the characteristic polyuria and hyposthenuria.

Studies were performed in four patients with diabetes insipidus and five with chronic renal disease (4 chronic glomerulonephritis, 1 chronic pyelonephritis) in order to establish points of physiological similarity and dissimilarity between these two groups of patients. Following the onset of intravenous hydration with 5% glucose (10 cc./min.) both patients with chronic renal disease and those with diabetes insipidus showed the absence of a peak diuresis (normally occurring at 85 minutes). During these experiments the patients with chronic renal disease excreted from 15.1 to 41.5% of the filtered water load (mean = 23.8%) on the basis of measurements of inulin and endogenous creatinine clearance. In response to the intravenous administration of 0.57 mU/Kg Pitressin to patients with diabetes insipidus and to those with chronic renal disease both groups demonstrated an equally shortened duration of antidiuresis ( $37 \pm 11$  and  $36 \pm 6$  minutes, respectively), but the "per cent inhibition" of diuresis was smaller in the renal group, an average of 13% as compared with a mean of 29% for the patients with diabetes insipidus.

It would appear that patients with chronic renal disease may excrete even greater proportions of the filtered water load than some with diabetes insipidus; moreover, the "per cent inhibition" of diuresis to ADH is decreased. It is therefore suggested that chronic renal disease may result in impaired renal response to ADH, an "end-organ defect" type of diabetes insipidus in contrast to the syndrome caused by deficiency of ADH which may be designated "central" diabetes insipidus.

*Blood Flow and Transcapillary Exchange in Hemorrhagic Shock.* ARNOLD H. WILLIAMS, HELEN K. SHEERWOOD and ALBERT EINHEBER, Washington, D. C. (Introduced by John R. Smith).

Conventionally both transcapillary exchange and capillary permeability are measured by determining the rate at which concentrations of injected solutes fall in plasma. The methods are based on the assumption that a fixed proportion of the solute leaves the vascular tree in each circulation. If the assumption were incorrect agreement between consecutive measurements would be impossible; as it is correct the rate of extravascular transfer must be affected by the speed with which solutes are carried to permeable areas by the blood stream. Since flow is involved it is important to determine if, and by how much, measurements are in error because of omission of the flow factor.

While a few studies of the effects of flow on transcapillary transfer have been made recently in isolated organ systems, to our knowledge this is the first in the intact animal. In the first ten minutes after injection there

was a linear relationship between the loss of deuterium from the circulation, computed on the basis of volume dilution in excess of blood volume ( $Fe^{58}$ ), and cardiac index (pressure-pulse contour) in dogs subjected to the Walcott hemorrhagic shock procedure, both before and after hemorrhage ( $p = 0.86$  and  $0.93$  respectively). Extravascular loss of T-1824 tagged albumin, however, was not related to flow nor was the later deuterium exchange.

After hemorrhage conventional measurements of permeability to deuterium showed a decrease and little or no change to albumin. However permeability actually was increased. When suitable corrections were made for the differences in blood volume and cardiac output induced by hemorrhage it was evident there was a relative increase of extravascular transfer. However exemia did not occur. Currently the effects of flow (Fick) on transfer of intermediate sized molecules are being studied.

*Sodium Maldistribution in Acute and Chronic Disease.*

GRAHAM WILSON, LAURA BROOKS and FRANCIS MOORE,\* Boston, Massachusetts.

The concept that a lowered plasma sodium concentration is due to a sodium deficiency must be reviewed. Our studies of this subject have been based on isotope dilution, both for total body water and total exchangeable sodium, with additional background study supplied by metabolic balances. The patients have fallen into three groups: heart disease without surgery, heart disease with surgery, and surgery without heart disease. We find that marked lowering of the plasma sodium concentration may occur in the presence of a normal or high total exchangeable sodium in all three groups of cases. In some instances, the extracellular sodium has clearly been diluted. In other instances the data suggest extensive disappearance of sodium from the extracellular phase. In addition, there is the small group of patients who have lost sodium by extrarenal routes and a very interesting though small group of patients who have uncontrolled renal sodium losses. And finally, there are patients suffering extensive trauma who, in the presence of normal renal sodium conservation and without extrarenal losses, show a depression of the plasma sodium concentration. Findings and theoretical interpretations will be discussed.

*Experimental and Clinical Studies on Small-Colony (G) Variants of Micrococcus pyogenes Obtained with Antibiotics.* ROBERT I. WISE and DORIS SERSTOCK, Minneapolis, Minn. (Introduced by Wesley W. Spink).

In attempting to select penicillin-resistant organisms from broth cultures containing penicillin-sensitive cultures of staphylococci by the Kirby technique, minute colonies were isolated on agar plates which were detectable by magnification. Pursuing this phenomenon further with 47 strains of staphylococci, 8 yielded G forms under the influence of penicillin, and 9 G variants were obtained from 10 cultures with erythromycin, magnamycin and bacitracin. Reverted large colonies were obtained from G cultures in the absence of antibiotics.

Bacteriophage typing\* and comparative cultural, metabolic and virulence studies were carried out with parent, small colony variants and reverted cultures. The parent and reverted strains were hemolytic; grew readily; coagulated plasma; did not produce penicillinase; resisted the killing action of human blood; and proved to be virulent when injected intradermally into rabbits and intravenously into mice. The G forms were nonhemolytic; the majority were coagulase negative; they grew less readily; possessed increased penicillin resistance, but without demonstrable penicillinase; were killed readily by human blood; and were avirulent for mice and rabbits. Typing with bacteriophage revealed loss of sensitivity by the G cultures to most bacteriophages but reversion was accompanied by return to a lytic pattern comparable to that of the parent strain.

This biologic phenomenon may be of considerable significance in the therapy of bacterial infections. Since the small colonies on agar plates are not readily apparent, sterile cultures of body fluids and tissues may be reported while patients are receiving antibiotics. These avirulent forms may be harbored in the tissues without symptoms and subsequent relapse may occur due to reversion to the virulent variety. Preliminary clinical studies support this contention in that 7 cultures of small colony variants of staphylococci have been isolated from 6 of 200 patients receiving antibiotics.

#### *Toxic Effects Following Placebo Administration.*

STEWART WOLF,\* and RUTH PINSKY, Oklahoma City, Okla.

The demonstration that major changes in vasomotor, glandular, smooth muscle and other physiological functions follow the administration of placebos has emphasized the need for caution in interpreting therapeutic effects. There is an equal need for caution in interpreting the occurrence of toxic reactions.

In an elaborately controlled "double blind" study of the effects of Tolserol on anxiety and tension on 31 patients in which 6 batches of "unknowns" were used and in which each patient acted as his own control during 6 separate two-week periods of observation; the effects of placebos were found to be almost identical with those of Tolserol. Twenty-five per cent showed improvement, 60% were unchanged and 15% became worse.

An equal number of minor or equivocal complaints such as light headedness, drowsiness and anorexia occurred with both Tolserol and placebos. Three patients had major reactions. One of them developed sudden overwhelming weakness, palpitation and nausea within fifteen minutes of taking her tablets. Identical reactions occurred following placebos and Tolserol. A second patient developed a diffuse itchy erythematous maculopapular rash after 10 days of taking pills. A skin consultant considered the eruption to be a typical dermatitis medicamentosa. After the pills were discontinued the eruption quickly cleared. The patient firmly refused to try an-

other batch of pills. Later it was learned that she had developed the rash while taking placebos. A third patient, within ten minutes of taking her pills, developed epigastric pain followed by watery diarrhea, urticaria and angioneurotic edema of the lips. After 48 hours and again after 96 hours, a second and third trial of pills produced the same reaction. This patient was shifted to another batch. When the same reaction followed again, she was given no further pills. When the batches were finally identified, it was found that she had developed her severe reactions on both Tolserol and placebos.

#### *The Influence of Enhanced Sodium Reabsorption by DOCA and Compounds E and F on the Rates of Excretion of Water, Potassium and Ammonia.* RICHARD WOMERSLEY, OSCAR A. THORUP and LOUIS G. WELT,\* Chapel Hill, N. C.

Renal tubular reabsorption of water may occur as a passive consequence of the active reabsorption of solutes (presumably in the proximal tubule) or, independently, under the influence of ADH (presumably in the distal tubule). Moreover, the exchange of potassium or ammonia for sodium is thought to occur only during the reabsorption of sodium in the distal tubule.

The influence of an enhanced reabsorption of sodium promoted by DOCA or Compounds E and F on the rates of excretion of water and electrolytes was studied in normal human subjects under conditions designed to eliminate the influence of ADH on the reabsorption of water. This state of physiologic diabetes insipidus was achieved by maintaining a positive balance of water of about one liter throughout each study.

The administration of DOCA decreased the rates of excretion of sodium, chloride, and water with only minimal increases in the rate of excretion of ammonia. The excretion of potassium was augmented in only one study. The administration of Compounds E and F decreased the rates of excretion of sodium with little or no decrease in the rate of excretion of water, and increased the rate of excretion of potassium. The rate of excretion of ammonia was minimally increased. The decrease in the rate of excretion of chloride was less than that for sodium.

These data may be interpreted to mean that DOCA promotes the retention of sodium in all portions of the tubule, and to the extent that this occurs proximally an obligatory reabsorption of water ensues. In contrast, Compounds E and F appear to influence the reabsorption of sodium in the distal tubule. These data suggest an explanation of the failure of DOCA to improve the so-called "water defect" in adrenal cortical insufficiency.

#### *Localization of Cardiac Defects by Dilution Curves Recorded Following Injection of T-1824 at Multiple Sites in the Heart and Great Vessels During Cardiac Catheterization.* E. H. WOOD\* and H. J. C. SWAN, Rochester, Minn.

Cardiac catheterization can not be relied on as a means of localizing right-to-left shunts and certain other cardiac

\* Performed with the cooperation of Dr. John E. Blair, New York City.

anomalies. This deficiency can be overcome by recording dilution curves of T-1824 in arterial blood following injections of dye via the catheter. Dilution curves were recorded photographically by means of earpiece and cuvette oximeters in 25 patients with acquired, 30 patients with cyanotic and 25 patients with acyanotic, congenital heart disease. When no right-to-left shunt was present, injection sites from pulmonary artery peripherally to brachial vein produced curves similar in contour but with time components progressively prolonged and concentration components reduced.

When, in the cyanotic patients, the injection of dye was made distal to the site of a defect, a dilution curve approaching normal contour was recorded. If, however, the injection was made at or proximal to the site of a defect, the appearance time was short and there was an abnormal initial deflection due to the passage of dyed blood into the arterial circulation through the defect. Thus it has been possible to localize veno-arterial shunts occurring through a patent ductus, a ventricular or an atrial septal defect.

In suspected tricuspid atresia it is possible, by comparing curves following injections into both atria, to decide whether a functional right ventricle passageway exists. Further, distinctive curves typical of the injection site may be recorded following injection of dye into aorta or pulmonary artery. Injections into the right and left pulmonary arteries or pulmonary veins (if entered) may allow differentiation of atrial septal defect and anomalous pulmonary venous drainage. The intracardiac injection technique is an established routine part of diagnosis and study of congenital heart disease in this laboratory.

#### *The Heterophile Agglutination Test and Its Interpretation.*

CHRIS J. D. ZARAFONETIS and VIRGINIA F. COLVILLE, Philadelphia, Pa. (Introduced by Richard A. Kern).

Studies in this laboratory indicate that certain discrepancies encountered in heterophile agglutination (HA) tests are due to factors which are not generally appreciated. For example, erythrocytes (SRBC) from different sheep may give widely different titers with the same serum specimen. It is, therefore, unsound (a) to compare titers of tests done with SRBC from different animals and (b) to make an arbitrary division between "normal" and titers which are "diagnostic" of infectious mononucleosis (IM). Furthermore, a false change in HA titer may result through use of different lots of SRBC on successive sera from a given patient.

It was also found that cold agglutinins (CA) for SRBC are often present in normal sera, as well as sera from patients with IM and other disorders. Laboratories recording HA titers after overnight refrigeration therefore report many false-positive results. Some CA for SRBC are absorbed by guinea-pig kidney suspensions, but all are dispelled simply by rewarming the tubes.

Finally, HA results must be interpreted in relation to the illness in question to have diagnostic value. Specifically, antibody dynamics during the course of illness and convalescence have to be demonstrated in IM in the same

manner as is required for the serologic diagnosis of other acute infectious diseases. The serologic diagnosis of IM cannot, therefore, be established by the HA test on a single serum specimen. Two or more sera from each patient must be tested simultaneously against the same suspension of SRBC in order to obtain a valid antibody curve.

*Rate of Disappearance and Metabolism of Hydrocortisone and Cortisone in the Synovial Cavity.* MORRIS ZIFF, HILDEGARD WILSON, DOMINICK SCIALABBA, EDWARD SCULL and JOHN GLYN, New York, N. Y. (Introduced by Currier McEwen).

The local effectiveness of hydrocortisone acetate when injected into arthritic joints has suggested study of the intra-articular processes which accompany the therapeutic response. At various time intervals after intra-articular injection of hydrocortisone, cortisone, and their acetates, the synovial fluid was aspirated completely and the synovial cavity washed with saline. The steroid compounds in the combined synovial fluid and washings were extracted and submitted to quantitative micro assay by three different methods.

It has been found that both hydrocortisone and cortisone are rapidly removed from synovial fluid since an average recovery of only about 10% of the amount injected was obtained after one hour. There was no consistent difference in the rate of removal of the two compounds, suggesting that the relative ineffectiveness of cortisone injected locally cannot be ascribed to failure of absorption. Three hours after intra-articular injection of hydrocortisone and cortisone as the free alcohols, only 1 to 2% could be found in the synovial fluid by Porter-Silber assay.

Evidence for the transformation of injected hormones to metabolic products was provided by paper chromatographic analysis of the post-injection synovial fluid extracts, which indicated three metabolites of hydrocortisone and two of cortisone. These were not found on chromatograms of extracts of synovial fluid obtained either before injection of hormone or from the uninjected contralateral joint. It is believed that the compounds found represent metabolites formed intra-articularly.

*Control of Cardiac Rhythm by an External Electric Pacer.* PAUL M. ZOLL, LEONA R. NORMAN and ARTHUR J. LINENTHAL, Boston, Mass. (Introduced by A. Stone Freedberg).

Electric impulses applied externally across the intact chest produced effective heart beats in dogs and in humans. Repeated electric stimulations over several weeks were not associated with cardiac or systemic injury. The intensity of current necessary to evoke cardiac responses depended on the resistance between the electrodes and the voltage and duration of the electric stimuli. The cardiac responses consisted of intermittent ectopic beats or regular tachycardia of supraventricular or ventricular type, depending on the frequency of the stimuli, their timing in the cardiac cycle and the location of the electrodes.

The method has been applied in 9 patients with cir-



culatory arrest. In 4 of 5 patients with complete heart block and Stokes-Adams attacks, regular effective ventricular beats were evoked by the external electric pacemaker. One patient was kept alive by the artificial pacemaker for 5 days of ventricular standstill until the intrinsic idio-ventricular pacemaker revived. In another patient multifocal ectopic ventricular beats and runs of ventricular tachycardia-fibrillation were replaced by the artificial externally controlled rhythm for 24 hours until the ventricular irritability subsided. In 1 of 3 dying, non-cardiac patients, a slow ventricular rhythm was replaced for a short time by a more rapid externally-paced ventricular

beat. Ventricular fibrillation occurring during surgery in 1 patient was uninfluenced by electric stimuli of maximal intensity.

In summary, the external, electric cardiac pacemaker behaves like a natural, intracardiac parasystolic focus but is under complete control with regard to frequency and duration of discharge and location of cardiac response. With this method various aspects of cardiac rhythmicity, the refractory period and paroxysmal arrhythmias have been studied. Its usefulness in the treatment of Stokes-Adams attacks due to ventricular asystole or tachycardia has been demonstrated.